Potential Rhizosphere Bacteria Originated From Potato Var. Hartapel From Buru Island As Plant Growth Promoters

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Abstract: Plant Growth Promoting Rhizobacteria (PGPR) are a group of bacteria that colonize the rhizosphere and can enhance plant growth directly or indirectly. Bacteria rhizoshere can induce substances like IAA and GA that can contribute to the improvement of potato growth, is crucial for sustainable potato cultivation. The present study was undertaken to screen the rhizosphere bacteria isolated from potato var. Hartapel growing regions of Buru Island for their physiological characteristics, including IAA and GA production. Of these isolates, 36 isolates were capable of producing IAA, and GA. Among the selective isolates, HB8 produced the highest amount of IAA (5.816 mg l^{-1}), while isolate HB32 produced the highest amount of GA (6.879 mg l^{-1}).

Key words : Buru Island, Bacteria, GA, IAA, Plant growth promoters, Potato var. Hartapel, Rhizosphere

1. Introduction

Potato yield in medium altitude areas is very low because of factors that are not suitable for potato tuberization. To overcome this can done by increasing induced systemic tolerance in potato plants against abiotic stresses through regulation of induced systemic tolerance. In Buru Island potatoes have been cultivated for hundreds of years at altitude of 700-1000 meters above sea level, so it can be used as a source of potential rhizosphere agents to be assembled as PGPR inducer expression changes of ROS-scavenging enzymes and photosynthetic activity, in the regulation of induced plant growth and development. The rhizosphere is a thin layer of soil immediately surrounding plant roots. This is an extremely important and active area for root activity and metabolism. A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant among them [1]. One effort that can be done is through the use of microbes as biostimulant effort, biofertilizer and bioprotectant. This is due to different types of microbes such as bacteria have been known to be used as a biocontrol agent to enhance the growth and production of plants known as the Plant Growth Promoting Rhizobacteria (PGPR), these bacteria are actively colonize the rhizosphere around the root surface and provide a positive influence to spur growth plants by providing nutrients and hormones to the plant and can be antagonistic to bacterial and fungal pathogens.

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These bacteria have the ability to provide and facilitate the absorption of various nutrients such as nitrogen and phosphate in the soil as well as synthesize phytohormones hyper growth [2]. Plant Growth Promoting Rhizobacteria (PGPR) can provide benefits through a variety of mechanisms such as helping plant the induced systemic resistance [3] and induced systemic tolerance [4]. Some strains of Bacillus have been isolated from the rhizosphere of potato [5,6,7], but there has been no research reports that discuss the role of the bacterial strains in abiotic stress tolerance in potato plants. The present study was undertaken to screen the rhizosphere bacteria isolated from potato growing regions of Buru Island for their physiological characteristics, including IAA and GA production.

2. Materials And Methods

2.1. Source of Bacteria

Bacteria were isolated from the rhizosphere soil samples Hartapel varieties of potato plants that grow on the altitude of 700 m above sea level in Leksula, South Buru-Maluku, Indonesia. In each sampling point, one sample consisted of rhizosphere soil (soil around the root zone) plants. Soil samples have been taken at a depth of 0-20 cm in the four quadrants stands potato varieties Hartapel then were combined.

2.2. Isolation of Rhizosphere Soil Samples of Potato

Isolation of rhizosphere bacteria carried by serial dilution method. Ten grams of rhizosphere soil was weighed and dissolved in 90 ml of sterile water, then shaked for 30 minutes. One ml of rhizosphere soil suspension was added to a test tube containing 9 ml of sterile water to obtain a suspension with a 10^{-2} dilution level. Dilution was done so in the same manner until a 10^{-8} suspension. Subsequently 0.1 ml of the suspension was grown on NA medium in a petri dish. NA medium which already contains rhizosphere bacteria were incubated for 24 hours at room temperature. Every single colonies were grown to reisolated and made as pure culture.

2.3. Production of indole acetic acid (IAA)

Production of auxin indole -3-acetic acid (IAA) by bacteria was tested using nutrient broth and Salkowski reagent [8]. PGPR isolates cultured in NB is equipped with L-tryptophan (0,1g

l^{-1}) at room temperature in the dark for five days, and the supernatant was taken after centrifugation. One ml of the supernatant was added to one ml of Salkowski reagent (12 g l^{-1} $FeCl_3$ in 429 ml l^{-1} H_2SO_4) [9] and incubated in dark for 24 hours at room temperature. The intensity of pink colour developed was read at 535 nm using a UV-VIS spectrophotometer. From a standard curve prepared with known consentration of IAA, the quantity in the culture filtrate was determined and expressed as mg l^{-1}.

