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K. A. Hannah; W. Mangunwardoyo ✉; I. Saskiawan

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Supplementation of Bacterial Indole-3-acetic Acid to Increase Growth and Productivity of White Oyster Mushroom (*Pleurotus osreatus* (Jacq.) P.Kumm)

K. A. Hannah[1], W. Mangunwardoyo[1, a)] and I. Saskiawan[2]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Indonesia, Depok 16424, Indonesia*
[2]*Microbial Ecology and Physiology, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong 16911, Indonesia*

[a)]Corresponding author: wibowo.mangun@ui.ac.id

Abstract. The research aims to understand the effect of supplementation of bacterial IAA produced by *Bacillus aryabhattai*, *Lysinibacillus boronitolerans* and *Pseudomonas putida* on the growth and productivity of white oyster mushroom (*Pleurotus ostreatus* (Jacq.) P.Kumm) through in vitro and in vivo assay. In vitro experiment was done through an addition of bacterial supernatant into PDA and measured the diameter (mm) of the mycelia. In vivo treatment was carried out through the addition of 50 % bacterial supernatant into growth media of mushroom cultivation (baglog) followed by measuring the mycelia length throughout the baglog for growth and number of fruiting bodies, wet weight of fruiting bodies, diameter of pileus, and stipe length for productivity. In vitro results revealed that there was a significant difference ($p > 0.05$) of mycelia growth between treatments on 4^{th}–6^{th} days of incubation. Supernatant of *L. boronitolerans* gave the best mycelia growth with a diameter of 84.86 ± 5.45 mm. In vivo results showed that there was a significant difference of mycelia growth between treatments at 2^{nd}–5^{th} weeks of incubation. Supernatant of *P. putida* gave the best effect on enhancing *P. ostreatus* productivity with the average of 6.89 ± 4.59 for number of fruiting bodies, 116.33 ± 22.58 g for wet weight of fruiting bodies, 8.84 ± 2.44 cm for the diameter of pileus and 7.80 ± 1.72 cm for the stipe length. Experiment results showed that bacterial supernatant which contains IAA have the ability to enhance the mycelia growth and it corresponds with the IAA concentration produced, while supplementation of bacterial supernatant did not show any significant effects on the productivity of *P. ostreatus*.

Keywords: *Bacillus aryabhattai*, growth, indole-3-acetic acid, *Lysinibacillus boronitolerans*, *Pleurotus ostreatus*, productivity, *Pseudomonas putida*

INTRODUCTION

Microorganisms can colonize a specific habitat and interact with the surrounding organisms. Secondary metabolites are known to play a role in mediating the interactions between microorganisms to regulate their coexistence and defend mechanisms. Interaction between bacteria-fungi and bacteria-plants are one of the example of interactions in nature [1]. Soil bacteria are known to have a role in plants' growth and shows positive interaction with surrounding plants. Such bacteria are known as plant growth-promoting bacteria (PGPB) [2]. Interactions between bacteria and fungi can be found in growth media of mushroom cultivation (baglog). The presence of bacteria in growth media of mushroom is predicted to play a role as mushroom growth-promoting bacteria (MGPB) to enhance mushroom growth [3].

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Microorganisms such as bacteria have the ability to produce metabolites such as indole-3-acetic acid (IAA) that can enhance plant and mushroom growth. IAA is known to regulate plant growth and development but still a little informations of its role in mushroom growth and productivity [4]. Studies on MGPB start to increase in recent years, especially in exploring beneficial bacteria that produces IAA and uses its supernatant to enhance mushroom growth and productivity. Various species of bacteria within the genus of *Bacillus* and *Pseudomonas* are explored, known to produce IAA and have beneficial effect of promoting plant and mushroom growth [2, 5].

There is still a number of understanding on the role of IAA in fungal growth compared to plant growth and development. Therefore, the objectives of this research are to study the effect of IAA produced by *Bacillus aryabhattai*, *Lysinibacillus boronitolerans* and *Pseudomonas putida* supplemented in growth media of *Pleurotus ostreatus*.

EXPERIMENTAL

Microorganisms

Bacterial isolate of *B. aryabhattai* and *L. boronitolerans* and fungal isolate of *P. ostreatus* are the collection of Laboratory of Food Microbiology, Indonesian Institute of Sciences (LIPI) Cibinong and *P. putida* is a collection of Indonesian Culture Collection (InaCC).

