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Full Length Research Paper

Apple pomace increases mycelial growth of *Pleurotus ostreatus*

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Apple pomace is a by-product from the apple processing industry and has the potential to support the growth of microorganisms. In this study, the effect of apple pomace on the growth rate of *Pleurotus ostreatus* mycelium was investigated. The mycelial growth dramatically increased by 34.5, 20 and 26% in solid culture, liquid culture, and solid-state fermentation, respectively, by adding 2.5% apple pomace. However, the growth of *P. ostreatus* mycelia was slightly inhibited by adding 5 or 10% compared to 2.5% apple pomace. Our findings reveal that apple pomace utilization can become a model for the valuable addition of similar wastes, and for the development of a solid-state fermenter.

Key words: Apple pomace, growth rate, *Pleurotus ostreatus.*

INTRODUCTION

Food industry, in general, generates a large quantity of biodegradable waste such as peels, seeds, pomace, rags, and kernels (Joshi and Attri, 2006). Several million tons of apple pomace are generated during the processing of apple products, including apple juice, jelly, and cider (Bhushan et al., 2008). Apple pomace is a rich source of nutrients like carbohydrates, dietary fiber, minerals, and vitamin C (Joshi, 1998). As it is rich in carbohydrates, dietary fiber, and minerals, this waste is a good substrate for fermentation, and thus has the potential to support the growth of microorganisms. In addition, apple pomace has been used as a raw material in several applications such as the production of enzymes, organic acids, and ethanol, as well as pectin recovery and production of animal feed.

Pleurotus ostreatus (white-rot fungi), also known as the oyster mushroom, is commercially important in the world market for mushroom. In addition to its importance in food production, *P. ostreatus* has recently received increasing attention for its use in bio-bleaching, for catalysis of difficult chemical conversions, and in the pharmaceutical industry (Vyas et al., 1994). Cultivation of oyster mushroom (*P. ostreatus*) on different substrates was found to influence its growth (Shah et al., 2004), and its growth on various substrates, including paddy straw, maize stalks/cobs, and vegetable plant residues, has been studied (Hassan et al., 2011). These results suggest that mushroom production can play an important role in managing farm organic wastes, whereby the by-products of agriculture and food processing industries are used as media for growing edible fungi.

The aim of this study was to investigate the effect of apple pomace on the growth rate of *P. ostreatus* mycelium since sustainable food production and value addition of wastes is the most important issue in the agro and food processing industry.

MATERIALS AND METHODS

P. ostreatus was obtained from the Korean Agricultural Culture Collection (Suwon, Korea) and cultured on mushroom complete medium (MCM; 0.46 g KH_2PO_4, 0.5 g $MgSO_4$, 1 g K_2HPO_4, 2 g yeast extract, 2 g Bacto peptone, 20 g glucose, and with or without 20 g/L agar) at 25°C. Apple pomace was collected from Chungbuk Wonye Nonghyup, Chung-buk, Korea. It was washed 5 times with sterilized distilled water to remove any adhering substances, freeze dried, powdered, and passed through a sieve to get fine uniformly sized particles. It was dissolved in distilled water (100 g per L, pH adjusted to 4.5), and autoclaved.

The growth rate of mycelia was determined by measuring its dry

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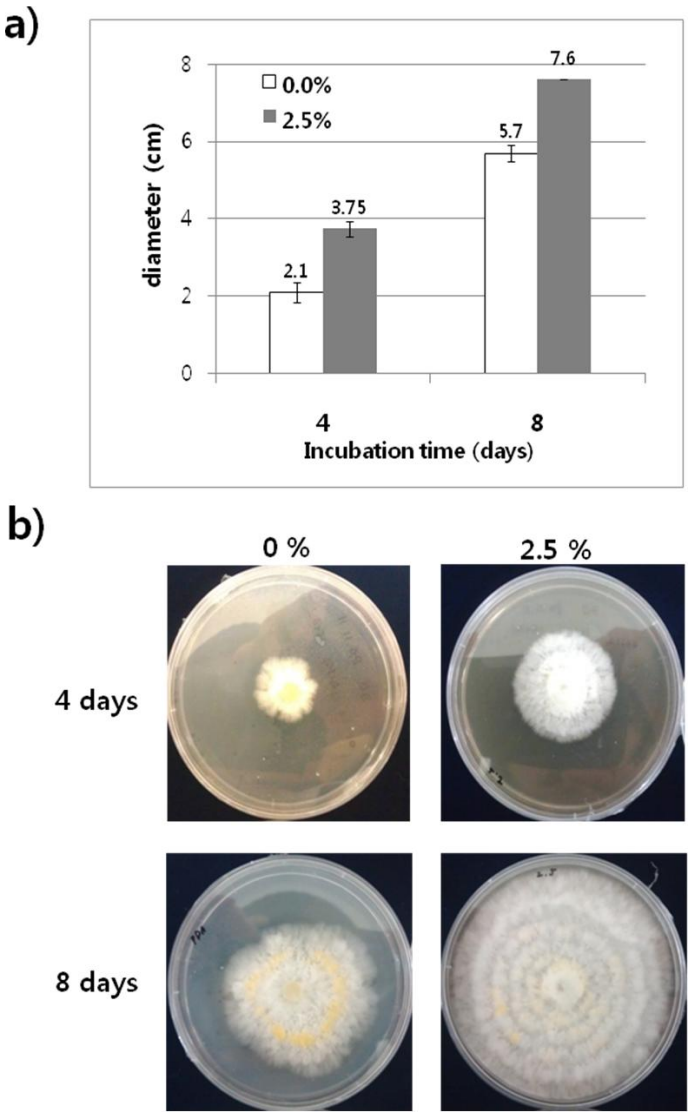

