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Isolation and Physiological Characterization of PGPR from Potato Plant Rhizosphere in Medium Land of Buru Island

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Abstract

A total of 70 bacteria were isolated from the rhizosphere of potato cv. Hartapel that grew at an altitude of 700 m above sea level on the island of Buru-Maluku. Of these isolates, 36 isolates were capable of producing IAA, GA, Siderophore and phosphate solubilization. Among the selective isolates, isolate 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). Isolate HB18 had the best ability in producing salicylate type siderophore (4.214 mg l⁻¹) and isolate HB3 showed the highest phosphate solubilization (14.237 mg l⁻¹). There were three isolates (HB3, HB8 and HB31) positively produced HCN. All 36 isolate showed physiological characters which revealed the potential use for biostimulant, biofertilizer and bioprotectant against soil borne pathogens.

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Key words : Potato cv. Hartapel, PGPR, Isolates, physiological characters, Bioprotectant, Medium land.

INTRODUCTION

Potatoes are one of the world's major food after rice, wheat, and corn but potatoes is relatively insensitive to losses due to soil salinity, drought, and low nutrient availability [1]. Potatoes in Indonesia are generally cultivated in the highlands, it is an obstacle in preserving nature. Cultivation of potato in the highlands can constantly damage the environment, especially the occurrence of soil erosion and lowered productivity. Therefore, the expansion

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step in land medium potato planting is one of the alternative measures that could be pursued, on the other hand these efforts will have problems due to environmental factors such as high temperature changes can hinder the process of tuberization.

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. 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 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 purpose of the study is isolate and characterize the physiological rhizosphere bacterial isolates potential as biostimulant, biofertilizer and bioprotectant which can to growth plants and confer protection against soil borne pathogens.

MATERIALS AND METHODS

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, Buru South-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 Hartapel potato varieties then were combined.

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 shaken 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.

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 ($0,1\text{g l}^{-1}$) 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 concentration of IAA, the quantity in the culture filtrate was determined and expressed as mg l^{-1} .

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 hydrochloric acid and the mixture was incubated at 27°C for 75 minutes. The blank was prepared with five percent hydrochloric acid. Absorbance was measured at 254 nm in the UV-VIS spectrophotometer. From a standard curve prepared by using gibberellic acid solution of known quantities, produced of GA by the culture was calculated and expressed as mg l^{-1} .

Production Siderophore Type Salicylate

Production of siderophore type salicylic by bacterial isolates were tested by the method as described in previous research [11]. The medium used were nutrient broth (NB). One ml of bacterial isolates were added to each flask and incubated at 37°C for seven days. After seven days of incubation, the culture broth were centrifuged at 10,000 g for 20 min. The supernatant was used for estimation of siderophore. Twenty ml of culture supernatant was taken and the pH was adjusted to 2.0 with dilute HCl. Into 20 ml of the supernatant was added 20 ml of ethyl acetate and extraction twice. Five ml of test solution was added to five ml of reagent

Hathway (one ml of 0.1 M ferric chloride and 1 ml of 0.1 N HCl was added to 100 ml of distilled water and to this one ml of 0.1 M potassium ferricyanide was added) and the absorbance was determined at 560 nm with sodium salicylate as a standard for estimation of salicylate. From the absorbance value of sample, the quantum of siderophore type salicylate produced was calculated and expressed as mg l^{-1} .

Phosphate Solubilization Test

Solubilization of phosphate was tested following the method of described by Pikovskaya [12,13]. Suspension 24-hour-old bacterial isolates grown on solid media containing tricalcium phosphate Phikovskaya (Ca_3PO_4) with dispersive method. The formation of transparent halos around each bacterial colonies showed solubilization activity. The resulting halos zone around the colonies after incubation for 3 days showed the presence of bacterial activity in solubilization phosphate. The ability of phosphate dissolving bacterial isolates using a quantitative liquid Phikovskaya media. Thirty ml of culture isolates were cultured in media Pikovskaya for 7 days and in the shaker. Bacterial suspension was filtered (Whatman No.1) and then centrifuged for 15 minutes at 10,000 g. Five ml of the supernatant was pipetted and added 0.5 ml of P concentrated reagent (P concentrated reagent : 12 g of ammonium molybdate, 0.277 g potassium antimol tartrate). The mixture was shaken for several minutes and then allowed to stand for 30 minutes. The absorbance was measured at 693 nm in the UV - VIS spectrophotometer. From a standard curve prepared by using PO_4 (Titrisol) solution of known quantities, phosphate solubilization was calculated and expressed as mg l^{-1} .

