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Effect of *Bacillus aryabhattai* extract addition on growth and productivity of *Pleurotus ostreatus* ((Jacq ex Fr.) P. Kumm)

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Abstract

Pleurotus ostreatus (White oyster mushroom) is one of the most widely cultivated mushrooms in the world. Nutrients provided in the media are one of the most important factors affecting the growth of mushrooms. The present study mainly focuses on the isolation of mushroom growth-promoting (MGP) bacteria and studies its effects on the growth of mushroom fruiting bodies. *Bacillus aryabhattai* was isolated from the growth media of *Pleurotus ostreatus*, identified based on 16S rDNA sequencing and phylogenetic tree construction by the neighbor-joining method using MEGA version 6.06 and its effect on fruiting body growth of the *P. ostreatus* was investigated. The results of this research strongly suggest that the addition of specific bacteria supernatant into the mushroom growth media has beneficial applications for mushroom production.

1. Introduction

Mushroom production has been regarded as the second most important commercial microbial technology after yeast (Pathak *et al*., 2009) because the mushroom has a composition of chemical compounds that are very attractive when viewed from the nutritional side (Dündar *et al*., 2008). The nutritional value of mushrooms can be equated with eggs, milk and meat (Oei, 2003). Mushrooms contain vitamins and amino acids in large amounts (Sanchez, 2004). Cultivation and consumption of mushrooms of the *Pleurotus* genus, especially the species *Pleurotus ostreatus*, have always been at a lower level than the other species which are more competitive in the market, such as *Agaricus bisporus* and *Lentinula edodes*. However, cultivation and consumption of mushrooms of this genus in the western region have increased markedly (Lee *et al*., 2002; Royse *et al*., 2004). One way to increase the production of mushrooms is to optimize the potential of the medium (compost) to provide the needs of the mushrooms. A further review of the media content which includes elements of carbon, azoth, growth-regulating hormones, composting microorganisms, and checking their ability to increase mycelium growth and subsequently also increase the nutrient absorption required by the fungus, can be an influential factor in mushroom productivity. Some growth hormones in different concentrations can affect the size of the mushroom cap. These hormones play a significant role in mushroom mycelia which is grown in the laboratory (Nasr and Mahdipour, 2013). There are several commercial plant growth-promoting bacteria known as trigger growth through the mechanisms of suppressing plant diseases (bioprotectant), increasing nutrient absorption (biofertilization) or the formation of phytohormones (biostimulants). The application of these bacteria has been tested and shows considerable results. However, a deeper understanding of microbial interactions needs to be done to improve success in field applications (Burr *et al*., 1984). The purpose of this study was to examine the additional effect of specific bacteria that were isolated from the growth media (baglog) of *Pleurotus ostreatus* and study its effect on the mushrooms fruiting body growth.

2. Materials and methods

2.1 Fungal and bacterial isolates

The pure culture of *Pleurotus ostreatus* was obtained from the Laboratory of Food Microbiology, Research Center for Biology, Indonesian Institute of Sciences. The cultures were grown on potato dextrose agar (PDA) in petri dishes at a temperature of 25°C for 5 days. The pure culture of *B. aryabhattai* was obtained from an active baglog sample from the Laboratory of Food Microbiology, Research Center for Biology, Indonesian Institute of Sciences.

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2.2 Cell-free extract preparation and indole acetic acid assay

The *B. aryabhattai* culture was centrifuged for 30 mins at 4°C and 8000 rpm to separate the extract from the precipitate. The cell-free extract of *B. aryabhattai* was determined using a spectrophotometer as reported by Saitou and Nei (1987) to determine the levels of the IAA hormone produced by the bacteria. The indole acetic assay (IAA) content assay was performed by dripping Salkowsky reagent into a bacterial supernatant with 2:1 ratio. The presence of the IAA hormone will be seen through the pink color that appears. The reaction was then observed with a spectrophotometer to obtain the absorbance value of the IAA hormone, and then the value was converted with the IAA standard curve to obtain the IAA content value in mg/L.

