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The measurement of indole acetic acid from rhizosphere bacteria

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ABSTRACT

In Indonesia, synthetic chemical fertilizers are generally used to spur plant growth. The use of synthetic chemical fertilizers is known to reduce soil quality, causing resistance to pests and plant diseases. One of the natural potencies that can be used as growth promoters is Indole Acetic Acid (IAA) producing bacteria. The objective of this study was to measure the levels of Indole Acetic Acid (IAA) from the rhizosphere bacterial isolate of green beans which is thought to be able to produce Indole Acetic Acid (IAA). The method used was a descriptive approach, including the isolation of green bean rhizosphere bacteria, measurement of Indole Acetic Acid (IAA) levels in bacterial isolate, and data analysis. Green bean rhizosphere bacterial isolate produced Indole Acetic Acid (IAA) with an indication of a color change after the addition of the Salkowski reagent, and quantitatively the results of calculations in the standard curve equation for Indole Acetic Acid (IAA) obtained a value of Indole Acetic Acid (IAA) levels was 50.91 ppm.

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INTRODUCTION

In Indonesia, synthetic chemical fertilizers are generally used to spur plant growth. The continuous use of synthetic chemical fertilizers is known to reduce soil quality, cause resistance to plant pests, and reduce crop production (Hadisuwito, 2007). This is also stated by Atmojo (2007) that the development of agricultural practices in Indonesia, particularly the use of chemical fertilizers with high concentrations and doses for a long period caused a decrease in soil fertility. One of the attractions of conventional agriculture towards organic farming is triggered by the desire to achieve an eco-friendly lifestyle so that it may shift the use of non-natural chemicals, such as fertilizers, synthetic chemical pesticides, and growth hormones in producing agricultural

products. The healthy lifestyle has become internationally popular with a note that agricultural products should have high nutritional attributes, food safety attributes, and eco-labelling attributes (Mayrowani, 2012). In order to achieve a healthy lifestyle, biological fertilizers are used to support the production of agricultural products.

The main principle of biological fertilizers is to utilize microorganisms in the soil as decomposers of organic matter, assisting the mineralization process both by symbiosis in plants and non-symbiosis. One example of soil microorganisms capable of producing Indole Acetic Acid (IAA) is *Azospirillum* sp. (Dewi, Arum, Imamuddin & Antonius, 2015). Bacteria that can increase plant growth are classified as Plant Growth Promoting Rhizobacteria (PGRP). One of the PGPR mechanisms in increasing plant growth is by producing phytohormones such as Indole Acetic Acid (Wahyudi, Astuti, Widyawati, Meryandini & Nawangsih, 2011).

Indole Acetic Acid (IAA) or auxin is a growth-stimulating hormone, physiological process regulators such as cell division, tissue differentiation, plant response to light, and gravity. The IAA can be synthesized by plants in actively differentiated tissues, including root and shoot tip meristems (Istiqomah, Aini & Abadi, 2017). However, the production of IAA in plants cannot meet its needs for optimum growth, thus additional exogenous IAA is needed to spur its growth. For example, the production of IAA by rhizobacteria is known to stimulate plant growth (Taghavi, Garafola, Monchy, Newman, Hoffman, Weyens, Barac, Vangronsfeld & Van der Lelie, 2009).

Indole Acetic Acid (IAA) production in media requires a precursor in the form of tryptophan. IAA biosynthesis includes Indol-3-Acetonitrile (IAN), Indol-3-Acetamide (IAM), and indole-3-pyruvic acid (IpyA). The pathways that are generally found in plant tissue are IAM and IpyA, while in bacterial cells, IAA biosynthesis can pass these three pathways. Tryptophan in the rhizosphere may come from two sources, namely from root exudates and microbial cells (Spaepen, anderleyden & Remans, 2007). The amount of tryptophan in plant root exudates varies depending on the plant species. Although the amount of tryptophan in root exudates is rather low (Retnowati, 2015), exogenous tryptophan can be absorbed efficiently by bacteria (A'ini, 2015)

