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Cultivation of Lingzhi Mushroom, *Ganoderma lucidum*, by Using Sugarcane Bagasse

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Abstract

Efficiency of sugarcane bagasse used as substrate on cultivation of Linzhi mushroom, *Ganoderma lucidum* in comparison with sawdust was investigated. The effects of these substrate on growth, cellulace activity and mushroom yield were analyzed. Growth of *G. lucidum* strains in sawdust was faster than in sugarcane bagasse. *G. lucidum* strains grown in sugarcane bagasse showed higher cellulase activity than in sawdust. Biological efficiency of *G. lucidum* strain cultured in sugarcane bagasse substrate was higher than in sawdust, indicating that the sugarcane bagasse could be substitute of sawdust substrate for *G. lucidum* production.

Keyword: Lingzhi, *Ganoderma lucidum*, Mushroom cultivation, Sugarcane bagasse

Introduction

Lingzhi mushroom, *Garnoderma lucidum* (Fr.) Krast, has been use as a traditional medicine to improve health and prolonging life in East Asia including China, Japan and Korea for over 2,000 years (Zhou *et al.,* 2012). Nowadays, several bioactive compounds regarding to therapeutic properties from Lingzhi have been discovered. For instance, several types of polysaccharide exhibited anticancer, antitumor, antiviral and antioxidant activity and able to activate immune system. Triterpenoids, unsaturated hydrocarbon namely Ganoderic acids, showed cytotoxicity against cancer cells and are considered to be anticancer agents. Besides,

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they revealed antiviral, antioxidant and antimetastatic activity, as well as affected on lower urinary tract symptoms (Bishop *et al*., 2015).

Since Lingzhi has a huge demand as a because of medicinal and cultural significance, mushroom in nature is certainly not enough. Therefore, cultivation of Lingzhi is necessary. Cultivation of mushroom is considered to be sustainable development by reason of organic wastes are normally used as a substrate to produced food without damaging the environment. Techniques for mushroom cultivation have been developed. Lingzhi can be cultivated using both log and bag culture technique. In Thailand, Lingzhi is generally produced by bag culture technique using a Para rubber wood sawdust as main substrate. Cultivation of mushroom required the sawdust from the Southern of Thailand. As fuel is used for transportation, price of the sawdust is increased according to distance from source to destination. The used of local organic waste for mushroom cultivation is an alternative to save cost. There are numbers of organic waste were reported for mushroom cultivation. These are included straw, husk, water hyacinth, banana pseudo-stem, corn cob, cotton seed hull, coffee bean hull, spent beer grain and sugarcane bagasse.

Sugarcane bagasse is a waste left from sugar industry as well as sugarcane juice mart. The waste has been used as a fuel for electricity generation or raw material for fertilizer. It would be better if they are used as a substrate for mushroom cultivation to produce such a food before converted to electricity or fertilizer. Therefore, the aim of this study was to evaluate growth, cellulase activity and yield of Lingzhi when sugarcane bagasse was used as a substrate for mushroom cultivation.

Materials and Methods

1. Mushroom strains and cultivation

Ganoderma lucidum (Fr.) Krast strain A1, G2, DOA, G45, G5A, G5Z, HHK and SUN were kindly provided by Dr. Surapol Rukpathum. The mushroom mycelia were maintained on potato dextrose agar (PDA) by periodic subculturing.

2. Mycelial growth rate

To determine mushroom mycelium growth rate, a 5mm diameter agar plug of each mushroom strain was inoculated to a PDA plate. Then, the plates were incubated at 30 ^{o}C. Diameter of mushroom colony was measured daily. Growth rate was calculated by size of diameter of colony (mm.)/day.

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3. Mushroom cultivation

To make mushroom grain spawn, sorghum grains were washed, submerged overnight and boiled. Then, damp sorghum grains were filled in bottle and sterilized by autoclave. The mushroom mycelium was transferred to sorghum grains and incubated at 30 °C until the grains were fully covered by mycelium.

