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Screening of Auxin-Producing Bacteria from Several Crops Rhizospheres

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Abstract: Plants have natural compounds that can stimulate plant growth and development called phytohormones. Phytohormones can be produced endogenously by plants themselves and are not optimal. Therefore it requires exogenous phytohormones that come from outside the plant. In some cases, non-pathogenic bacteria can promote plant growth and be used in agriculture as biofertilizers. Soil rhizospheres from crops are suspected that the soil in the root area (rhizosphere) contains bacteria that stimulate plant growth. Hormones that play a role in plant growth function in division, enlargement of sprout cells, and division of cells at the root growth point. This research aims to determine bacteria that potentially produce the hormone auxin. This research identifies bacterial isolates from microscopic and macroscopic characterization. Screening isolates of auxin-producing bacteria using colorimetric methods and spectrophotometric methods. The results of characterization and screening from 5 samples showed that the bacterial samples that had the potential to produce auxin were samples isolated from eggplant soil (e) with a concentration of 6.5 ppm. The auxin produced is auxin IBA (Indole-3-butyric acid). The IBA auxin content is high enough to be applied as a plant growth-promoting bacteria.

Keywords: Soil, bacteria isolates, and auxin IBA

INTRODUCTION

Phytohormone compounds are non-nutrient organic substances produced by plants in some areas of the plant and then distributed to other areas of the plant. The transplanted plant parts will react morphologically, biochemically, and physiologically but, depending on the plant species, these chemicals are only effective in moderate doses (around 1 mM). Phytohormones are regulatory substances needed to promote plant growth and to control growth and development. Auxins, gibberellins, and cytokinins are the three main classes of chemicals that form phytohormones in nature. [1].

Plant growth regulators are compounds that play a role in triggering plant growth. As an organic compound produced by plants, it can be used in other parts of the plant, production locations and works in different parts of the plant, and actively works at low concentrations [2]. In plants, the presence of the auxin hormone is found in young leaves, shoot apical meristems, and seed embryos. This auxin hormone has several physiological effects on plants, including resulting in cell enlargement, abscission, inhibition of lateral buds, root growth, and activity of the cambium [3].

In sustainable agriculture, plant growth-promoting bacteria (PGPB) plays an important role as improving soil properties, reducing the growth of phytopathogens, reducing biotic and abiotic stress, and soil biodiversity [4]. PGPB has beneficial properties that can stimulate plants both directly and indirectly [5]. Some of the PGPB

can bind or produce auxin (Indole-3-Asetic Acid (IAA)), cytokinin, gibberellin production, aminocyclopropane-1-carboxylic acid deaminase (ACC) production, atmospheric nitrogen fixation, phosphorus solubilization, lytic enzyme production (chitinase, cellulase, protease, glucanase), siderophore production, induced systemic resistance (ISR), and antibiotic production of lipopeptides [6]. Therefore, this research was conducted to determine the concentration of auxin in isolated crop rhizosphere bacteria.

METHOD

This research conducted at Laboratory of Biology and Laboratory Biotechnology, Faculty of Agriculture, Universitas Muhammadiyah Jember.

Rhizospheres soil sampling

Five samples were collected from five different crops of rhizospheres. This method takes suspected soil from the crop's rhizospheres with diagonal methods at the five different points on the crop field. 300 grams of soil.

Bacteria isolation

Soil samples were isolated using a selective method "Ohba and Aizawa" with the following stages: The test tube was filled with 9 ml of sterile aqudest and 1 gram of soil was added to all samples. The soil suspension was then shaken using a vortex until homogeneous. After the suspension is formed, dilute it to 10-2 and take 0,1 ml to inoculate on the NA media using the spread method.

Macroscopic and microscopic characterization of bacteria isolates

Bacterial isolates that have the potential to produce auxin were characterized macroscopically by observing colony morphology including color, shape, and margins. On the other hand, microscopic is characterized microscopically by observing bacteria cell's shape and gram staining.