HCN Test

Screening of bacterial isolates for the production of hydrogen cyanide (HCN) is performed according to the method described as previous research [14]. The medium used is NA containing 4.4 g per liter of glycine. The production of cyanide was detected using a solution of cyanide detection solution (CDS) containing 2 g of picric acid and 8 grams of sodium carbonate dissolved in 200 ml of sterile distilled water. Pieces of filter paper the size of 1 x 1 cm was immersed in a solution of CDS and placed on the bottom of a petri dish, incubated at room temperature for 4 days. Discoloration of the filter paper from orange to brown after incubation was considered as microbial production of cyanide.

Thermotolerant Test

Resistant to temperature test with temperature levels of 50 and 70⁰C. A total of 100 µl isolates were added to 10 ml of NB medium were then incubated for 48 hours. After incubation the suspension isolates were reisolation in NB medium + agar then observed of growth after 24 hours. Isolates were grown indicated that these isolates were thermotolerant to temperature levels tested [15,16].

RESULTS AND DISCUSSION

Isolation of rhizosphere bacteria

From rhizosphere soil samples of varieties Hartapel from South Buru at an altitude of 700 m above sea level as much as 70 bacterial isolates were obtained. The test results of the physiological characteristics of bacterial isolates include production test phytohormones (IAA and GA), siderophores, phosphate dissolution, HCN production and resistance to temperature, is presented in Table 1 .

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 [17], and gene regulation [18]. Indole acetic acid is the common natural auxin that shows all auxin activity and extensively affects plants physiology [19]. Indole acetic acid is a phytohormone which is known to be involved in root initiation, cell division and cell enlargement [20]. 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).

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 promotory rhizobacteria are scanty [21]. Yet, it has been reported to be produced by certain

rhizospheric bacteria's like *Bacillus licheniformis* and *Bacillus pumilus* [22]. Gibberellins also can alter the plant morphology by the elongation of stem tissues [21]. Gibberellic acid produced isolates is presented in Table 1. The maximum production was recorded in isolate HB32 (6.879 mg l⁻¹) and the minimum amount was produced by isolate HB3 (2.866 mg l⁻¹).

Production of Siderophore Type

Siderophore classified into functioning to chelate ligand of iron. The main group siderophore include catechol (phenolat), hidroksamat and carboxylic acid (a derivative of citric acid). Siderophore stimulated the plant growth directly by increasing the availability of iron in the soil surrounding the roots or indirectly inhibiting the growth of plant pathogens with less efficient iron-uptake system [23]. Production siderophore of both salicylic and catechol type from rhizosphere isolates can stimulate the growth of plants as a form of contribution microbial properties [11]. Isolate HB18 showed the best ability in producing salicylate type siderophore (4.214 mg l⁻¹) while isolate HB20 had the lowest ability in producing salicylate type siderophore (2.772 mg l⁻¹).

Production of HCN

Three isolates (HB3, HB8 and HB31) positively produced hydrogen cyanide. The role of hydrogen cyanide in disease suppression has been demonstrated by several scientists in various crops [24,25,26]. Hydrogen cyanide is well known as a secondary metabolite produced by rhizosphere *Pseudomonas* [27]. Production of hydrogen cyanide by certain strains of *Pseudomonas fluorescens* has been involved in suppression of soil borne pathogens [25,28]. Hydrogen cyanide is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens [26].

Phosphate Solubilization Test

Phosphate solubilization ability is marked by the formation of transparent halos around the colony bacteria in media containing Phikovskaya tricalcium phosphate (Ca₃PO₄). The isolate that showed the best capability in solubilizing phosphate was isolate HB3 (14.2376 mg l⁻¹) and isolate HB18 also was able to solubilize phosphate but resulted in lower concentration of soluble phosphate (4.457 mg l⁻¹). Bacteria rhizosphere phosphate solvent as agents of plant growth promoters, which along its use as inoculants can increase phosphate uptake by plants [29,30]. Improving soil fertility is one of the most common strategies to increase agricultural

production. Microorganisms are biological agents capable of dissolving inorganic phosphate land and make it available to plants. The ability of some microorganisms to convert phosphate to form solution is available, such as orthophosphate, is an important trait PGPR to increase crop yields [29,31].