Figure 1. Effect of apple pomace on mycelial growth in solid culture. (a) This assay was performed 3 times, and the average ± SD values were calculated for each repetition of the experiment. b) Mycelial growth aspects in solid cultures. Top-left; MCM agar medium (4 days after inoculation), top-right; MCM agar medium containing 2.5% apple pomace (4 days after inoculation), bottom-left; MCM agar medium (8 days after inoculation), bottom-right; MCM agar medium containing 2.5% apple pomace (8 days after inoculation).

weight in liquid culture. One-week-old mycelium agar discs (5 discs, diameter = 0.5 cm) from the MCM plates were inoculated into a 300-mL flask containing 50 mL of the inoculum medium, inoculated for 11 days at 120 rpm, and used to inoculate a 2-L flask containing 500 mL of fresh medium. Cultures were filtered through Whatman filter paper (grade 2, 110 mm; GE Health Care, USA). The mycelia were washed with distilled water, and then placed on a previously weighed filter paper. The filter was placed overnight in a drying oven at 60°C. Further, solid-state fermentation (SSF) was carried out in a test tube. The sawdust was mixed with 2.5% apple pomace solution (500 g per L). Approximately 74 g of sawdust medium (SM) was packed in each test tube (20 cm × 3 cm). The tubes were sealed using silistopper, and covered with a piece of paper tied down with a rubber band around the neck of the test tube.

The bags were autoclaved, and allowed to cool for 24 h. Inoculation was performed using a sterile cork borer, in 1-week-old mycelium agar discs (diameter, 0.6 cm). The fermentation was carried out in a controlled environment for 28 days at 25°C.

RESULTS AND DISCUSSION

To evaluate the growth rate of *P. ostreatus* mycelia, we used various concentrations of apple pomace, including 1, 2, 2.5, 5 and 10% (data not shown). After that, we selected the optimal concentration of apple pomace (2.5%), which showed the highest growth rate of *P. ostreatus* mycelia. Interestingly, the growth of *P. ostreatus* mycelia was slightly inhibited by adding 5 or 10% compared to 2.5% apple pomace (data not shown). The discrepancy between these results may be explained by 2 reasons. First, apple pomace is a rich source of carbohydrate polymers; it contains pectin (12.7%), lignin (12.8%), hemicelluloses (5%), cellulose (17.6%), and starch (17.9%) (Kennedy et al., 1999). The most remarkable feature of white-rot fungi is its ability to mineralize completely lignin to CO_2 and water (the only organisms known to do this). However, lignin degradation also poses potential hazards to the fungus, because some of the oxidative intermediates (unstable radicals such as aryl cation and phenoxy radical intermediates) can be fungitoxic (Deacon 2006). Second, apple pomace contains several mineral nutrients, including carbon (44.56%), oxygen (44.78%), hydrogen (6.18%), chlorine (1.02%), and nitrogen (0.57%) (Kennedy et al., 1999). An adequate supply of mineral nutrients is essential for composting as well as the decomposition of organic matter, in general. Nitrogen supply is particularly important, and can often be the rate-limiting factor (Deacon 2006). In addition, the nutritional content of the substrate can be improved by nitrogen supplementation to enhance the yield of oyster mushrooms (Curvetto et al., 2002). Supplementing the substrate with various nitrogen-rich supplements is widely used approach for enhancing yield (Bonatti et al., 2004). However, Baysal et al. (2003) reported that the slower spawn running may be attributed to excess nitrogen content, which is known to inhibit mycelial growth.

To evaluate mycelial growth rate, the mycelial disc (formed using a sterile cork borer) was inoculated on an MCM agar plate, with and without apple pomace. Eight days after inoculation, the average diameter of mycelia on the MCM agar plate containing 2.5% apple pomace was 7.6 cm (covering the entire plate), whereas that on the MCM agar plate without apple pomace was 5.7 cm. In the growth rate assay with a medium containing 2.5% apple pomace, the growth rate increased by approximately 34.5% over a total of 5 days (from 4 to 8 days after incubation) (Figures 1a and b).