A Para rubber wood sawdust and find chopped sugarcane bagasse were used as main substrate for mushroom cultivation. The mushroom substrate was prepared mixing of 10 kilograms of either sawdust or sugarcane bagasse, 300 gram of rice bran, 10 gram of lime (Calcium oxide), 20 gram of sodium sulfate, 100 gram of gypsum salt (magnesium sulfate) and 70% moisture content of water. 900 gram of substrate was packed in polypropylene bag and sterilized by autoclave. After cooling down to room temperature, 10 gram of grain spawn was transferred to each bag and incubated at room temperature until the bags were fully colonized by mycelium. Colonization period of mycelium in substrate bags was recorded. Then, the mushroom was induced by transfer the bags to chamber at 30±3 °C and 80-90% RH. Fruiting bodies were collected after 50 days. Mushroom productivity was expressed as biological efficiency (fresh weight of mushroom/ dry weight substrate used × 100).

4. Cellulase activity assay

To assay cellulase activity, 50 gram of substrate colonized by mushroom mycelium was mixed with 100 ml of 0.2 M sodium acetate buffer pH 4.5 and shaken at 180 rpm for 1 hour. Then, the culture filtrate was obtained by filtration and centrifugation at 4 °C, 8000 rpm for 10 minutes. 0.25 ml of culture filtrate was mixed with 0.25 ml of 1% Carboxymethyl cellulose (CMC) in 0.05 M sodium acetate buffer, pH 4.5. For blank, 1% of CMC was added after incubation. The reactions were incubated at 50 °C for 50 minutes. The cellulase activity was determined by using DNSA method. One unit (U) of enzyme activity was defined as the amount of enzyme that produced 1 µmol of reducing sugar per minute under the conditions assayed.

5. Statistical analysis

The experiment was set-up in triplicate. The data was analyzed by Analysis of Variance (ANOVA) and Turkey's Test in SPSS software.

Results and Discussion

The mycelium of *G. lucidum* strains were inoculated on potato dextrose agar (PDA) and incubated at 30 °C. The growth rate of mycelium was determined by measurement of colonial diameter daily. The growth rate of *G. lucidum* strains could be divided into 6 groups. *G. lucidum* strain G2 was the most grown, while strain G5Z was the least (Fig. 1). The different growth characteristics found in these strains may be due to genetically difference (Sawetsuwannakun and Bangyeekhun, 2011).

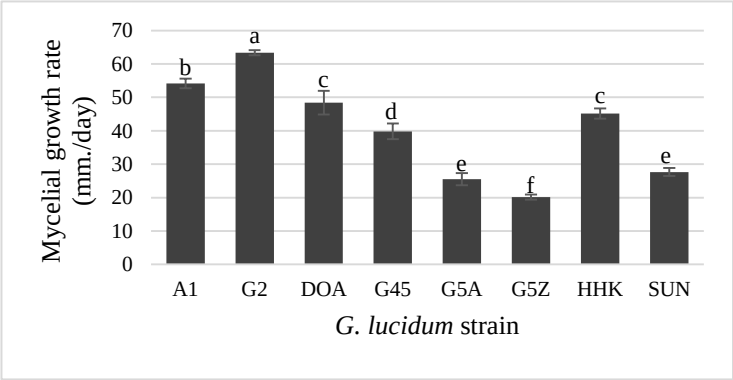

Figure 1. Growth rate of *G. lucidum* strains on PDA at 30 °C for 5 days. The values represent means of triplicate culture. Error bars indicate SD. The alphabet a-f shows the statistic different.

Cultivation of *G. lucidum* strains was performed using bag culture. The sawdust and sugarcane bagasse were used as main substrates for mushroom cultivation. Colonization period of mycelium in substrate bags was determined. Growth of *G. lucidum* strains in sawdust substrate was faster than in sugarcane bagasse substrate. *G. lucidum* strain A1 took 32-34 days for fully colonization in sawdust or sugarcane bagasse bags. Whereas strain G2 ran through 52 days in bag containing sugarcane bagasse. Strain G2, G45, G5A and G5Z spent time in sugarcane bagasse more than in sawdust substrate, while strain A1 was vice versa (Fig. 2).