Bacterial Supernatant Preparation

Three bacterial isolates were inoculated in 200 mL nutrient broth media supplemented with 300 ppm tryptophan as precursor and incubated in a shaker incubation at 30 °C and 120 rpm. After 48 h bacterial culture was collected and centrifuged at 10,000 rpm for 30 min. Cell-free supernatant was then stored in a sterile Erlenmeyer.

Quantitative determination of bacterial IAA: Amount 1 mL of cell-free supernatant was added to a reaction tube 10 mL and 2 mL of Salkowski reagent (H_2SO_4 95–97 %, 0.5 M $FeCl_3$•$6H_2O$ and aquadest). The mixture was stored in a dark room for 30 min to obtain discoloration to pink to indicate a positive reaction of IAA production. The IAA concentration was then measured by using spectrophotometer (λ 535 nm) and calculated from linear equation of standard IAA [6].

In vitro Assay

In vitro assay was done to understand the effect of bacterial supernatant on *P. ostreatus* growth. Amount 10 % of bacterial supernatant of *B. aryabhattai*, *L. boronitolerans* and *P. putida* was added to 15 mL of PDA separately, then poured into 90 mm petri dish and control treatment petri dish only contains PDA, six replicates each treatment. Hardened media was then inoculated with *P. ostreatus* using cork borrer 5 mm, placed in the middle of the petri dish, incubated at 30 °C until petri dish fully covered by mycelia. Measurement of the mycelia diameter (mm) was done every 24 h using a digital calliper.

In vivo Assay

In vivo assay was done to understand the effect of bacterial supernatant on *P. ostreatus* growth and productivity. This assay contains of four treatments; growth media with 50 % supernatant of *B. aryabhattai*, *L. boronitolerans* and *P. putida* and control treatment (without bacterial supernatant). Amount of 50 % of bacterial supernatant was mixed into the growth media of *P. ostreatus* (sawdust 85-90 %, $CaCO_3$ 1–2 %, bran 10–15 %, corn powder 2–4 % and gypsum 1–2 % and 240 mL water), packed into polyethylene plastic (1 kg) separately and sealed with plastic ring, then sterilized at 90–110 °C for 4–5 h. Sterilized media was then inoculated with 5 g of mother spawn *P. ostreatus* (corn and $CaCO_3$ 1 %) and incubated at 27–30 °C in incubation room until growth media was fully colonized. Spawn run was measured every week by measuring the length (cm) of mycelia growth from top of the ring to the bottom of media. After the growth media was fully colonized, it was then moved to mushroom production room at lower

temperature (25–27 °C) and higher humidity (80–90 %) to initiate the production of fruiting bodies. Productivity was obtained from measuring time of harvest (days after inoculation/day) of the fruiting bodies, number of fruiting bodies, wet weight (g), pileus diameter (cm), and stipe length (cm). All the parameters of each treatment were compared to control treatment.

Statistical Analysis

Complete Randomized Design (CRD) was used as experimental design for in vitro and in vivo assay, with each assay containing four treatments and a minimum of 6 replicates. Data were then analyzed using one way Analysis of Variance (ANOVA) ($\alpha = 0.05$) and continued with Least Significant Different (LSD) analysis ($\alpha = 0.05$). Software IBM SPSS Statistics Version 20 was used for all statistical analysis.

RESULTS AND DISCUSSION

IAA Production

The result of quantitative determination of bacterial IAA showed that *L. boronitolerans* produced the highest IAA concentration was 7.51 ± 1.38 ppm, followed by *B. aryabhattai* and *P. putida* with IAA concentration of 1.30 ± 0.12 ppm and 1.02 ± 1.38 ppm, respectively.

In vitro Assay

After 8 days of incubation, the supplementation of bacterial IAA into PDA resulted in a significant increase of mycelia growth of *P. ostreatus* for 4th–6th days of incubation. Supernatant of *L. boronitolerans* gave the best effect on enhancing mycelia growth with the longest mycelia diameter of 84.86 ± 5.45 mm on the 6th days of incubation, followed by *B. aryabhattai* and *P. putida* with mycelia diameter of 81.99 ± 2.89 mm and 80.35 ± 5.12 mm, respectively (Table 1 and Fig. 1).

In vivo Assay

After 6 weeks of incubation, mushroom cultivation media (baglog) was fully covered with mycelia. One-way ANOVA and LSD test revealed that there was a significant difference of mycelia growth between treatments on 2nd–5th weeks of incubation (Table 2 and Fig. 2).

TABLE 1. Average diameter of mycelia *P. ostreatus* (mm).

