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Submission date: 25-Aug-2021 11:56AM (UTC+0700)

Submission ID: 1635620955

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Word count: 2378

Character count: 12607



Original Research Article

Production of Indole Acetic Acid (IAA) Hormone from Fungal Isolates Collected from Rhizosphere of Aromatic Rice in Tana Toraja

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Abstract	Keywords
South Sulawesi, especially Tana Toraja district is an aromatic rice center area that has potential to be developed. Applied cultivation in this area is still traditionally and based on local wisdom, i.e. no use of fertilizers, pesticides and other chemicals. Based on this fact, it is suggested that there are a high diversity of microorganisms from the rice rhizosphere, especially fungus that produce Indole Acetic Acid (IAA) hormone. A series of dilution method was done for isolation of fungal isolates. Purified isolates were cultivated on Potato Dextrose Agar (PDA) until further use. Measurements of IAA production were performed using standard methods. From rhizosphere of aromatic rice Pare Kaloko, 19 fungal isolates were successfully collected, whereas from 15 isolates were collected from Pare Bau. Average production of IAA was ranged from 0.556-2.190 mg /l among isolates of Pare Kaloko, and 0.048-1.810 mg/l among fungal isolates from Pare Bau.	Aromatic rice Fungal isolates Indole Acetic Acid Rhizosphere

Introduction

Tanatoraja is one of the districts in South Sulawesi as the regional development of local rice. Several types of aromatic rice, such as Pare Bau, Pare Tallang, Pare Laloda, Pare Kaloko, Pare Birrang, Pare Ambo, Pare Kobo, Pare Lea, and Pare Bumbungan grow in this area.

Cultivation systems that applied in this area is still conducted traditionally, that resulting very low production. Farming system without the use of

inorganic fertilizers, pesticides and other chemical medicinal drugs, so expect a natural ecosystem can be maintained and rich of microorganisms diversity, especially rhizosphere fungus that are biofertilizer, bio-activator and biocontrol.

Types of fungi in the rhizosphere of aromatic rice done by isolation and identification of both morphological and molecular process. Identification is an activity that is very important because many types of fungi are

unknown in their number and type. The number of fungus species has been already known up until now only about 69,000 of the estimated 1.5 million species that exist in the world. It can be ensured that Indonesia is very rich with diversity of plants and animals. It also has a very high diversity of fungus, considering the humid environment and tropical temperatures that favor the growth of fungus (Rifai, 1995)

Many types of fungi can be isolated from the rhizosphere of cultivated plants such as rice, peppers, potatoes, tobacco and maize, this fungus can stimulate the growth of plants that belong to the Plant Growth Promoting Fungi (PGPF). Regardless of synthesis by plants, IAA also produced by fungi (Yurekli et al., 2003; Maor, 2004; Bose et al., 2013; Usha and Padmavati, 2013). These fungi can improve plant growth on root colonization functionally (Usha and Padmavati, 2013). Fungal isolates have been shown to produce siderophores, IAA, catalase enzyme activity and has the ability as a biocontrol agent.

Therefore, the rhizosphere fungi have an important role in increasing the availability of the hormone auxin in plants, so it needs to be studied on the isolation and identification of aromatic rice fields that live on the natural ecosystem that is expected to obtain a variety of fungal species, especially those that are Plant Growth Promoting Fungi (PGPF).

Materials and methods

Isolation of rhizosphere soil fungi

Soil samples taken near the root or roots attached to the 9 types of health aromatic rice plants. Each type of uptake at random at the intersection of the diagonal so it will get 5 samples of soil at each planting site of aromatic rice. The samples were then mixed together and put into a plastic bag (Gams et al., 1987). Aromatic rice rhizosphere taken as much as 10 g then suspended in 100 ml of sterile distilled water and shaken for 20 minutes, after which 1 ml of suspension was transferred into 9 ml of sterile distilled water in a test tube, and then shaken until homogeneous (dilution phase I / 10^{-1}), the same dilutions performed until 10^{-4} and 10^{-5} dilution. Dilutions from 10^{-1} to 10^{-5} were respectively taken 1 ml put into a sterile Petri dish using measuring pipette aseptically, then the PDA medium was diluted (temperature 45°C) has been added chloramphenicol poured into a Petri dish, and

then homogenized by shaking the Petri dish until the suspension is spread evenly in the media. After it was incubated at room temperature ($22-25^{\circ}\text{C}$) for 5-7 days. To obtain pure cultures, performed purification fungus obtained (Affandi et al., 2001). Colonies of fungus grown on dilutions 10^{-3} - 10^{-5} were subjected for purification. Purification was done by transferring the fungal colonies on new sterile PDA medium.

In vitro screening of isolates

Purification of fungus spore isolates was done by single spore isolation. Each cultured isolates fungi grown on PDA slant medium and incubated for 5 days. Cultures of fungus that have been aged 5 days was added 4 ml of sterile distilled water. Spores growing scraped with a needle to release it from the agar and vortex to obtain a spore suspension. Spore suspension was diluted with sterile distilled water to achieve a 10^{-3} dilution. A total of 0.1 ml of the suspension spread evenly on the surface of a Petri dish containing PDA medium and incubated at room temperature ($26-28^{\circ}\text{C}$). Single colonies were grown in PDA slant was then transferred to working culture and stock culture. Purification of fungus isolates that do not form spores done by taking a single hyphae were grown on PDA medium with the aid of a microscope.

