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Effects of Aeration of Sawdust Cultivation Bags on Hyphal Growth of *Lentinula edodes*

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The effects of aeration through lid filters on the hyphal growth of *Lentinula edodes* (oak mushroom) in sawdust cultivation bags were investigated. The aeration treatment levels were traditional 27 mm hole cotton plugs, cotton balls and combinations of seven hole sizes × two hole positions (up and under) in the lids covering plastic bags containing 1.4 kg sawdust medium at 63% moisture that had been autoclaved for one hour and inoculated with sawdust spawn of *L. edodes* strain 921. Aeration treatment effects were measured based on the CO_2 concentration at the 15th wk, as well as the hyphal growth rate and degree of weight loss of bags every 14 days for 15 wk. In bags with traditional cotton plugs, the CO_2 concentration was 3.8 ± 1.3%, daily mean hyphal growth was 2.3 ± 0.6 mm and daily mean weight loss was 0.84 ± 0.26 g. In the bags with 15 mm diameter holes, the CO_2 concentration was 6.0 ± 1.6%, daily hyphal growth was 2.8 ± 0.2 mm and daily weight loss was 0.86 ± 0.4 g. The bags with 15 mm holes had a higher CO_2 concentration and lower water loss than bags with other hole sizes, but the hyphal growth was not significantly different from that of other bags. The weight loss of bags increased proportionally relative to the lid hole sizes. Taken together, these results indicate that traditional cotton plugs are economically efficient, but 15 mm hole lids are the most efficient at maintaining hyphal growth and controlling water loss while allowing CO_2 emissions.

KEYWORDS : CO_2 concentration, Hyphal growth, *Lentinula edodes*, Medium weight loss, Sawdust bag cultivation

Introduction

Lentinula edodes (Shiitake) is the second most popular edible mushroom in the world market [1, 2] and contains polysaccharides and substances for immune enhancement and antitumor activity [3, 4].

L. edodes mycelium does not grow well where oxygen is limited, and when it grows actively the O_2 demand becomes much higher than that of other mushrooms. O_2 and CO_2 are important factors in the cultivation of mushrooms. In general, mushrooms consume O_2 to secure the chemical energy needed for mycelial growth, and fruiting bodies then generate CO_2 [5]. Higher concentrations of CO_2 lead to elongation of the stipes of mushrooms, inhibited pileus development [6, 7] and hindered basidiospore development [8, 9].

Growing Shiitake in sawdust medium takes about 100 days, including about 60 days for mycelia growth and about 40 days for browning of the medium surface [5]. This cultivation time is relatively long when compared to the 20 to 30 days required for *Flammulina velutipes* and *Pleurotus ostreatus* [10]. Because oxygen is in high demand during the mushroom fruiting period, the air permeability of the filters or plugs attached to the culturing bags greatly affects development of the mushroom [11]. Insufficient ventilation due to small or clogged holes (Fig. 1) can result in poor mycelial growth, contamination and decreased mushroom production. Conversely, improving ventilation in shiitake cultural bags can shorten the mycelial growth period and thus reduce cultivation cost.

This study was conducted to investigate the effects of ventilation of cultivation bags on carbon dioxide concentration, mycelial growth and medium weight change in the bags.

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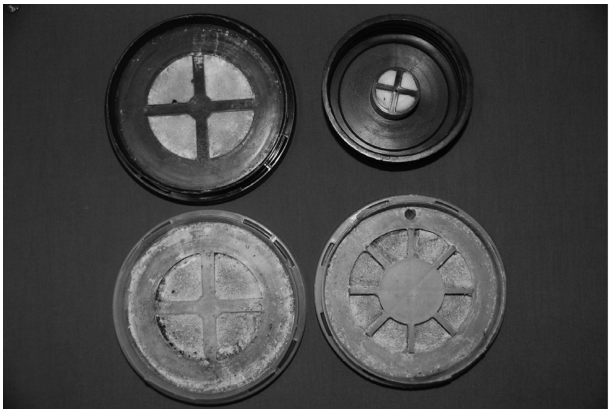

Fig. 1. Various plastic lids generally used for mushroom cultivation. Ventilation holes vary in size (15 to 45 mm) and number (4 to 8 holes), and can become clogged with growing hyphae.