Screening of auxin-producing bacteria isolates

Screening of 5 isolates bacteria based on their ability to produce auxin. Each isolate was cultured into a test tube as a culture taken from the colony on NA media to NB media incubated at 370 C for 16 hours. 0.1 ml of culture was taken and cultured in 100 ml of NB media which was added 1 mM L-Tryptophan as an inducer or precursor and incubated at 370 C for 24 hours. 2 ml of isolates was centrifuged 20 minutes at 4.000 rpm to obtain the supernatant. A total of 0,5 ml supernatant was reacted with 2 ml of Salkowski reagent and incubated in the dark room for 15 minutes. Isolates auxin-producing bacteria were seen by observing the color change.

Measurements of auxin concentration

The concentration of auxin produced by isolates was measured using spectrophotometer at a wavelength (λ) of 450 nm. The reaction solutes of the 5 bacterial isolates culture was equalized (OD= 0,4). The auxin standard curve was made from a synthetic auxin IBA stock solution. The regression equation is shown in (Figure 3), where y = 0.1677x - 0.1358 and the regression value is 0.9903. The regression equation is obtained from the absorbance value of IBA standard and sample.

RESULTS AND DISCUSSION

Macroscopic and microscopic characterization of bacteria isolates

The five isolates that have the potential to produce IBA hormone were characterized macroscopically and microscopically based on Tabel 1 in Nutrient Agar (NA) media. While the microscopic characterization observed cell shape and gram staining using a 400x magnification microscope on the Figure 1 and Figure 2 the colony and cell morphology of 5 potential auxin hormone-producing bacteria isolates.

Table 1. Macroscopic and microscopic characterization of bacteria isolates

Sample Code	Color	Shape	Margin	Elevation	Cell shape	Gram Strain
a	Yellowish	Circular	Entire	Raised	Coccus	Positive
b	White	Circular	Entire	Flat	Bacillus	Positive
c	White	Circular	Endu-late	Flat	Bacillus	Positive
d	Yellowish white	Circular	Entire	Raised	Bacillus	Positive
e	White	Circular	Entire	Flat	Bacillus	Positive

Notes: Sample code; (a): Paddy soil; (b): Edamame soil; (c): Tomato soil; (d): Chili soil; (e): Eggplants soil

