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Comparison of fruiting body formation techniques of the king oyster mushroom *Pleurotus eryngii*

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Abstract

The objective of this study was the comparison of fruiting body formation techniques of the king oyster mushroom (*Pleurotus eryngii*). Wild type (PE) and mutant strains (PET1, PET2, PET3 and PET4) were used. The cultivation and fruiting body formation techniques were performed in 4 conditions; 1) bag culture 2) bag culture with casing 3) tray culture 4) tray culture with casing. The result showed that number of flush was not different (1-2 flush per bag) in all methods. The bag culture gave a highest yield. Due to this method, there was small area for fruiting body penetration and gave one of big fruiting body. Yield of tray culture with casing was higher than non-cased. It implied that the cultivation with casing enhanced the biological efficiency, fresh weight and dry weight. But, the bag culture with casing method gave low yield. However, BE of PET4 was highest when cultivated on tray culture with casing.

Keywords: King oyster mushroom, Fruiting body formation, Casing, Biological efficiency

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Introduction

A king oyster mushroom (*P. eryngii*) is popular edible mushroom. This mushroom was found for the first time at the root of Eryngium and named as Eryngii (Gyorfi and Hajdu, 2007). *P. eryngii* began cultivated at northern Italy and Switzerland. The demand of this mushroom was increased due to good texture, nutritional and pharmaceutical values. In japan, the production of *P. eryngii* was increased from 2000 ton in 1995 to 10070 ton in 2001 (ohga and Royse, 2004). This trend was also similar in many countries such as China, US and Korean (Estrada and Royse, 2007; Kim et al., 2013).

For the cultivation, *P. eryngii* was cultivated on several cellulose substrates such as log and wheat straw but sawdust is common used (Stamets, 1993). There are the difference methods for mushroom cultivation such as log, bag and tray cultures. Currently, the bag culture is the popular method of Pleurotus spp. and give the highest yield when compare with another methods (Mandeel et al., 2005; Gregori et al., 2007).

In previous study, the mutant strains of *P. eryngii* were generated by Agrobacterium tumefaciens-mediated transformation. The fourth mutant strains (PET1-4) were obtained and cultivated in bag culture. Yield and BE of PET4 were significantly higher than wild type (Romruen and Bangyeekhun, 2017). However, bag cultivation technique did not suitable for other mutant strains which may be contained difference characteristic from wild type. In this study, the methods of fruiting body induction were studied to compared the yield and BE of mutant and wild type strains.

Materials and Methords

1. Mushroom strains and spawns production

P. eryngii (wild type; PE) and mutant strains (PET1-4) were used in this study (Romruen and Bangyeekhun, 2017). The mycelia were maintained on potato dextrose agar (PDA) and incubation at 25 ^{o}C.

The spawns were prepared on sorghum gains. The gains were soaked overnight in water, rinsed and boiled in water. The cooled gains were distributed into the bottles and sterilized by autoclave. Each bottle was inoculated with agar plug of 10-days-old mycelium and incubated at 25^{o}C. until the substrate became fully colonized.

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2. Mushroom cultivation

Wild type and transformants were inoculated into sawdust substrate. Sawdust substrate was prepared by mixing 10 Kg of sawdust, 0.8 Kg of bran, 0.1 Kg of lime (Calcium oxide), 0.1 Kg of calcium chloride, 20 g of epsom salt (magnesium sulfate) and 65-70 liters of water.

900 g of substrate was packed into heat-resistant bag and autoclaved at 121°C. for 20 minutes. The cooled substrates were inoculated with spawn and incubated at 25 °C. until the mycelium fully covered the substrate. For bag culture (BC), these bags were kept open at 18±2 °C. for fruit body formation. For bag culture with casing (BCC), colonized substrates were removed from bags, placed in a basket and covered with soil before fruiting body formation.

For tray culture (TC), the plastic bag was used as tray. The sterilized substrate was compacted in the plastic bag (3 cm in depth). Spawn was inoculated and covered with second layer of substrate. This step was repeated twice. These bags were incubated as describe above. The colonized bags was made a hole and kept at 18±2°C. for fruiting body induction. For tray culture with casing (TCC), soil was overlaid on the surface (3 cm in depth) before fruiting body induction.

3. Yield and biological efficiency (BE)

Weight of fresh harvested mushroom was measured (wet weight). Then, the fruiting bodies were incubated at 80 °C. until the constant weight (dry weight) was obtained. Biological efficiency (BE) was calculated by using the total mushroom yield from each flush as follows: Biological efficiency (BE) = (weight of fresh harvested mushroom/weight of wet substrate used) x 100

4. Statistical analysis

The statistical difference of data was analyzed by one-way ANOVA and Turkey's test using the program SPSS 11.0.