2.3 Baglog preparation

Baglog as a mushroom-growing medium is made by mixing sawdust, bran, corn, lime, gypsum and water.

2.4 Statistical analysis

The difference in the *P. ostreatus* growth parameter was determined using ANOVA with significant differences ($p<0.05$).

3. Results and discussion

The result of IAA content assay from *B. aryabhattai* shows that the bacteria produce the highest amount of IAA content (3.17 mg·L^{-1}) on the 7th day of incubation (Figure 1). IAA is a common product of L-tryptophan metabolism produced by several microorganisms including plant growth-promoting bacteria (PGPR) (Lynch, 1985). IAA stimulates cell elongation by modifying certain conditions, such as an increase in osmotic contents of the cell, an increase in permeability of water into the cell, a decrease in wall pressure, an increase in cell wall synthesis and inducing specific RXA and protein synthesis. It promotes activity that inhibits or delays the abscission of leaves, and induces flowering and fruiting (Zhao, 2010).

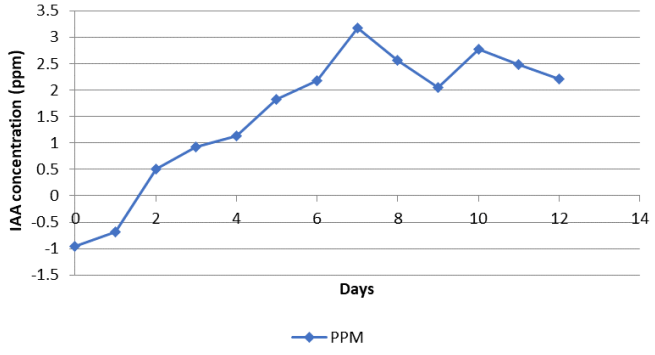

Figure 1. The content of IAA produced by *B. aryabhattai* and the time of production.

The addition of *B. aryabhattai* extract into baglog showed an increase in the growth of white oyster mushroom mycelia on each day of observation, but statistically, there was no significant effect ($P<0.05$) from the addition of bacterial extract on the growth of white oyster mushroom mycelia on each day of observation. *Bacillus aryabhattai* extract was tested for its effect on the growth of white oyster mushroom mycelia, showing the results that baglog with the addition of bacterial extract with a concentration of 50% triggered the growth of white oyster mushroom mycelia faster than baglog given a mixture of extracts with a concentration of 75% and control baglog (Figure 2).

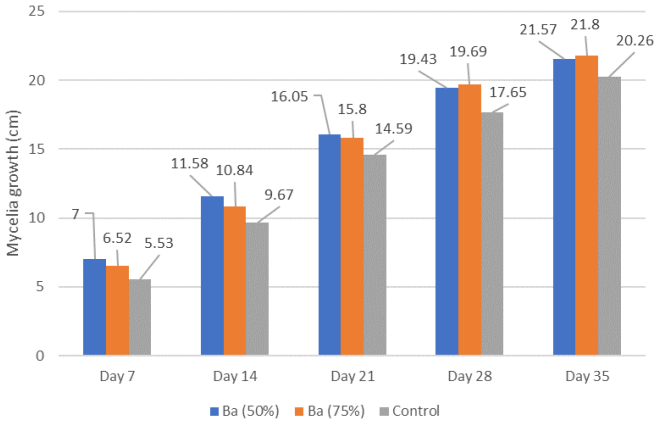

Figure 2. Mycelia growth of *P. ostreatus* in baglog with a mixture of *B. aryabhattai* extract with concentrations of 50% and 75%.