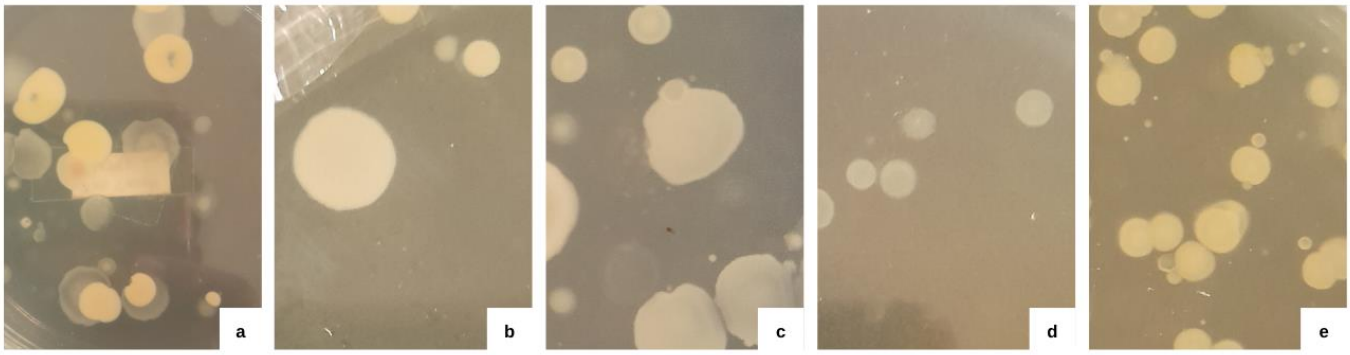

Figure 1. Colony morphology of bacteria isolates

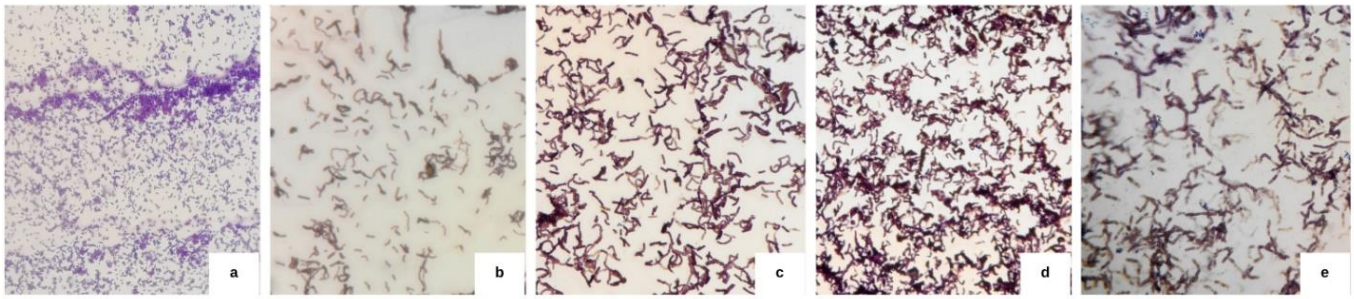

Figure 2. Gram staining

The five isolates that have the potential to produce IBA hormone were characterized macroscopically and microscopically based on Tabel 1 in Nutrient Agar (NA) media. While the microscopic characterization observed cell shape and gram staining using a 400x magnification.

On the Tabel 1 sample (a) Paddy soil has different cell shape. Rice paddy fields are dry-wet alternation ecosystems, resulting in the soil or water ecotones which are ideal environments for soil microorganisms [7]. Paddy field is the major natural CH4 source [8]. Therefore, rice fields have a diversity of microorganisms because of the living environment in the soil

Screening of auxin and Measurements of auxin concentration

Based on Figure 3 color change when these compounds were mixed with Salkowski reagent. IBA resulted in a color change to orange with a wavelength of maximum absorbance increase around 450 nm [9].

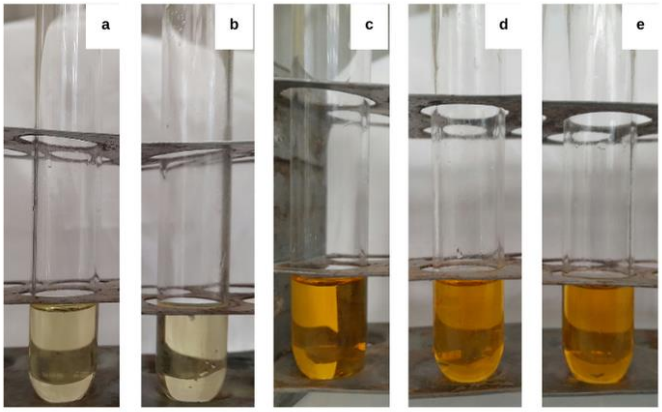

Figure 3. Colorimetric results

Table 2. Measurements of auxin concentration

Sample Code	Average of IBA Concentration (ppm)
a	1,722
b	1,657
c	6,373
d	6,455
e	6,502

Based on Table 2 the highest measurements of auxin IBA concentration from sample (e) eggsplants soil around 6,50 ppm, otherwise the lowest sample was produced by the (b) edamame soil sample around 1,65 ppm.

Auxin can induce ethylene production via the up-regulation of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase 4 (ACS4) gene, which was found to be an early auxin-induced gene [10, 11]. IBA production is synthesized from isobutanol and acetyl-CoA by alcohol-O-acetyl transferase. In addition, two NADHs are in excess to produce one IBA from glucose. To simultaneously solve carbon loss and cofactor imbalance, we designed a novel pathway to produce the isobutanol and acetyl-CoA moieties from glucose and acetate, respectively [12].

CONCLUSION

There were 5 isolates of crops rhizospheres that produced the IBA hormone with concentrations ranging from 1,72 – 6,50 ppm. Based on macroscopic and microscopic characterization of bacteria isolates had varied elevations, gram staining, and colony margin. On the other hand, based on microscopic characterization bacteria isolates had varied cell shape.

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