Some species of bacteria are known to be able to increase plant growth by producing phytohormones such as *Azotobacter* sp. (Widyastuti, Siswanto & Suharyanto, 2010), *Bacillus* sp. (Wahyudi et al., 2011), *Bradyrhizobium, Azospirillum,* and *Pseudomonas* (Tangapo, 2020). Determination of levels of Indole Acetic Acid (IAA) from bacteria can be performed in various ways, including GC-MS, and calorimetric using Salkowski reagent (Hidayatullah, Rahayu & Lisdiana, 2017). The objective of this study was to measure the IAA level of rhizosphere bacterial isolate which is thought to be capable of producing IAA. This study needs to be conducted because the exploration of rhizosphere bacteria in the Sorong region, West Papua has never been conducted, particularly on the roots of green beans.

RESEARCH METHODS

Research Design

The research design used was to describe the observations descriptively based on the color change of the rhizosphere bacterial isolate after adding the Salkowski reagent, then the absorbance value of the sample was calculated based on the regression equation from the IAA standard curve, thus the IAA concentration was obtained in the bacterial isolate.

Population and Samples

Samples were taken at the SP2 plantation of Sorong Regency. Soil samples were taken from the rhizosphere area of healthy green beans. The sample was taken using a sterile spatula, then the sample was inserted into sterile plastic and taken to the laboratory for further testing.

Procedures

The procedure in this study consisted of two stages, namely the Isolation of Rhizosphere Bacteria and Measurement of IAA levels. At the Rhizosphere Bacterial Isolation stage, the samples were isolated using nutrient agar (NA) media. The method used was the pour plate method. In this study, the sample was diluted from the 10^{-1} to the 10^{-3} dilution factor. The bacterial isolate was incubated for 48 hours at room temperature. After the isolation process, the bacterial isolate was purified on the agar slant. Furthermore, the bacterial isolate was cultured on Nutrient Broth media for 72 hours at room temperature. Subsequently, the sample was prepared for measurement of the IAA levels.

The stage of IAA measurement was carried out using the Salkowski reagent. The Salkowski reagent consisted of 150 ml concentrated H_2SO_4, and 7.5 ml of $FeCl_3.6H_2O$ (0.5M). These materials were dissolved in 250 ml of distilled water. The auxin used in this study is synthetic indole acetic acid (IAA). Synthetic auxins were used as the standard for IAA measurements. The concentration of the IAA stock solution was made at 0.1 mg/ml or 100 ppm. The IAA stock solution was made in various concentrations starting from 0 ppm to 60 ppm. The samples used were isolates of rhizosphere bacteria from green beans. Each bacterial culture was taken 1.5 ml and then centrifuged at 10000 rpm for 10 minutes. Furthermore, the supernatant was taken and used as a sample for measuring the IAA levels. Analysis of IAA levels was carried out using a spectrophotometer with a wavelength of 520 nm.

A total of 0.5 ml of the IAA standard solution, blank (aquades), and supernatant as samples were taken and then added with 2 ml of Salkowski reagent. All of these solutions were homogenized using vortex slowly until homogeneous, then incubated for 15 minutes in a dark room at room temperature (27 ^{0}C). Furthermore, the absorbance of each solution was measured using a spectrophotometer at a wavelength (λ) of 520 nm. A standard curve was made for the absorbance value of the IAA standard solution, thus the regression value was obtained (Figure 1). Finally, the absorbance value of the sample was calculated using the standard curve equation formula.

Data Analysis

The absorbance value of the sample is calculated using the following formula:
The calculation of indole acetic acid (IAA) for the sample using the IAA standard curve solution equation ($y = a\,x + b$) is as follows:

Where,

The absorbance value of the sample	= c
The absorbance value of the IAA standard for a concentration of 0	= d
The final absorbance value of the sample	= c – d
	= e

Then, the final absorbance value of sample e is calculated by the formula for the standard curve equation $y = a\,x + b$

y e	= a x + b
-a x	= e –b
x	$= \frac{e-b}{a}$
= …. ppm.	

