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Production of IAA hormone in rhizosphere bacterial isolates of community forest stands

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Abstract. The rhizosphere is the soil around plant roots, which is directly affected by soil microbes, and exudation of plant roots have an essential role in plant health and soil fertility. The IAA (Indole Acetic Acid) hormone or known as the auxin hormone, is a major member of the auxin group that controls important physiological processes, including cell enlargement and division, tissue differentiation, and responding light and gravity. The purpose of this study was to determine the ability of rhizosphere bacteria isolates to produce IAA hormone associated with tree roots at community forest stand. This study comprised rejuvenating bacterial isolates to obtain pure and uncontaminated isolates and testing IAA concentrations. The results showed that rhizosphere bacterial isolates around the roots in community forest stands could produce IAA hormones. The BR 2 (*Bacillus*) bacteria isolates produced the highest IAA, which indicated with the color change of isolates to pink, while BB 3 and JS 1 isolates produced the lowest IAA.

1. Introduction

The rhizosphere is the soil around plant roots, which is directly affected by soil microbes and exudation of plant roots [1]. According to Susilawati (2015) [2], the rhizosphere part is known to have a crucial role in plant health and soil fertility. Some rhizosphere microbes are needed during nutrient cycling and the process of soil formation, plant growth, influencing microbial activity as well as biological control of root pathogens [3].

IAA hormone, also known as auxin hormone, is a major member of the auxin group that controls many critical physiological processes, including cell enlargement and division, tissue differentiation, and responding light and gravity [4]. Auxin hormone produced by plants is called endogenous IAA, while exogenous IAA is auxin hormone produced by organisms other than plants. Endogenous auxin is produced in plant meristem, needed during cell elongation, a process before cell differentiation. Auxin also serves to increase cell elasticity, while a low concentration of exogenous IAA is needed in multiplication to improve budding and root hair formation. Root hair growth is stimulated by the presence of rhizosphere bacteria that produce IAA [5].

Exploration tree and identification of rhizosphere microbe in some community forest stand in South Sulawesi, such as Mahogany [6,7], Bitti, Ebony [8,9], Teak [10], Jabon merah [11–13], and Uru [14] plant stands have been conducted. Based on Jufri's research (2017) [15], the number of



isolates of rhizosphere bacteria in bitti, Teak, and red jabon stands were 37 isolates, while in mahogany, uru, and ebony stands were 11 isolates [16]. Bacterial isolates from the previous studies are still in the form of collections. Therefore further research is needed to select rhizosphere bacterial isolates from community forests with potential for IAA hormone production. Furthermore, this study is expected to obtain superior isolates that can be applied to increase plant growth.

2. Research methodology

The samples used were 29 pure isolates from the isolation of soil samples from Bitti, Teak, Uru, Ebony, and Jabon stands that had been rejuvenated.

2.1. Culture Media

The purpose of making microbial culture media is to grow bacterial cells. For bacterial growth, the NA media is used (Schlegel, 1993) in [17]. Procedures for making culture media were as follow:

2.1.1. Making NA (Nutrient Agar). The process of making Nutrient Agar (NA) media carried out as follows:

- a. Nutrient Broth was weighed for 4 grams, then poured into an Erlenmeyer together with 5 grams of sugar, 10 grams of agar, and 500 ml of aquadest.
- b. The Erlenmeyer tube was closed with using plastic wrap and aluminum foil, then homogenized by using a hotplate stirrer.
- c. The solution was sterilized at 121°C for 15 minutes with an autoclave
- d. The solution was allowed to stand to a temperature of $\pm 50^\circ\text{C}$ in LAF (Laminar Air Flow Cabinet).
- e. The solution poured into the sterile petri dish; 500 ml of solution was poured into 20 Petri dishes evenly.
- f. After the solution had hardened, the Petri dishes were covered with plastic wrap

2.1.2. Rejuvenation of Bacterial Isolates. Bacterial isolates were grown on NA media in Laminar Air Flow. The steps were as follows:

- a. Sterilization of Laminary Air Flow before rejuvenation. LAF was sterilized by tuning on the UV Laminary lamp for 15 minutes
- b. NA media that has been stored for \pm three days and isolating existing bacteria were prepared
- c. Bacterial colonies were taken by using an ose needle on the tup of bacterial isolates collection and then rubbed to the NA media
- d. The tup of bacterial isolates was closed and glued with parafilm paper
- e. Petri dishes were closed with plastic wrap and then stored in a box that had been sterilized before