Fig. 3. Measurement of CO_2 (%) in a 1.3 kg *Lentinula edodes* cultivation sawdust bag using a gas sampler (Model GV-110S; Gasteko, Ayase, Japan) and CO_2 core sword (2H, 2HH; Gasteko).

Materials and Methods

***L. edodes* sawdust cultivation bag preparation.** The *L. edodes* sawdust cultivation bags used in this study were a round pillar type, 10 cm in diameter and about 20 cm height Bags were filled with 1.4 kg medium composed of 8 : 2 mixtures of oak sawdust and rice bran, including 63% water content. Bags were inoculated with strain *L. edodes* 921. For ventilation, the top of the bag had a traditional 27 mm diameter cotton plug, a 23 mm round ball, or a 8 cm diameter plastic lid with a 15 to 45 mm hole on or under each lid and was plugged with a sponge. A total of 16 types of ventilation lids were used (Fig. 2) with three replicated bags each ventilation lid type and the bags were incubated at 25°C to 30°C for four months.

Measurements of CO_2 concentration, mycelial growth, and medium weight loss. The CO_2 concentrations were measured at wk 15. Specifically, the concentration of CO_2 in the middle part of the bags (Fig. 3) was measured with a gas sampler (Model GV-110S; Gasteko, Ayase, Japan) and CO_2 core sword (2H, 2HH; Gasteko). In addition, the mycelial growth and change in medium weight were measured under a dissecting microscope every one or two weeks for 15 wk.

Results and Discussion

Ventilation hole sizes and CO_2 concentration. The CO_2 concentration was 3.83 ± 1.33% in the bags with a traditional cotton plug and 3.76 ± 1.18% in the bags with a cotton ball plug (Fig. 4). The average CO_2 concentration was 6.05 ± 3.57% in the bags with a 15 mm hole and 3.64 ± 1.23% in those with a 5 mm hole. The CO_2 concentrations were generally 0.06 to 1.17% lower in bags with holes on both the upside and underside on the lid than in bags with holes only on the underside.

The carbon dioxide concentrations in the medium changed by 1.73~7.00% over 15 wk in the bags with the traditional cotton plugs and 1.50~7.28% in those with 5 mm or 10 mm ventilation holes. The CO_2 concentration in bags with only one ventilation hole underneath the lid was about 13.13%, while that of bags with holes on both sides of the lid was 11.35%. These findings indicate that

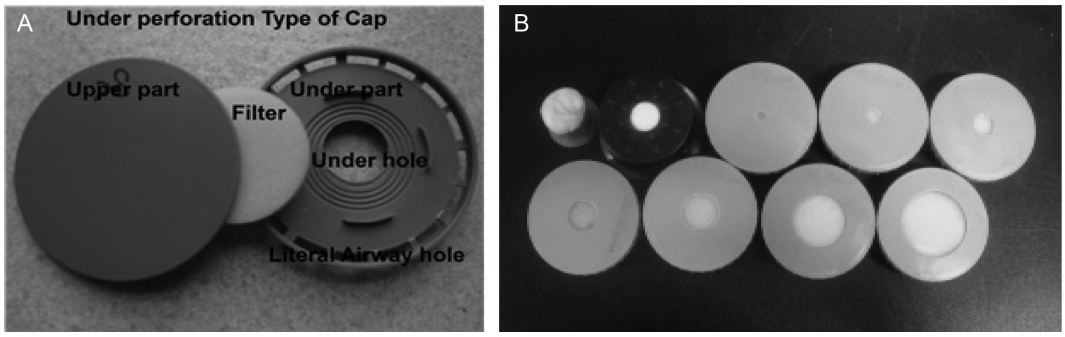

Fig. 2. Eight centimeter diameter lid structure with upper part, filter and lower part (A) and lids with traditional 27 mm cotton plug and various size (15 to 45 mm) ventilation holes (B).