2.4. Production of Gibberellic acid (GA3)

This test used nutrient broth media [10]. One ml of bacterial isolates were added to the media and incubated at 37˚C for seven days. The cultures then were centrifuged at 8000 g for 10 min to remove the bacterial cells. Fifteen cultures were added to 5 ml of zinc acetate. Account after 2 minutes was added 2 ml of potassium ferrocyanide solution and centrifuged at 8000 g for 10 min. Five ml of the supernatant was added to five ml of 30 per cent hydrocloric acid and the mixture was incubated at 27^0C for 75 minutes. The blank was prepared with five percent hydrocloric acid. Absorbance was measured at 254 nm in the UV-VIS spectrophotometer. From a standard curve prepared by using gibberellic acid solution of knwon quantities, produced of GA by the culture was calculated and expressed as mg l^{-1}.

3. Results and Discussion

3.1. Isolation of Rhizosphere Bacteria

From rhizosphere soil samples of varities Hartapel from South Buru at an altitude of 700 m above sea level as much as 36 bacterial isolates were obtained. The test results of the physiological characteristics of bacterial isolates include production IAA and GA.

3.2. Production of Indole Acetic Acid (IAA)

The ability of the bacterial isolates to produce IAA was detected by the development of pink colour after the addition of salkowski reagen to the culture. Some species of bacteria have the ability to produce as IAA. Much evidence suggests that PGPR can affect plant growth and development as it can produce phytohormones. Phytohormones such as auxin is known to stimulate cell elongation and cell division differentiation [11], and gene regulation [12]. Indole acetic acid is the common natural auxin that shows all auxin activity and extensively affects plants physiology [13]. Indole acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement [14]. In our study, all 36 isolate were able to produce IAA growing in medium addition of triptophan. Maximum IAA production was recorded in isolate HB8 (5.816 mg l^{-1}) as compared to other isolates. The minimum amount of IAA production was recorded in isolate HB27 (0.240 mg l^{-1}) (Figure 1).

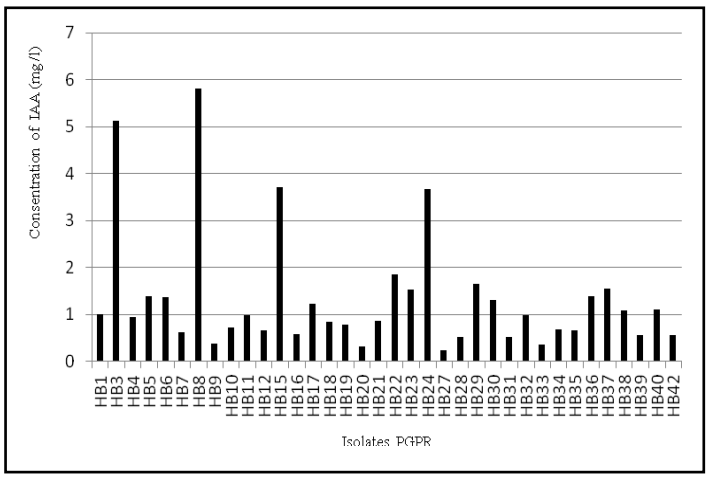

Figure 1. *IAA production by isolates bacteria rhizosphere*

3.3. Production of GA

Plant growth and development is also regulated by phytohormone producing PGPR's. Phytohormones such as auxins and cytokine production by PGPR's have been reported by many researchers, but evidence regarding production of gibberellins by the plant growth promotroy rhizobacteria are scanty [15]. Yet, it has been reported to be produced by certain rhizospheric bacteria's like *Bacillus licheniformis* and *Bacillus pumilus* [16]. Gibberellins also can alter the plant morphology by the elongation of stem tissues [17]. The maximum production was recorded in isolate HB32 (6.879 mg l^{-1}) and the minimum amout was produced by isolate HB3 (2.866 mg l^{-1}) (Figure 2).

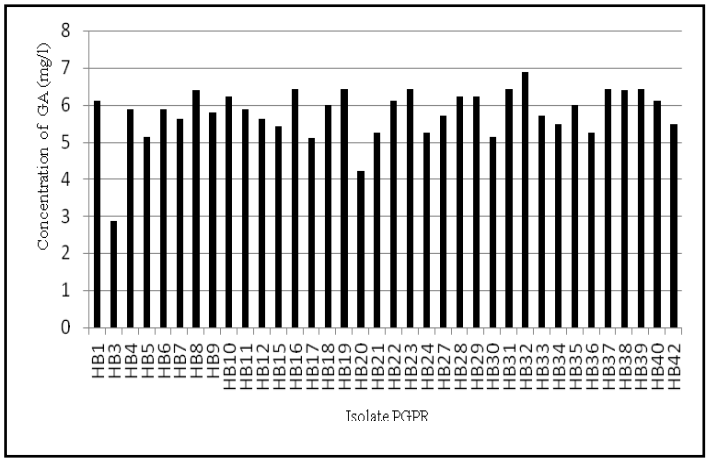

Figure 2. *GA production by isolates bacteria rhizosphere*

4. Conclusion

Bacterial isolated from rhizosphere of potato var. Hartapel Buru Island had the capability of producing phytohormones as plant growth promoters. Isolates HB8 produced the highest level of IAA concentration, and HB32 secreted the highest number of GA concentration.

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