Results and Discussion

Mutant strains of *P. eryngii* were cultivated in 900 g of sawdust substrate which is the best substrate for this mushroom (Stamets, 1993). In this study, bag and tray cultures with or without casing were performed. In all treatments, the mycelium was grown on substrate at 25°C. for 49-52 days. Then, the fruiting body was induced at 18 °C. In all strains and methods of cultivation, 1-2 flushes were obtained (Fig. 1). Yield (wet and dry weight) and BE was

investigated (Fig. 2, 3 and 4) and revealed that the maximal yield was found in the first flush (data not shown).

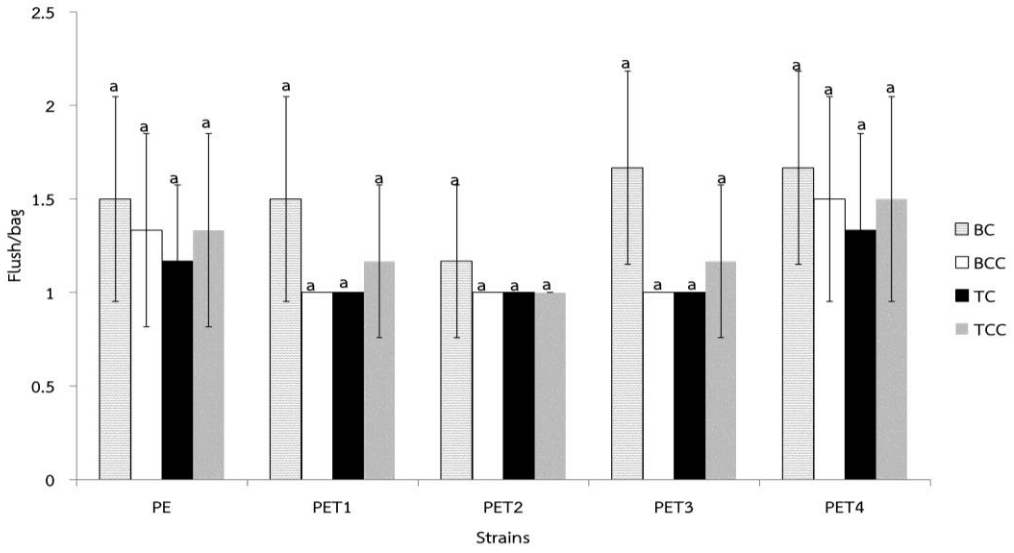

Figure 1. The average numbers of flush per bag of wild type (PE) and transformants (PET1-4) when cultivated in bag culture (BC), bag culture with casing (BCC), tray culture (TC) and tray culture with casing (TCC). The values were means of triplicates experiments, error bars were SD and the letter indicates the statistic group.

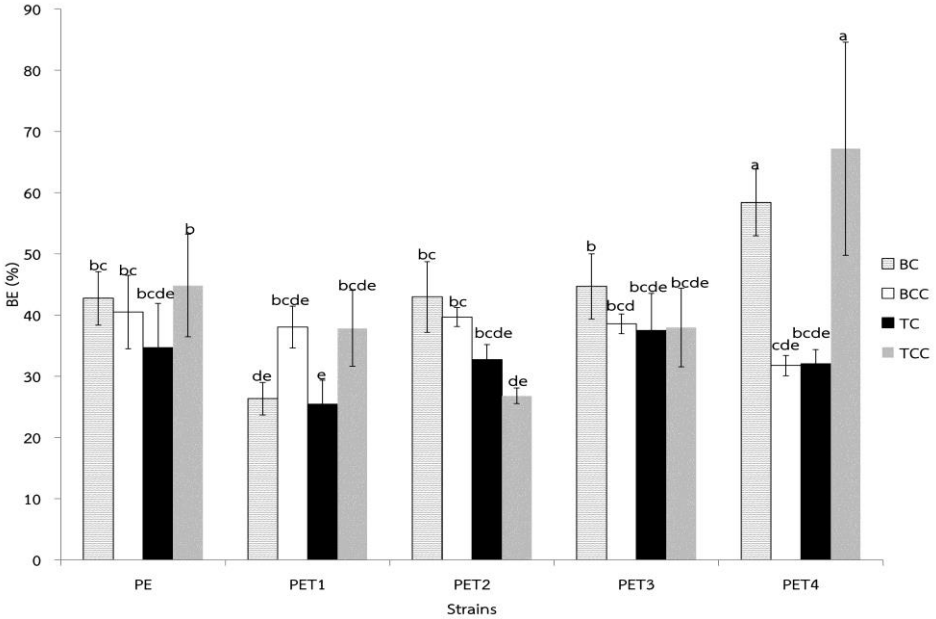

Figure 2. The percentage of biological efficiency (%BE) of wild type (PE) and transformants (PET1-4) when cultivated in bag culture (BC), bag culture with casing (BCC), tray culture (TC) and tray culture with casing (TCC). The values were means of triplicate experiments, error bars were SD and the letter indicates the statistic group.