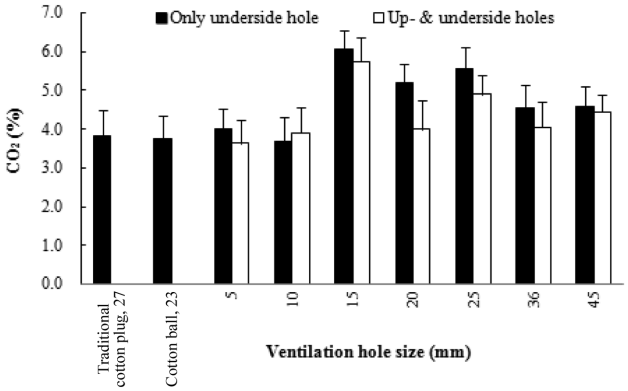

Fig. 4. Ventilation hole size and CO_2 (%) in *Lentinula edodes* cultivation bags over 15 wk. The black bars correspond to bags with only one hole on the underside of the lid, while the white bars indicate bags with holes in both the up and underside. Error bars represent the standard error of the mean (n = 3) per treatment.

as mycelia digest the substrate large amounts of CO_2 are produced [12].

Ventilation hole sizes and mycelial growth. As shown in Fig. 5, mycelial growth was greatest in bags with a 36 mm hole in only the underside of the lid (3.05 ± 0.17 mm/day), and slowest in those with a 5 mm hole (2.06 ± 0.53 mm/day). Except in the bag with 36 mm hole, the mycelial growth was higher in bags with holes on both sides of the lid than in bags with holes underside only. Mycelial growth in bags with a traditional cotton plug did not differ from that of bags with cotton balls, as indicated by values of 2.35 ± 0.64 mm/day vs. 2.44 ± 0.56 mm/day (data for Fig. 5). Mycelial growth in bags with 5 to 10 mm small holes, traditional cotton plugs or cotton balls for ventilation was greatest at wk 2, after which it decreased.

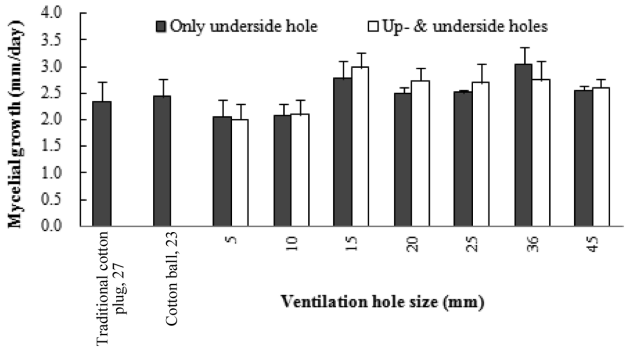

Fig. 5. Ventilation hole size and daily mycelial growth in *Lentinula edodes* cultivation bags. Black bars correspond to bags with holes only in the underside of the lid while white bar correspond to those with holes on both sides of the lid. Error bars represent the standard error of the mean (n = 3) per treatment.

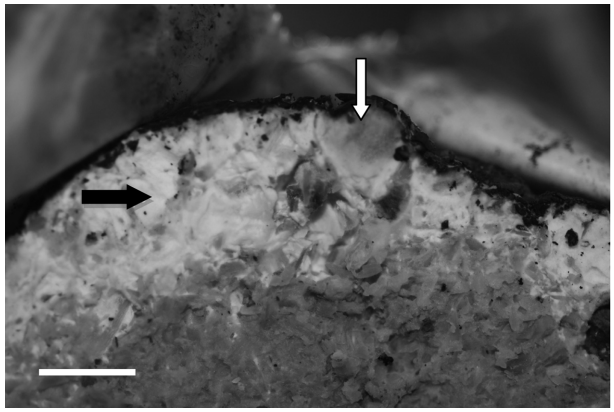

Fig. 6. White hyphal mass at the edge of *Lentinula edodes* sawdust medium (black arrow) with primordia (white arrow) on the top (scale bar = 1 cm).