The growth-inducing effect by bacteria was observed by measuring fungal mycelia in baglog, and baglog with the addition of 50% and 75% *B. aryabhattai* extract showed an increase in mycelia, growth faster than control baglog. Mycelia growth from baglog with 50% concentration (7.00 cm) was greater than that of 75% baglog (6.52 cm) and control baglog (5.53 cm) on day 7 growth. Mycelia growth from baglog with 50% concentration on days 14 and 21 (11.58 cm and 16.05 cm) is bigger compared to 75% baglog mycelia on days 14 and 21 (10.84 cm and 15.80 cm) and control baglog (9.67 cm and 14.59 cm). Mycelia growth observed on days 28 and 35 showed baglog with the addition of *B. aryabhattai* extract at a concentration of 75% showed the best results (19.69 cm and 21.8 cm) when compared to baglog with the addition of 50% bacterial extract (19.43 cm and 21.57 cm) and control baglog (17.65 cm and 20.26). Overall observations showed that baglog with the addition of bacterial extract showed an increase in mycelia growth when compared to the control baglog.

The effect of adding *B. aryabhattai* extract into baglog was further observed in the fruiting body phase (Figure 3), and the results showed an increase in the

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number of white oyster mushroom fruiting bodies (Figure 4) and weight (Figure 5) for every day of observation, but statistically there was no significant effect of the addition of bacterial extract on the number of white oyster mushroom fruiting bodies and weight on each day of observation.

Figure 3. White oyster mushroom fruiting bodies resulted from the addition of 75% (A), 50% (B) and control (C) extracts of *B. aryabhattai*.

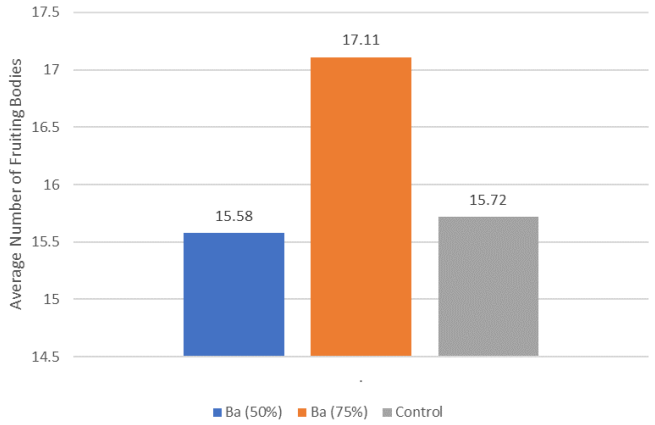

Figure 4. The average number of white oyster mushroom fruiting bodies in baglog with a mixture of *B. aryabhattai* extract with a concentration of 50% and 75%.

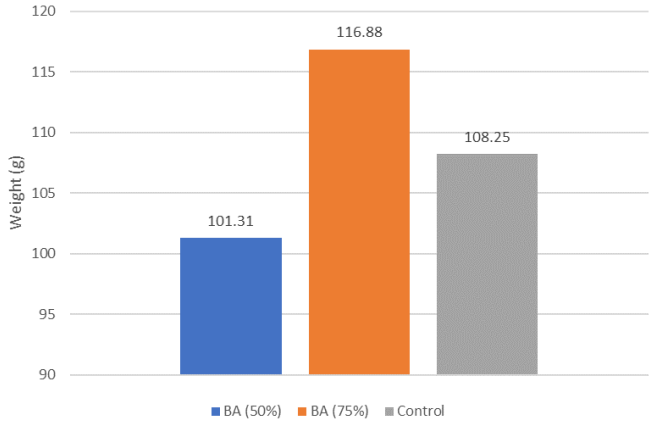

Figure 5. Weight of white oyster mushroom fruiting bodies in baglog with a mixture of *B. aryabhattai* extract with concentrations of 50% and 75%.

4. Conclusion

The addition of *B. aryabhattai* extract into baglog affected the growth and productivity of white oyster mushrooms, although there was no statistically significant effect. Several test parameters such as number of fruiting bodies and weight showed an increase after being given *B. aryabhattai* extract with concentrations of 75% and 50% into baglog, this indicates that *Bacillus aryabhattai* has the potential to trigger the growth of white oyster mushrooms.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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