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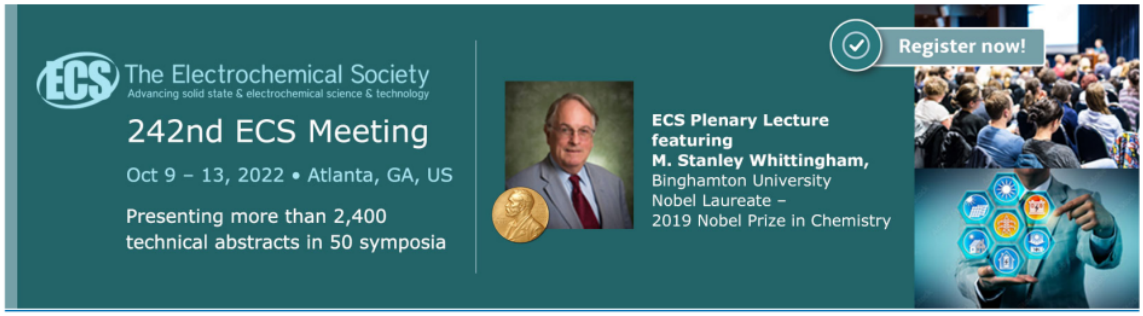

Selection of endophytic fungi from Sinjai local red rice as producer of IAA (Indole Acetad Acid) hormone

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Abstract. South Sulawesi, specifically Sinjai area, is an aromatic rice development area that has the potential to be developed. The applied cultivation was still traditional and based on local wisdom which was without the use of fertilizers, pesticides and other chemical drugs, so it was expected that there was a high diversity of microorganism biodiversity in plant tissues, especially endophytic fungi could produce Indole Acetic Acid (IAA) hormone. The purpose of this study was studying the potential of fungi isolates as producer of IAA hormone from Sinjai local red rice. Stages of research included macroscopic and microscopic observations of endophytic fungi and IAA hormone analysis. The fungus which had the potential to produce the highest IAA hormone was P31 BMB 16E isolate (15.17 ppm).

1. Introduction

Indole Acetic Acid (IAA) hormone is the main hormone in almost all types of plants, which controls many important physiological processes such as cell enlargement, cell division and tissue differentiation [1]. *IAA* hormones are divided into two groups namely endogenous *IAA* and exogenous *IAA*. Endogenous *IAA* comes from the plant itself, while exogenous *IAA* is produced by microbes such as fungi [2]. *IAA*, which is produced by fungi, is known to produce more lateral roots, root hairs and root hair branches, thereby increasing the ability of plants to obtain nutrients.

Modern agriculture is currently very dependent on the use of chemicals including synthetic fertilizers, fungicides and pesticides which can actually cause pressure on the environment. Biotechnology products have been developed to solve these problems, one of which is the development of endophytic microorganisms that produce *Indole Acetic Acid (IAA)* phytohormone which can stimulate plant growth.

This study aimed to obtain endophytic fungi that had the potential to produce *IAA* hormones in Sinjai local red rice plants that was used as bio fertilizer agents for plants, so that if there were positive isolates that produced *IAA* hormones when applied to plants it could be beneficial for plant growth.

2. Materials and methods

2.1. Time and location

This research was conducted in July 2019. The research was carried out at Laboratory of Pests and Plant Diseases Department, Hasanuddin University.

2.2. Tools and materials

The used tools in conducting this research were test tubes (pyrex), erlenmeyer (pyrex), beaker glass (pyrex), drop pipettes, volume pipettes, micro pipettes (gilson), autoclaves (UL Model 25X-2), analytical scales (AND HF-300), spectrophotometer (Genesys 10S UV-Vis), centrifuge (Hitachi CTI 5R ε), shaker incubator, refrigerator, microwave, oven (655f), aluminum foil, tube rack, pH paper, bunsen, ose needle, bottle, stir bar, digital cameras and stationery.

The used fungus isolates were those obtained from Sinjai local red rice with 18 fungus isolates. The used ingredients were tryptophan, methanol, aquades, Salkowski reagents (concentrated of H2SO4, FeCl3 6H2O, aquades), 70% alcohol, spiritus.