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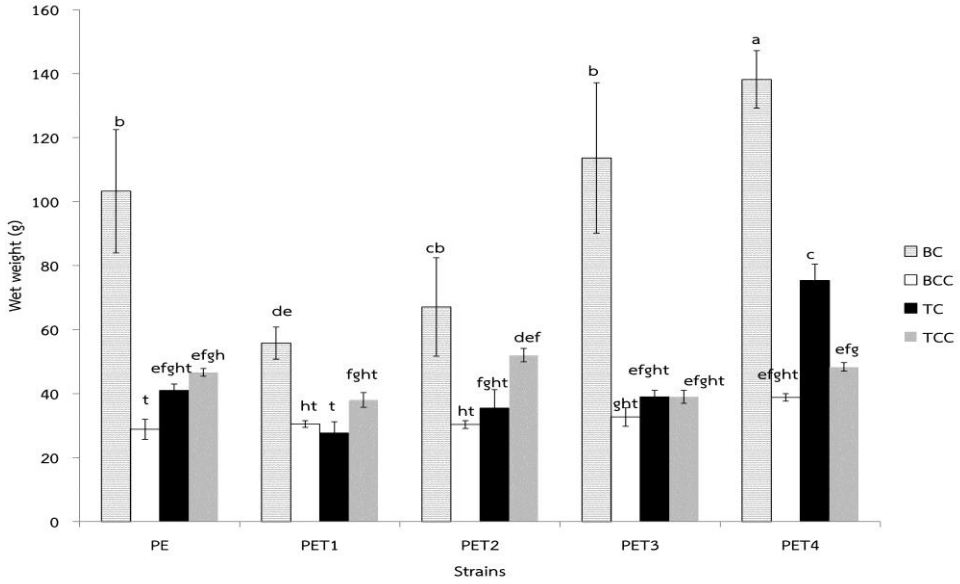

Figure 3. Wet weight of wild type (PE) and transformants (PET1-4) when cultivated in bag culture (BC), bag culture with casing (BCC), tray culture (TC) and tray culture with casing (TCC). The values were means of triplicate experiments, error bars were SD and the letter indicates the statistic group.

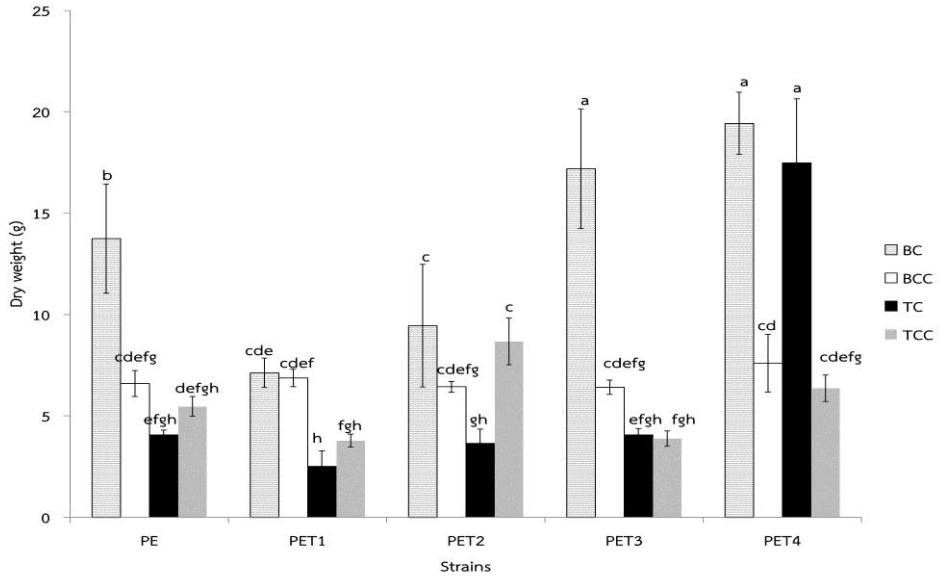

Figure 4. Dry weight of wild type (PE) and transformants (PET1-4) when cultivated in bag culture (BC), bag culture with casing (BCC), tray culture (TC) and tray culture with casing (TCC). The values were means of triplicate experiments, error bars were SD and the letter indicates the statistic group.