Conversely, mycelial growth in bags with 15~45 mm holes was maintained at 2.15 to 3.22 mm/day from week 1 to 5. In bags with 5~20 mm holes, mycelial growth was highly positively correlated with carbon dioxide concentration (r = 0.85~0.99). Additionally, more active hyphal growth was associated with higher CO_2 concentrations. Massive amounts of CO_2 can stimulate mycelial growth [12], but the relationship between high CO_2 contents by active hyphal growth and relatively less aeration in the bags was complex. Dikaryotic mycelium of *L. edodes* can be repressed under highly dissolved oxygen conditions [13]. At various CO_2 levels, 0.03%, 12.5% and 25.0%, *Rhizopus oligosporus* grow best at the lowest 0.03% CO_2 [14].

After about five weeks, the medium gradually formed protrusions on its side owing to the growth of pure hyphal mass around the edges inside the medium. At about 10 weeks, the surface of the medium gradually became brown from the top. Inside the brown layer, there was a white hyphal mass 1.0 to 1.5 cm thick that was dense and compact at the edge with mushroom primordia (Fig. 6). Generally mushroom primordia formation responds to a reduction in CO_2 [12], while oxygen concentration affects the quality of stored mushrooms. For example, *Flammulina velutipes* mushrooms stored in a package with 80% oxygen showed a delayed senescence process and decreased postharvest quality loss when compared to those with lower than 50% oxygen [15].

Ventilation hole sizes and medium weight loss. Larger hole sizes are associated with greater medium weight loss (Fig. 7). The medium weight loss was 0.84 ± 0.26 g/day in bags with traditional cotton plugs and 0.75 ± 0.22 g/day in those with cotton balls. Media with ventilation holes of 45 mm lost 1.76 ± 0.54 g/day, while that with 5 mm holes lost only 0.55 ± 0.20 g/day. Generally, media with holes on both sides of the lid lost more weight than that with holes on only the underside of the lid.

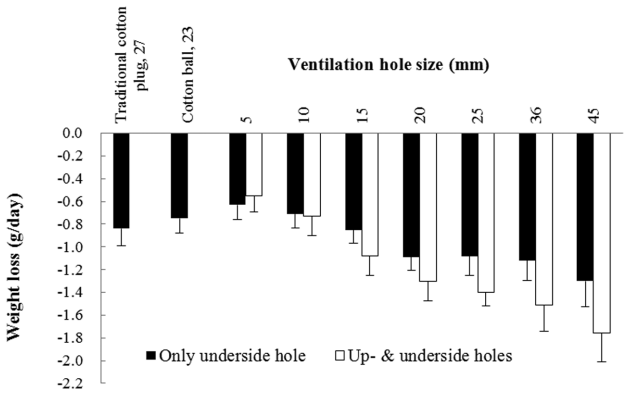

Fig. 7. Ventilation hole size and daily medium weight loss in *Lentinula edodes* cultivation bags. Black bars correspond to bags with holes in the underside only and white bars to those with holes in both the up- and underside. Error bars represent the standard error of the mean (n = 3) per treatment.

The rate of medium weight loss differed depending on hole size and cultivation period. The greatest weight loss period occurred earlier in bags with larger holes. Bags with smaller holes such as cotton plugs, cotton balls or holes 5 to 10 mm size lost the greatest amount of weight at wk 15, whereas those with holes larger than 20 mm lost the most weight at week 10. At wk 10, weight loss rates in bags with holes 5 to 10 mm and with 45 mm holes were about 0.7 and 2.7 g/day and 1.5 and 2.0 g/day, respectively (data not shown).

In conclusion the effects of aeration through lid hole filters on the hyphal growth of *Lentinula edodes* in sawdust cultivation bags were investigated. An appropriate ventilation hole size and position for the mushroom hyphal growth was 15 mm on underside of the lid. Conventional cotton plugs can be effective for mycelial growth, ventilation of carbon dioxide and controlling water loss from the bags. The greater the hole size on the lid of the cultivation bags, the greater the water loss from the medium. However, mycelial growth was not proportional to the ventilation hole size, but rather to carbon dioxide production. Thus, the appropriate ventilation hole size for the greatest mycelial growth in the bags should be determined by considering gas diffusion and water evaporation from the bags.

Acknowledgements

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