2.3. Isolate rejuvenation

Pure isolates from Sinjai local red rice were rejuvenated by inoculating the fungus isolates used an ose needle into a petri dish that filled with PDA (*Potato Dextrosa Agar*) medium then incubated for 5-7 days.

2.4. Analysis of IAA production by fungi

The fungus inoculum was inoculated on 5 ml PDB (*Potato Dextrosa Broth*) which was enriched with tryptophan 100 µ g/ml and on PDB (*Potato Dextrosa Broth*) medium which was not enriched with tryptophan. Tryptophan was used as a precursor for *IAA* formation. Then incubated and agitated at room temperature with a speed of 150 rpm for 3 days in dark conditions.

Fungi cell cultur were centrifuged at 6000 rpm for 15 minutes to separate the microbial colonies from the medium. The supernatant of centrifugation result was transferred to a clean and sterile test tube of 1 ml, and then 4 ml of Salkowski reagent was added. The supernatant mixture and Salkowski reagent were incubated for 60 minutes in a dark room at room temperature. The solution changed become pink, this was an indication of *IAA* content in the solution. Then the absorbance of the solution was measured by using a spectrophotometer at a wavelength of 530 nm.

2.5. Making IAA Standard Curves

IAA standard curve making had done by the modified Pattern and Glick [3] method. The process of making an IAA standard solution could be carried out as follows:

1. Prepared 50 ml of methanol which had been dissolved 2.5 mg IAA synthesis concentration of 50 ppm
2. IAA synthesis solution was pipetted into test tubes of 20 µl (1 ppm), 100 µl (5 ppm), 200 µl (10 ppm), 300 µl (15 ppm), 400 µl (20 ppm),500 µl (25 ppm), 600 µl (30 ppm), 700 µl (35 ppm), 800 µl (40 ppm) dan 900 µl (45 ppm)
3. Added methanol so that the volume of each test tube becomed 1000 µl
4. Then added 4 ml of Salkowski reagent to each test tube then it was homogenized
5. Incubated for 30 minutes in a dark room with room temperature so the solution becomed turn pink

The IAA standard solution was measured for absorbance using a spectrophotometer at a wavelength of 520 nm. The results was obtained from the spectrophotometer which were made IAA standard solution curves which showed the relationship between IAA standard solution (x) and absorbance (y) and the obtained equation was y = 0.018x0.001. Calculations to find IAA concentration by replacing the variable of y in the standard curve equation with

the measurement result of the supernatant absorbance of fungi isolates from each sample. IAA standard curve result was obtained value of x which stated the concentration of IAA.

2.6. Data analysis

Data of mushroom test results which produced IAA hormone were presented in tables and figures, and then the data were analyzed descriptively. Observations were made on the ability of the fungus to produce IAA hormones, based on the absorbance values of the standard curves, which were tested median for the mid, high and low criteria.

3. Results and discussion

3.1. Isolate rejuvenation

Fungi isolate which was obtained from Sinjai local red rice amounted to 36 isolates, but those successfully rejuvenated were 18 fungi isolates. Each fungus isolate was tested for its ability to produce IAA hormone

3.2. Color changes in IAA test

The results of the study after a qualitative test obtained supernatant mushroom isolates that changed color to dark yellow, pink, and dark pink when fungus isolates that had been grown on the PDB (Potato Dextrosa Broth) liquid media added to the Salkowski reagent. The color changed in each isolate was observed 30 minutes after the isolate was incubated in a dark room. Most color changes were the color changed from yellow (control) to dark yellow, while the least color changed was the color changed to dark pink. The results of observations differed from those of [4] that showed color cngaed to pink in all endophytic fungi isolates whiich were isolated from aromatic rice of *pare kaloko* species after administration of Salkowski reagents which was compared with controls. The difference of the results, which was obtained, was thought to be due to the origin of the fungus isolates and the different types of fungi.