RESULTS

The IAA standard solutions were prepared with various concentrations starting from 0 ppm to 60 ppm (Table 1). The absorbance value of the IAA standard solution at a concentration

of 0-60 ppm can be seen in (Table 2). While the IAA standard curve had a regression value of R^2 = 0.9781 (Figure 1).

Table 1. Various concentrations of indole acetic acid (IAA)

No	The concentration of IAA (ppm)	Aquades (ml)	IAA stock (ml)
1	0	1	0
2	10	0.9	0.1
3	20	0.8	0.2
4	30	0.7	0.3
5	40	0.6	0.4
6	50	0.5	0.5
7	60	0.4	0.6

Table 2. The absorbance value of the IAA standard at the concentration of 0-60 ppm

No	Concentration (ppm)	Absorbance at λ 520 nm
1	0	0
2	10	0.207
3	20	0.216
4	30	0.386
5	40	0.514
6	50	0.679
7	60	0.734

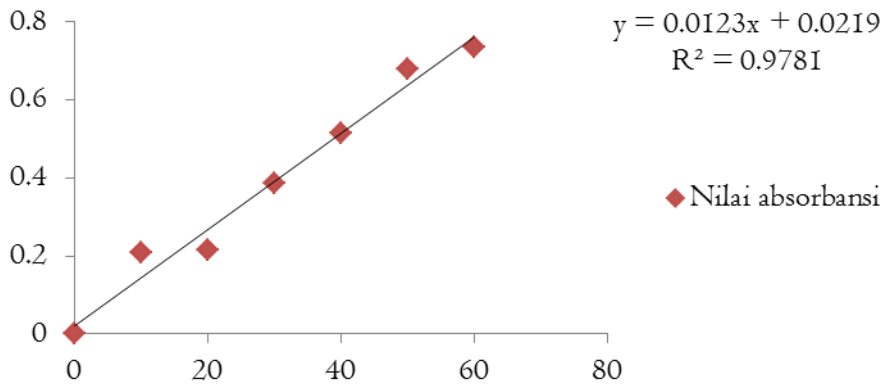

Figure 1. Standard curve of IAA

The results of IAA measurement in rhizosphere bacteria isolated from the root area of green beans showed IAA levels of 50.91 ppm (Table 3). This was evidenced by the change in the color of the bacterial culture after the addition of the Salkowski reagent (Figure 2).

Table 3. The results of IAA measurement in rhizosphere bacterial isolate

Rhizobacteria Sample	Absorbance Value	Absorbance Value of IAA Standard	IAA Level (ppm)
RK	0.697	0.065	50.91

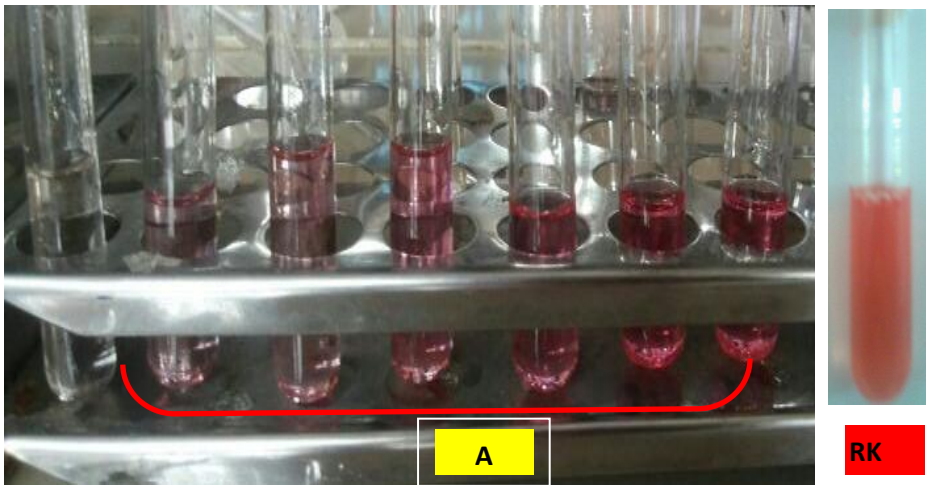

Figure 2. Color differences of the IAA standard solution from a concentration of 0-60 ppm and sample of Rhizosper bacteria (RK)