Measurement of Indole Acetic Acid (IAA)

IAA production measurements performed using standard methods, namely by culturing 1 loop full of rhizosphere fungi isolates first. In the Czapek Dox Cliquid (Cz) media with the addition of L-Tryptophan and incubated (shaker) for 48 h in a dark room at room temperature. IAA produced by rhizosphere fungi isolates were screened by using a filter paper and then centrifuged at 3000 rpm for 30 min, 2 drops of acid orthophosphate was added, then added with 4 ml of Salkowski reagent (50 ml, 35% sulfuric acid, 1 ml of 0.5 mol FeCl_3 solution). This was kept in dark for 24 h. The change of solution colour to pink means, isolated fungi has produced IAA. Furthermore, measurement of absorbance of IAA production by using a spectrometer (Spectronic 20) at a wavelength of 530 nm (Gutierrez et al., 2009).

Results and discussion

Totally 19 fungal isolates were successfully isolated and purified from rhizosphere of aromatic rice Pare

Kaloko and 15 isolates from Pare Bau. Isolates were screened based on their morphological characters i.e., colony colour, colony surface and colony edge. Further characterization is still in process to identify the Genus of each isolate.

Ability in IAA production differed between fungal isolates. From 19 fungal isolates collected from aromatic rice Pare Kaloko, the production of IAA was ranged from 0.556 to 2.190 mg/l, whereas isolates from Pare Bau IAA production ranged from 0.048-1.810 mg/l (Figs. 1 and 2)

Fig. 1: Quantitative measurement of IAA production (mg/l) of rhizosphere fungal isolates from aromatic rice, Pare Kaloko.

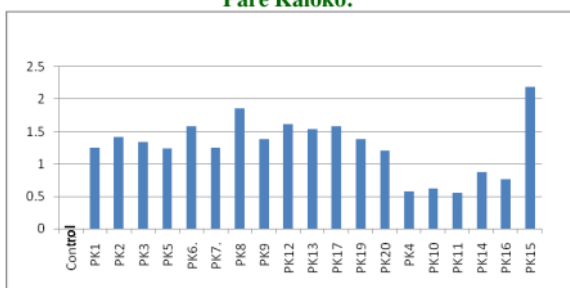
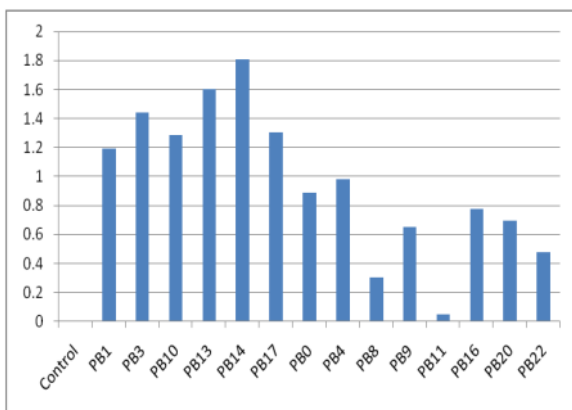


Fig. 2: Quantitative measurement of IAA production (mg/l) of rhizosphere fungal isolates from aromatic rice, Pare Bau.



Difference in IAA production among fungal isolates was observed by many researcher (Yadav et al., 2011), reported, that the fungus *Aspergillus niger* produces IAA by ($85 \mu\text{g ml}^{-1}$) and *T. harzianum* ($68 \mu\text{g ml}^{-1}$) and *Penicillium citrinum* ($52 \mu\text{g ml}^{-1}$) at 3 days of incubation at 30°C . The same report about IAA production in *Aspergillus niger* studied for 5-16 days

and a maximum production of 1.28 to $6.8 \mu\text{g ml}^{-1}$ was observed in Czapek-Dox broth medium with 0.1% tryptophan at 6 days of incubation (Bilkay et al., 2010). In the same study it reported that the production of IAA was found maximized at 28°C (Gunasekaran, 1978; Hasan, 2002). *Trichoderma atroviride* produces the highest level of IAA (6.2 , 9.8 and $38.55 \mu\text{g ml}^{-1}$) in the presence of $200 \mu\text{g ml}^{-1}$ tryptophan, tryptamine and tryptophol, respectively (Gravel et al., 2007). IAA is a secondary metabolite of the fungus, excreted by the microorganisms near the end of the growth phase or during plant dormancy phase. Therefore, it is expected that this plant regulator production time is long.

Many of fungi can produce auxin in sterile culture (Buckley and Pugh, 1971; Gruen, 1959). Most species use tryptophan to produce Indole-3-Acetic Acid (IAA), mainly through indole-3-pyruvate acid and tryptamine pathway (Tudzynski and Sharon, 2002). Tryptophan also increased biosynthesis of IAA 2.7-fold, that IAA biosynthesis enhanced by the availability of substrate. Enzymatic activity enhanced and IAA can be produced when the external tryptophan becomes available to the fungus, for the production of IAA. The fungus should be able to utilize tryptophan plant (Maor et al., 2004). IAA biosynthesis requires an external tryptophan. Therefore, tryptophan must be exported from the plant to the fungus during the biotrophic stage to support IAA biosynthesis, because the fungus does not infect cells at this early stage and does not have direct access to the cellular metabolic plant. If tryptophan is actively transported by plant or the specific-tryptophan transfer by simple diffusion needs to be further investigated (Maor et al., 2004). One of the roles suggested for the production of IAA by the fungus is to mediate the interaction between fungi and plants. A high concentration of IAA can inhibit the hypersensitive response (Robinette and Matthyse, 1990; Jouanneau et al., 1991) and can suppress the expression of plant defense genes (Yamada et al., 1985; Shinshi et al., 1987).

Conclusion

From Rhizosphere of aromatic rice Pare Kaloko 19 fungal isolates were successfully collected, whereas from Pare Bau 15 isolates were isolated. Average production of Indole Acetic Acid (IAA) was ranged from 0.556 - 2.190 mg/l among isolates of Pare Kaloko, and 0.048 - 1.810 mg/l among fungal isolates from Pare Bau.

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