Figure 2 shows the dry weight of *P. ostreatus* cultured in MCM media with and without apple pomace. In a

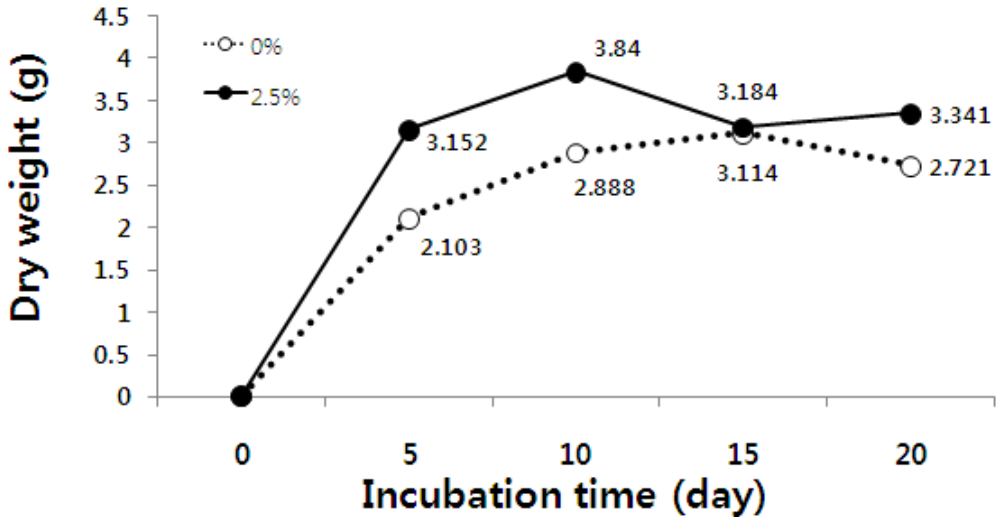

Figure 2. Effect of apple pomace on mycelial dry weight in liquid culture.

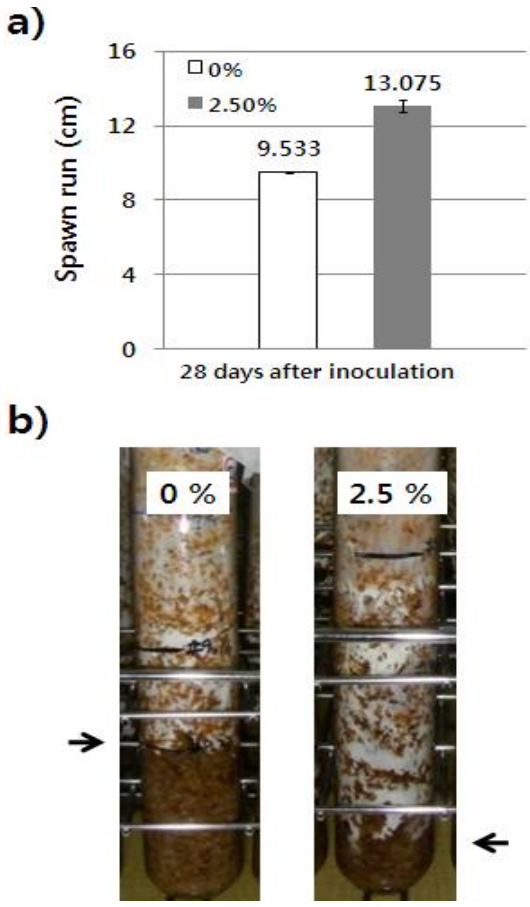

Figure 3. Spawn running of mycelia in sawdust-based substrate. (a) The assay was performed 3 times, and the average ± SD values were calculated for each repetition of the experiment. (b) Mycelial growth aspects in sawdust-based substrate alone (left) and in sawdust containing 2.5% apple pomace (right). Arrows indicate the end of the spawn running (28 days after inoculation).

shaker culture with a medium containing 2.5% apple pomace, the dry weight increased by approximately 20% over a total of 16 days (from 5 to 20 days after incubation). When 2.5% of apple pomace was added, the average growth rate and dry weight were 0.95 cm/day and 3.37 g, respectively.

Figure 3a shows the growth rate of *P. ostreatus* cultured in SM with and without apple pomace. The SM containing 2.5% apple pomace showed a faster rate of mycelial growth during the spawn run (Figure 3a). The spawn run rate in SM containing 2.5% apple pomace was 0.46 cm/day, while it was 0.34 cm/day for only SM. After 28 days of incubation, the length of mycelial growth in the SM containing 2.5% apple pomace was 3 cm longer than that of mycelial growth in SM alone (Figure 3b).

This study only addressed the use of mycelial growth (one of the 3 developmental stages: mycelial, primordial, and fruiting body) to ferment apple pomace. Prior to scale-up, other processes and determinant factors should be studied. Although the apple pomace is a seasonal agro-industrial residue, the high amount of pomace available annually renders the production of high quantities of oyster mushroom supplement possible within a short duration at a low cost.

Conclusions

Apple pomace utilization can become a model for the value addition of similar wastes and development of solid state fermenter. Because apple pomace is a waste material, its use in mushroom production should not only be cheaper than grains and other primary products, but also contribute to reducing solid waste disposal problems. It can be concluded from this study's result that apple pomace can be used as a good substrate for cultivating *P. ostreatus*. However, more work is needed before it can

be recommended. Experiments should be conducted with a wider range of isolates under commercial fruiting conditions. Therefore, further studies should be needed for this matter.

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