Composition of cellolose, lignin and nitrogen in substrate could be influence on colonization period in substrate. The sawdust contains cellulose and lignin with 58% and 41% respectively (Sornprasert and Aroonsrimorakot, 2014) whereas, sugarcane bagasse comprises of those with 44% and 24%, respectively (Pereira *et al.,* 2011). Mycelial growth of *G. lucidum* was efficient when cultured in high proportion of cellulose and lignin substrate (Sornprasert and Aroonsrimorakot, 2014). Moreover, it was suggested that the spawn running in high nitrogen

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substrate could be slower than in low amount of nitrogen (Yang *et al*., 2013). Nitrogen composition in sawdust is 0.30% while in sugarcane bagasse is 1.23%.

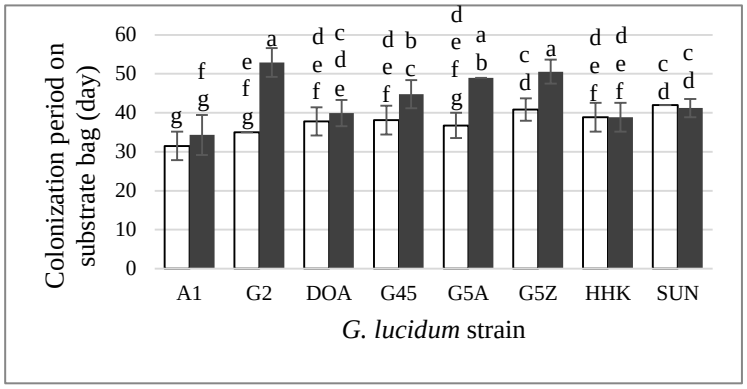

Figure 2. Colonization period of mycelium of *G. lucidum* strains in sawdust (whirt) or sugarcane bagasse (black) substrate. The values represent means of triplicate culture. Error bars indicate SD. The alphabet a-g shows the statistic different.

Total enzymes were extracted form mushroom cultivation bags containing either sawdust or sugarcane bagasse as the substrate. Cellulase activities were assayed by using DNSA method. The results revealed that cellulase activities of *G. lucidum* strain A1 G2 DOA G45 and G5Z grown in sugarcane bagasse substrate were higher than others (Fig. 3).

Analysis of *G. lucidum* cultivated in sugarcane bagasse revealed that the mushroom able to produced several lignocellulolytic enzymes, i.e. cellulases, hemicellulases and lignin degrading enzymes (Manavalan *et al*., 2012). In other mushrooms, prophenol oxidase and manganese peroxidase, the lignin degrading enzymes, were produced first and celluolytic enzymes were produced afterward in sugarcane bagasse degradation (Dong *et al.,* 2013). A number of studies revealed that cellulase efficiency in cellulose hydrolysis is usually reduced in the presence of lignin. This is probably due to the association cellulose with lignin, the access blocking of enzyme to cellulose, and the unproductive binding of the enzymes to lignin (Vermaas *et al*., 2015). Composition of lignin in sawdust and sugarcane bagasse is 41% and 24% respectively. Therefore, it is possible that low cellulase activity of *G. lucidum* grown in sawdust was a result of high percentage of lignin in the substrate.