2.1.3. Liquid Culture Media. The media used for bacterial growth and propagation is NB liquid media. The steps in making liquid media were as follow:

- a. 8 grams of NB media and 20 grams of glucose were put into an Erlenmeyer containing 1 liter of distilled water
- b. The solution was homogenized by using a hot plate stirrer, and 0.1 gram of L-Tryptophan was added
- c. NB liquid media put into each bottle for 20 ml per bottle, according to the number of isolates grown
- d. The media inserted into the autoclave for 15 minutes at 121°C
- e. Bacteria isolate was stroked with an ose needle for 2-3 times into a bottle containing NB liquid media
- f. Media bottles containing bacterial isolates were incubated using a shaker with a speed of 100 rpm for six days

2.1.4. Measurement of IAA Concentration

The procedures performed on measuring IAA concentrations were as follow:

- Measurement of IAA levels was done by taking 5 ml of culture, then put into centrifugation tubes, and centrifuged for 30 minutes at 7000 rpm.
- The supernatant filtered with filter paper, then 1 ml of supernatant added with 4 ml of salkowski reagent (150 ml H₂SO₄, 250 ml sterile aquadest, and 7.5 ml FeCL₃, 6H₂O 0.5 ml)
- After incubated in a dark room for 30 minutes. The test tube was removed from the incubator, and then color changes occurred in the sample
- The change in color of the sample to pink after incubation indicates that the isolate is able to produce IAA
- Measuring the concentration of IAA produced by isolates by using a spectrophotometer at a wavelength of 520 nm. IAA concentrations were calculated after compared with the absorbance of the IAA standard solution with a regression equation $Y = 0.018 X - 0.001$

Notes:

Y = Absorbance
X = Concentration

2.2. Data Analysis

The data were analyzed with descriptive analysis and qualitative methods by observing through the sample color during the testing stage, which indicates a reaction to bacteria, and a change in sample color to pink means isolates able to produce. The quantitative method measured the absorbance suspension of isolates by using a spectrophotometer to see the concentration of IAA produced.

3. Result and discussion

3.1. Result of Rejuvenation

Thirty-five isolates of bacterial isolates obtained from the soil samples isolation taken from the tree stand at community forests, but bacterial isolates that were successfully purified were 29 pure isolates which not contaminated. Bacterial growth in isolates often overlapping between each species (Figure 1), and some can also be contaminated with several types of fungi and unwanted bacteria. Therefore rejuvenation process is needed to obtain isolates that truly pure and free from contaminated bacteria.

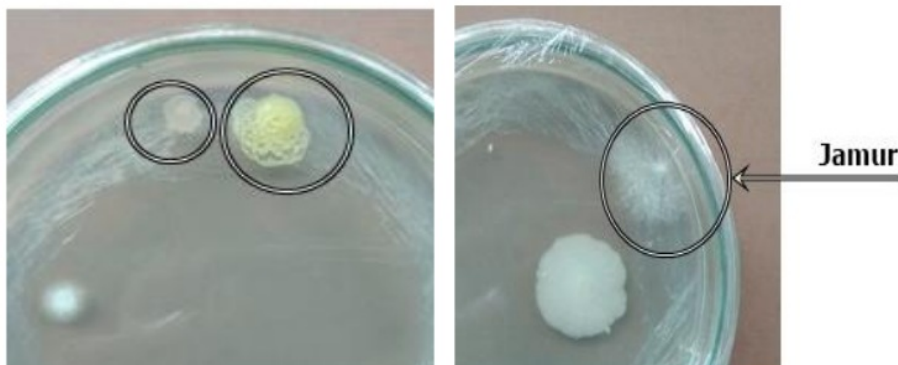


Figure 1. Bacterial isolate from rejuvenation result, (a) Overlapping bacteria, (b) Bacteria contaminated with fungus

Based on the results, 29 pure isolates from 35 isolates were rejuvenated, including three pure isolates from Uru stands, five isolates from *A.cadamba* stands, five isolates from Bitti stands, two

isolates from Ebony stands, and 14 isolates from Teak stands. The results of the rejuvenation of bacterial isolates are presented from Table 1 to 6.