Treatments	Incubation period (days)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Bacillus aryabhattai	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	23.84 ± 1.81^{a}	46.83 ± 3.22^{a}	66.07 ± 2.95^{a}	81.99 ± 2.89^{ac}	89.17 ± 2.04^{a}	90.00 ± 0.00^{a}
Lysinibacillus boronitolerans	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	23.69 ± 2.22^{a}	49.90 ± 2.69^{ab}	68.89 ± 4.55^{ac}	84.86 ± 5.45^{ac}	89.60 ± 0.99^{a}	90.00 ± 0.00^{a}
Pseudomonas putida	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	20.84 ± 3.96^{a}	46.08 ± 4.46^{ac}	63.71 ± 5.25^{ab}	80.35 ± 5.12^{a}	88.52 ± 2.52^{a}	90.00 ± 0.00^{a}
Control	5.00 ± 0.00^{a}	5.00 ± 0.00^{a}	20.89 ± 1.88^{a}	43.52 ± 1.26^{ac}	59.84 ± 1.81^{b}	75.79 ± 2.31^{ab}	86.61 ± 1.92^{a}	90.00 ± 0.00^{a}

Note: In one column, the mean values followed by the same letter are not significantly different from each other (p>0.05 followed by LSD test).

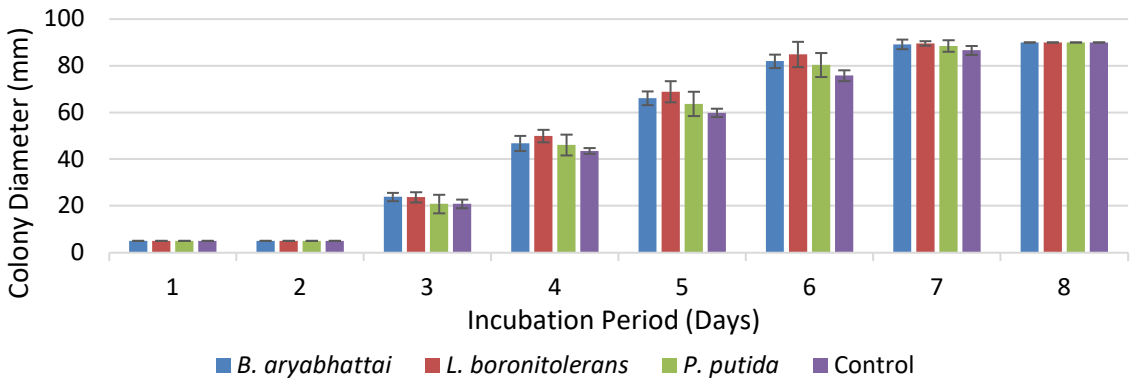

FIGURE 1. Colony diameter of *P. ostreatus* treated with three different bacterial IAA for 8 days if incubation.

TABLE 2. Means of mycelia length (cm) of *P. ostreatus* in baglog supplemented with 50 % bacterial IAA

Treatments	Incubation period (weeks)				
	1	2	3	4	5
Bacillus aryabhattai	4.56 ± 0.20[a]	9.31 ± 0.16[a]	13.13 ± 0.71[a]	15.24 ± 0.46[a]	17.96 ± 0.46[a]
Lysinibacillus boronitolerans	4.65 ± 0.42[a]	9.43 ± 0.21[a]	12.94 ± 0.26[ac]	15.10 ± 0.45[a]	17.45 ± 0.38[ac]
Pseudomonas putida	4.45 ± 0.21[a]	9.09 ± 0.20[b]	12.57 ± 0.29[bc]	14.9 ± 0.54[ac]	17.85 ± 0.79[a]
Control	4.49 ± 0.19[a]	9.05 ± 0.30[b]	12.34 ± 0.30[b]	14.64 ± 0.43[bc]	17.17 ± 0.69[bc]

Note: In one column, the mean values followed by the same letter are not significantly different from each other (p>0.05 followed by LSD test).