DISCUSSION

Measurement of indole acetic acid (IAA) levels is based on changes in color reactions in the IAA standard solution and sample RK after adding Salkowski reagent (Figure 2). Measurement of IAA levels was carried out using a spectrophotometer at a wavelength (λ: 520 nm) with variations in the concentration of the IAA standard solution (0-60 ppm) resulting in an absorbance value between 0 - 0.734 (Table 2). The higher the concentration of the solution, the more its absorbance value increases. This is also stated by Kai, Nakamura, Wakasa & Miyagawa, (2007) that the concentration of the IAA standard solution is directly proportional to the absorbance value.

IAA standard curve was made by plotting the absorbance values obtained for each concentration on the y-axis, while the IAA concentrations were plotted on the x-axis. The standard curve resulted in a regression of $R^2 = 0.978$ and an equation for calculating the IAA concentration in the sample was $y = 0.012x + 0.021$ (Figure 2). The final absorbance value of IAA obtained by culture of rhizosphere bacteria as RK isolates were 0.632. The absorbance value was entered into the equation of the IAA standard curve ($y = 0.012x + 0.021$) so that the IAA concentration of bacterial culture RK was 50.91 ppm.

The incubation treatment in a dark room is carried out because naturally, IAA is very sensitive to light. The formation of an indole ring is characterized qualitatively by a change in the color of the solution from yellow to pink, which indicates the formation of indole acetic acid (IAA) (Xu, He, Zhang, Mao, Wang, Li & Lian, 2018). However, measuring IAA levels using Salkowski reagent has a weakness, namely the presence of intermediate compounds that appear in the IAA biosynthetic pathway can react with Salkowski reagent and give color so that it is also measured (Glickmann & Dessaux 1995). Biosynthesis of IAA in media requires a precursor in the form of tryptophan. IAA biosynthesis includes Indol-3-Acetonitrile (IAN), Indol-3-Acetamide (IAM), and indole-3-pyruvic acid (IPA).

The mechanisms for the formation of IAA include: (1) tryptophan is converted to indole pyruvic acid through a transmission reaction, (2) indole pyruvic acid is then converted into indolasetaldehyde through a decarboxylation reaction, and (3) the final stage is the oxidation of indolasetaldehyde to produce indoleacetic acid (IAA). Tryptophan is decarboxylated into tryptamine, then tryptamine is oxidized and daemonized to produce indolasetaldehyde. The molecule will be further oxidized to produce indoleacetic acid. Meanwhile, the pathway for IAA formation in the absence of tryptophan will be through the tryptophan-independent route. IAA

production is influenced by the incubation period of bacteria, where the highest IAA production occurs in the stationary phase (Masciarelli, Urbani, Reinoso & Luna, 2013).

The potential of rhizosphere bacteria from green beans in producing IAA which has been carried out is in line with several previous research results. In general, IAA-producing bacteria come from around the root area of nuts. Silitonga, Priyani & Nurwahyuni, (2013) have identified five isolates of IAA-producing bacteria originating from the soybean rhizosphere area, where of the five isolates, the highest concentration is 24.1 ppm and the lowest concentration is 14 ppm. According to Sulistiono, Soemardi & Purwanto (2004), the difference in the amount of auxin in peanut fruit can change which is influenced by the need for fruit at each stage of its growth. Besides, the free auxin content in developing fruit was higher than in undeveloped fruit, whereas the auxin content in developing fruit was less than in undeveloped fruit. Fatmawati (2015) also states that Plant Growth Promoting Rhizobacteria (PGPR) are soil microbes that come from around or on the surface of plant roots, and these bacteria are involved in plant growth and development by producing and secreting various kinds of chemical compounds around plant roots.

CONCLUSION

The bacterial culture originated from the rhizosphere area of green beans produced Indol Asetic Acid (IAA) which was marked by a change in the color of the solution after the addition of the Salkowski reagent, and according to the results of quantitative calculations using the IAA standard curve equation, the IAA level was 50.91 ppm. Indole Asetic Acid (IAA) can be used as a plant growth regulator (PGRs).

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