Bag culture gave yields and BE more than tray culture in all strains. It probably due to the area of bag culture for fruiting body formation was small. The only one of big fruiting body was penetrated and had a higher yield and BE than smaller ones. In generally, the bag culture was used for cultivation this mushroom and gave yield about 90.5-141 g (Moonmoon, *et al*., 2010). In addition, weights of the used substrates in all strains were higher than other methods (data not shown). It implied that bag culture could be maintained the humidity of substrate more than another methods. Our results agree with Mandeel *et al*. (2005) who reported that *Pleurotus* spp. in bag culture resulted in a maximal yield when compared with plastic tray, pottery trays and polyester net.

However, tray culture with casing gave higher yield than bag culture with casing in all strains. Due to the substrate in bag culture was removed from the bag and placed in the basket and covered with soil. Although the substrate was cased but the area for fruiting production was large. Thus, it caused of a small fruiting body and low yield and BE. This probably due to the small area led to small size of fruiting body (Gyorfi and Hajdu, 2007; Bellettini *et al*., 2016).

In term of casing, BE of PE, PET1 and PET4 from tray culture with casing were higher than non-cased. Moreover, yields of PE, PET1 and PET2 from tray culture with casing were also higher than non-cased. However, yield and BE of PET3 from both method were equal. The results indicated that the casing improved yield and BE of mushroom which according to previous studies (Gyorfi and Hajdu, 2007; Estrada *et al*., 2009). On the other hand, yields from bag cultures were higher than bag culture with casing in all strains. It was due to the area for fruiting production as described above.

Interestingly, wet (138.17±8.99 g) and dry (19.43±1.54 g) weight of PET4 from bag culture were significantly higher than those of wild type (103.24±19.26 and 13.75±2.69 g for wet and dry weight, respectively). BE of PET4 (58.40%) was also higher than PE (42.73%) about 1.4 times which according to previous study (Romruen and Bangyeekhun, 2017). However, BE of PET4 from tray culture with casing gave the highest BE (67.19±17.41%). It probably due to the used substrate was very low weight (data not shown).

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Conclusion

P. eryngii was cultivated on sawdust substrate. By comparison of 4 differenced mushroom cultivation methods, 1-2 flushes were obtained. In term of casing, tray culture gave a higher yield than bag culture. Bag culture gave a highest yield and was the best method for wild type and mutant strains. However, BE of PET4 was highest when cultivated on tray culture with casing.

Acknowledgements

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References

Bellettini, M. B., Fiorda, F. A., Maieves, H. A., Teixeira, G. L., Avila, S., Hornung, P. S., Junior, A. M. & Ribani, R. H. (2016). Factors affecting mushroom *Pleurotus* spp. Saudi Journal of Biological Sciences. Article in press: 1-14.

Estrada, A. E. R., Jimenez-Gasco, M. M. & Royse, D. J. (2009). Improvement of yield of *Pleurotus eryngii* var. *eryngii* by substrate supplementation and use of a casing overlay. Bioresource Technology. 100: 5270-5276.

Estrada, A. E. R. & Royse, D. J. (2007). Yield, size and bacterial blotch resistance of *Pleurotus eryngii* grown on cottonseed hulls/oak sawdust supplemented with manganese, copper and whole ground soybean. Bioresource Technology. 98: 1898-1906.

Gregori, A., Svagelj, M. & Pohleven, J. (2007). Cultivation Techniques and Medicinal Properties of *Pleurotus* spp. Food Technology and Biotechnology. 45(3): 238-249.

Gyorfi, J. & Hajdu, Cs. (2007). Casing-material experiments with *Pleurotus eryngii*. International Journal of Horticultural Science. 13(2): 33-36.

Kim, M. K., Ryu, J. S., Lee, Y. H. & Kim, H. R. (2013). Breeding of a long shelf-life strain for commercial cultivation by mono–mono crossing in *Pleurotus eryngii*. Scientia horticulturae. 162: 265-270.

Mandeel, Q. A., Al-Laith, A. A. & Mohamed, S. A. (2005). Cultivation of oyster mushrooms (*Pleurotus* spp.) on various lignocellulosic wastes. World Journal of Microbiology and Biotechnology. 21: 601-607.

Moonmoon, M., Uddin, Md. N., Ahmed, S., Shelly, N. J. & Khan, Md. A. (2010). Cultivation of different strains of king oyster mushroom (*Pleurotus eryngii*) on saw dust and rice straw in Bangladesh. Saudi Journal of Biological Sciences. 17: 341-345.

Ohga, S. & Royse, D. J. (2004). Cultivation of *Pleurotus eryngii* on umbrella plant (*Cyperus alternifolius*) substrate. The Japan Wood Research Society. 50: 466-469.

Romruen, U. & Bangyeekhun, E. (2017). Yield improvement of the king oyster mushroom, *Pleurotus eryngii*, by transformation of its cellulase gene. Biologia. 72(2): 140-144.

Stamets, P. (1993). Growing gourmet and medicinal mushrooms, 3rd, pp. 301-304. Ten speed Press, California, USA