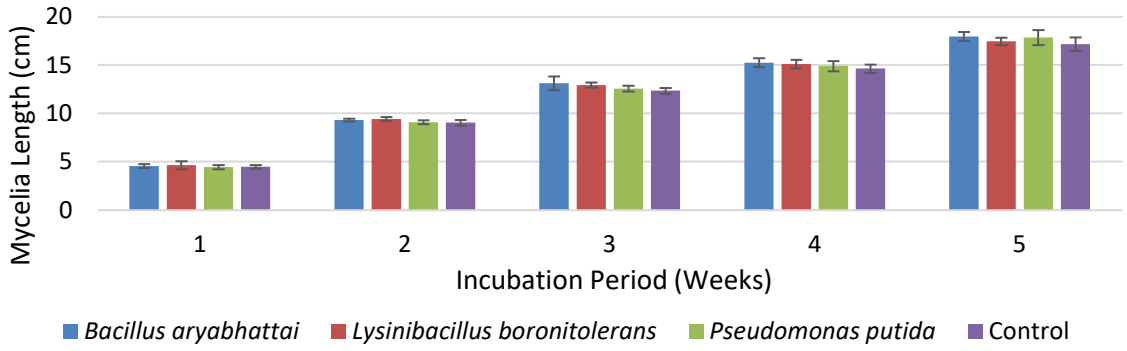

FIGURE 2. Mycelia length of *P. ostreatus* throughout baglog supplemented with different bacterial IAA for 5 weeks.

In vivo assay showed that supplementation of bacterial IAA enhances the growth of *P. ostreatus*. All bacterial isolates used in this experiment are known to be soil bacteria and have the ability to promote plant growth by elongating plant cells. Bacteria within the genus *Bacillus* and *Pseudomonas* also found inside growth media of *P. ostreatus* and was known that there are interactions between bacteria and fungi [7].

TABLE 3. Average number of fruiting bodies, wet weight (g), diameter of pileus (cm) and stipe length (cm) of *P. ostreatus* on different baglog supplemented with bacterial IAA.

Treatment	Total fruiting bodies	Wet weight (g)	Pileus diameter (cm)	Stipe long (cm)
Bacillus aryabhattai	5.13 ± 2.03[a]	89.38 ± 35.07[a]	7.90 ± 1.83[a]	7.60 ± 1.44[a]
Lysinibacillus boronitolerans	5.67 ± 1.94[a]	93.44 ± 46.52[a]	8.29 ± 1.20[a]	6.64 ± 1.76[a]
Pseudomonas putida	6.89 ± 4.59[a]	116.33 ± 22.58[a]	8.84 ± 2.44[a]	7.80 ± 1.72[a]
Control	5.00 ± 2.10[a]	82.17 ± 34.49[a]	8.21 ± 1.78[a]	6.78 ± 1.53[a]

Note: In one column, the mean values followed by the same letter are not significantly different from each other ($p>0.05$ followed by LSD test).

Mushroom cultivated from baglog supplemented with 50 % bacterial IAA of P. putida performed better in terms of number of fruiting bodies, wet weight (g), diameter of pileus (cm) and stipe length (cm) compared to other treatments (Table 3), but it does not differ significantly.

The results of productivity measurements have yet to explain the role of IAA on enhancing the productivity of *P. ostreatus* because there was no significant difference between all treatments. The experiment results also showed that concentration does not affect the performance of mushroom productivity, as seen by the results that bacterial IAA of *P. putida* with the lowest IAA concentration gave the highest performance on mushroom productivity [8].

The results of productivity measurements differ from growth performance of *P. ostreatus* on both in vitro and in vivo assay, in which bacterial supernatant enhances the mycelia growth and the degree of its performance corresponds with the concentration of IAA produced. Bacterial IAA of *L. boronitolerans* produced the highest IAA (7.51 ppm) gave the best results on mycelia growth on both in vitro and in vivo assay, followed by *B. aryabhattai* and *P. putida* with IAA concentration of 1.30 ppm and 1.02 ppm, respectively. This experiment revealed that bacterial IAA affects mycelia elongation only in the vegetative phase and did not show any significant effects on enhancing productivity (reproductive phase) [9]. Similar findings on mycelia growth enhancement matched with the study of Febriansyah et al reporting that bacterial IAA enhances mycelia growth on PDA [10].

CONCLUSION

Supplementation of bacterial IAA from *B. aryabhattai*, *L. boronitolerans* and *P. putida* showed significant effects on enhancing mycelia growth of *P. ostreatus* on 4^{th}–6^{th} days of incubation, with *L. boronitolerans* gives the best mycelia growth. The same effect in the increase of mycelia growth was also observed from baglog on 2^{nd}–5^{th} weeks of incubation, although no significant effect observed statistically. The best performance of mushroom productivity was obtained from baglog supplemented with bacterial IAA of *P. putida*, but it did not show any significant difference on the mushroom productivity.

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