Table . 1 Physiological Character PGPR isolates From Rhizosphere Potato Plant Hartapel varieties

No.	Isolates	Production of Hormone		Production of Siderophore Type Salicylat (mg l ⁻¹)	Production of HCN *)	Phosphate Solubilization		Resistance to Temperature (***)	
		Concentration of IAA (mg l ⁻¹)	Concentration of GA (mg l ⁻¹)			Halo Zone **)	Concentration of Phosphate Solubilization (mg P l ⁻¹)	50°C	70°C
1	HB1	1.015	6.102	2.973	-	+	12.433	-	-
2	HB3	5.114	2.866	3.228	+	+	14.237	+	+
3	HB4	0.950	5.884	3.968	-	+	10.498	-	-
4	HB5	1.401	5.139	3.568	-	+	7.494	-	-
5	HB6	1.381	5.878	3.203	-	+	12.132	-	-
6	HB7	0.617	5.618	2.858	-	+	11.564	+	-
7	HB8	5.816	6.389	3.347	+	+	13.210	+	+
8	HB9	0.393	5.784	4.121	-	+	12.492	+	+
9	HB10	0.720	6.237	3.182	-	+	11.685	-	-
10	HB11	0.986	5.879	2.748	-	+	12.515	-	-
11	HB12	0.657	5.618	2.949	-	+	11.830	-	-
12	HB15	3.719	5.412	3.080	-	+	10.889	-	-
13	HB16	0.594	6.413	3.886	-	+	11.857	+	-
14	HB17	1.226	5.095	3.039	-	+	12.662	-	-
15	HB18	0.857	5.986	4.214	-	+	4.457	+	+
16	HB19	0.789	6.413	3.366	-	+	11.742	-	-
17	HB20	0.329	4.217	2.772	-	+	12.302	+	+
18	HB21	0.875	5.240	2.879	-	+	12.641	-	-
19	HB22	1.861	6.103	3.588	-	+	11.076	-	-
20	HB23	1.541	6.413	3.272	-	+	11.873	-	-
21	HB24	3.671	5.240	3.183	-	+	12.684	+	+
22	HB27	0.240	5.698	3.942	-	+	11.375	+	-
23	HB28	0.523	6.236	3.525	-	+	4.882	-	-
24	HB29	1.649	6.235	3.848	-	+	11.238	-	-
25	HB30	1.315	5.139	3.443	-	+	10.935	-	-
26	HB31	0.522	6.413	3.893	+	+	12.372	+	-
27	HB32	0.991	6.879	3.162	-	+	12.911	-	-
28	HB33	0.354	5.698	2.927	-	+	12.175	-	-
29	HB34	0.684	5.476	3.074	-	+	12.973	-	-
30	HB35	0.657	5.986	2.873	-	+	12.635	-	-
31	HB36	1.399	5.240	3.812	-	+	12.417	+	-
32	HB37	1.544	6.413	3.815	-	+	11.238	+	-
33	HB38	1.082	6.389	3.006	-	+	5.102	-	-
34	HB39	0.567	6.413	3.203	-	+	8.691	-	-
35	HB40	1.104	6.102	4.067	-	+	9.289	+	-
36	HB42	0.562	5.476	3.583	-	+	11.904	-	-

Remarks : *) + Positive brownish discoloration

- No changes color

***) + Positive formation of transparent around colony

- Negative formation of transparent around colony

****) + Resistance at 50 and 70°C

- Not hold at 50 and 70°C

Thermotolerant Test

Thermotolerant test results showed that 13 isolates were resistant or be able to live at 50 °C for 48 hours of incubation and six isolates (HB3, HB8, HB9, HB18, HB20, and HB24) could survive at the temperature of 70°C (Table 1). Thermophilic microorganisms contain heat resistance and undenaturated protein to adapt to the environmental conditions of temperature extreme [32], because the protein components and structure of the cell membrane is resistant to heat.

CONCLUSION

Bacterial isolated from rhizosphere of potato cv. Hartapel Buru Island had more than one physiological characters. Isolates HB8 produced the highest level of IAA concentration, HB32 secreted the highest number of GA concentration, HB18 had the highest siderophore type salicylate production, HB3 had the best phosphate solubilization ability and isolates HB3, HB8 and HB31 had the ability to produce HCN. Isolates HB3, HB8, HB9, HB18, HB20, and HB24 grew at a temperature of 70°C.

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