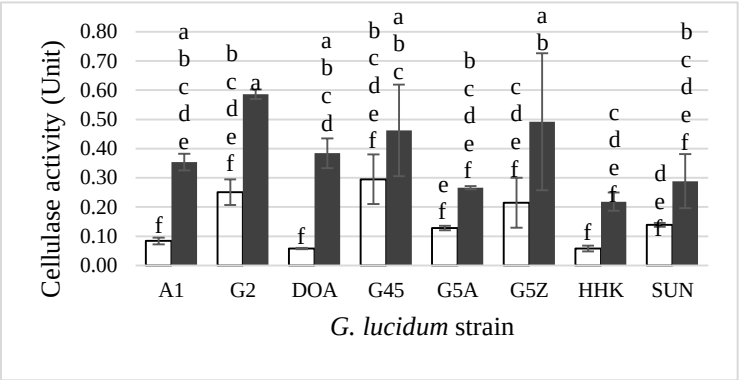

Figure 3. The cellulase activity of *G. lucidum* strains grown in sawdust (white) or sugarcane bagasse (black) substrate. The values represent means of triplicate culture. Error bars indicate SD. The alphabet a-f shows the statistic different.

Efficiency of substrate on yield of *G. lucidum* fruiting body was determined.Biological efficiency of *G. lucidum* strain G2, DOA, G45, G5A, G5Z and HHK cultured in sugarcane bagasse substrate was statistically higher than in sawdust substrate (Fig. 4).

It was suggested that C:N ratio of the substrate could influence on growth and development of mushroom. Condition with high C:N ratio was suitable for the mycelium growth, whereas low C:N ratio was appropriate for primodia and mushroom development (Yang *et al*., 2013). Substrate with C:N ratio of 70-80 yielded proper mushroom fruiting body, while with C:N ratio of 50 generated only the strip without cap and C:N ratio of lower than 50 gave no fruiting body formation. In this study, the C:N ratio was not determined in the substrates. Therefore, it could be merely speculated that C:N ratio in sugarcane bagasse was higher than that in sawdust and influenced on fruiting body production of *G. lucidum*. Sugarcane bagasse showed potential substrate for the mushroom production of many species, such as *Pleurotus ostreatus, Lentinus edodes* (Vetayasuporn *et al*., 2006; Salmones *et al*., 1999). According to this study, sugarcane bagasse could be substitute of sawdust substrate for *G. lucidum* production.

Yield of mushroom could be influenced from several factors. For instance, amount of appropriate nutrient in substrate would provide energy for mycelial growth and mushroom production. Physical structure of substrate would increase water-absorbing capacity (Yang *et al*., 2013). Hence, combination of several agricultural waste could make the best substrate formula for mushroom production. In future study, we will shed the light on finding the proper mixture of ingredients form several agricultural wastes in order to increase in mushroom yield.

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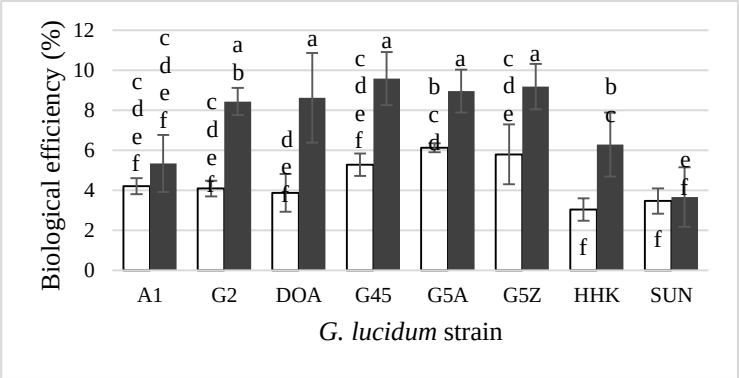

Figure 4. Biological efficiency of *G. lucidum* strains grown in sawdust (white) or sugarcane bagasse (black) substrate. The values represent means of triplicate culture. Error bars indicate SD. The alphabet a-g shows the statistic different.

Conclusion

Mushroom cultivation is one of the effective means to convert agricultural waste to food. Beside sawdust, several agricultural wastes such as rice straw, banana leaves, corn cob and sugarcane bagasse can be used as an alternative substrate for mushroom cultivation.The present study reveals that sugarcane bagasse is useful material for cultivation of Lingzhi mushroom, a medicinal mushroom. Therefore, cultivation of Linzhi by using a cheap substrate like sugarcane bagasse may be a profitable agribusiness for making an extra income.

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