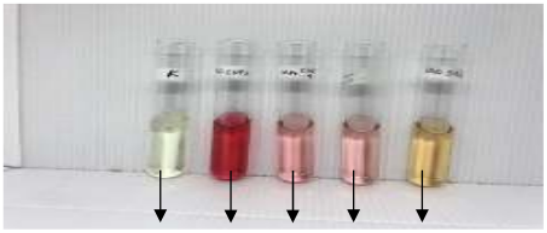

Figure 1. IAA test Qualitatively, (a) Control, (b) P31 BMB 16E isolates (c) P31 BMB 13E (d) P31 BMB 07 isolates and (e) P31 BMB 05 isolates

3.3. IAA hormone production by fungus

The tested fungus isolates were able to produce IAA hormones with varying levels. A total of 17 fungal isolates produced IAA with the addition of tryptophan. Based on the test of median, there were as many as 3 isolates produced a dark pink which was an indicator of the test color for the formation of IAA with high criteria. Another 9 isolates produced pink with medium criteria and 7 isolates produced transparent pink with low test criteria.

In Table 1 it can be seen that IAA hormone production which was included in the high criteria was in the range of 0.02 ppm (P31 BMB 08) to 15.17 ppm (P31 BMB 16E) in PDB medium which enriched tryptophan. In this study, the concentration had produced by P31 BMB 16E in producing IAA was still relatively low compared to *Phanerochaete chryosporium* mushroom which produced IAA with a high concentration of 840.46 µg / ml

containing *Jatropha seedcake* (JSC) in the basal salt medium (BSM) and addition of tryptophan 100 µg / ml for 15 days incubated which was agitated on a rotary shaker at a speed of 150 rpm at 30 ° C (Bose et al., 2013).

Table 1. Results of IAA concentration measurement of endophytic fungal isolates with a wave length of 520 nm

Isolate Code	Absorption Value	IAA Concentration (ppm)
P31 BMB 09AE	0.04	0.48
P31 BMB 16E	0.98	15.17
P31 BMB 05	0.12	1.73
P31 BMB 07	0.15	2.20
P31 BMB 17E	0.03	0.33
P31 BMB 18	0.03	0.33
P31 BMB 15	0.03	0.33
P31 BMB 06	0.03	0.33
P31 BMB 10	0.03	0.33
P31 BMB 14E	0.03	0.33
P31 BMB 13E	0.03	0.33
P31 BMB 19	0.16	2.36
P31 BMB 11	0.06	0.80
P31 BMB 12	0.1	1.42
P31 BMB 08	0.01	0.02
P31 BMB 17 AE	0.03	0.33
P31 BMB 04	0.02	0.17

P (Rice), BMB (Sinjai Red Rice)

According to Glick [6], obtaining fungi isolates that were able to produce IAA could provide new trand in increasing plant growth. Isolates that were able to produce IAA can be used as biological control through competition, antibiotic production, induction of plant resistance, phytohormone production and increased nutrient availability through N fixation or increased dissolution of organic and inorganic phosphates.

4. Conclusion

Based on the results and discussion, the conclusions of this study as follows: 1) there were 18 endophytic fungi isolates in Sinjai local red rice which had been successfully rejuvenated and all of them were able to produce IAA hormone. 2) the highest IAA hormone ability was obtained in P31 BMB 16E isolates with IAA concentration of 15.17 ppm at 48 hours incubation.

References

[1] Fahima T K and Enny Z 2015 Azotobacter potential as an IAA hormone producer *J. Sci Arts* **4** (2) 2337-20
[2] Suciamith 2006 Microfrora-soil of banana and cassava plants on peat lands and alluvial soils in Bengkulu *Bio-diversity* **7**(4) 303-06
[3] Pattern C L and Glick B R 2002 Regulation of indoleacetic acid production in *Pseudomonas putida* GR12-2 by tryptophan and the stationary-phase sigma factor RpoS *Can. J. Microbiol.* **48** 635–42
[4] Abri, Kuswinanti T, Lisan E and Sjahrir 2015 *Isolation of Rhizosfer Fungi Producing Indole Acetic Acid (IAA) Hormone on Tanatoraja's Aromatic Rice* Essay (Makassar:

Agrotechnology Department, 45 University Makassar)

[5] Bose A, Shah D and Keharia H 2013 Production of indole-3-acetic-acid (IAA) by the white rot fungus *Pleurotus ostreatus* under submerged condition of Jatropha seedcake *Mycology* **4** 103–11

[6] Glick 1995 The enhancement of plant Growth by Free living bacteria *Can. J. Microbiol.* **41** 109-117

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