Table 1. Characterization of Rhizosfer bacteria from rejuvenation in Uru stands







Isolate Code	Front Figure	Back Figure	Genus [14]
UT 1			<i>Streptomyces</i>
UT 2			<i>Bacillus</i>
UT 3			<i>Bacillus</i>

Table 2. Characterization of Rhizosfer bacteria from rejuvenation in Ebony stands





Isolate Code	Front Figure	Back Figure	Genus [14]
EB 2			<i>Bacillus</i>
EB 3			<i>Coryneform</i>

Table 3. Characterization of Rhizosfer bacteria from rejuvenation in Bitti stands

Isolate Code	Front Figure	Back Figure	Genus [14]
⁷ BB 2			<i>Coryneform</i>































BB 3			<i>Coryneform</i>
BB 4			<i>Bacillus</i>
BB 5			<i>Bacillus</i>
BB 6			<i>Clostridium</i>

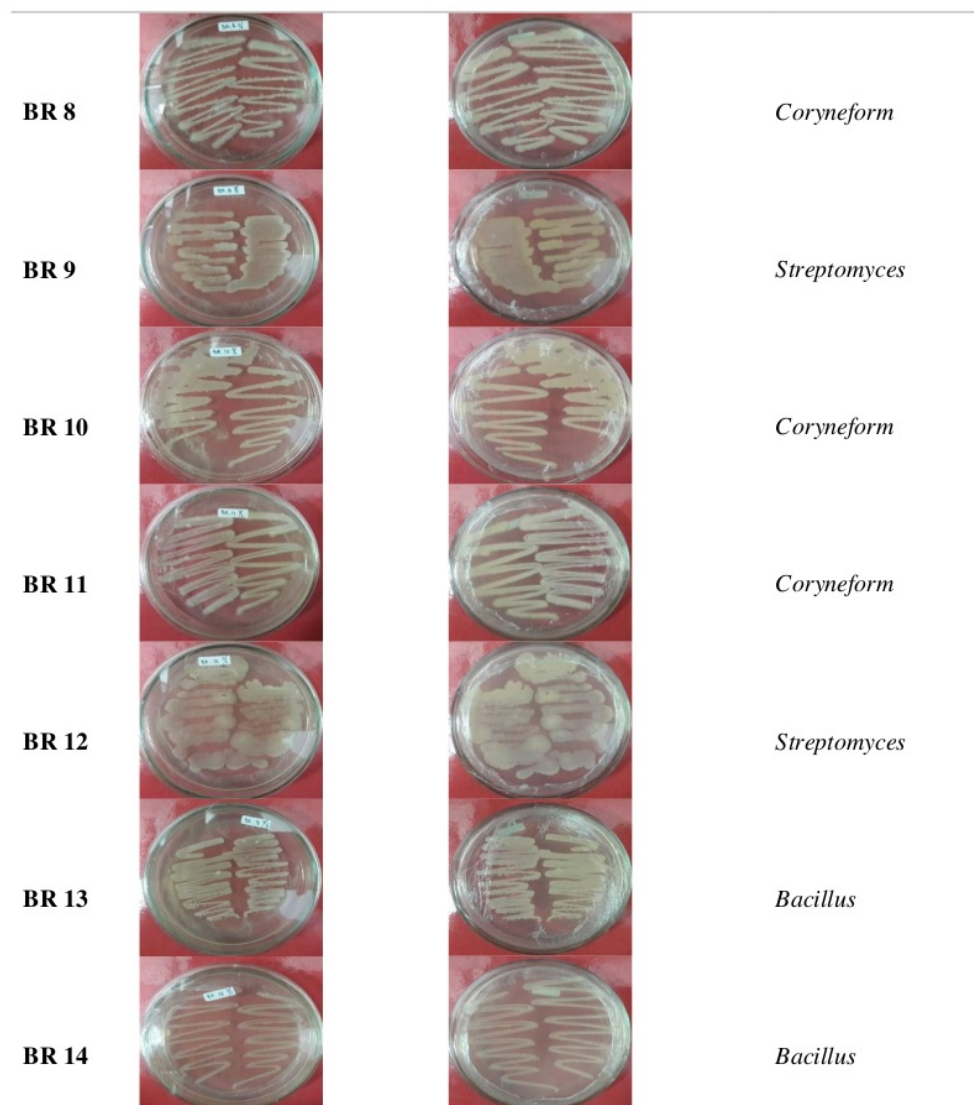
Table 4. Characterization of Rhizosfer bacteria from rejuvenation in Jabon stands

Isolate Code	Front Figure	Back Figure	Genus [15]
JS 1			<i>Agrobacterium, Ralstonia</i>
JS 2			<i>Clostridium</i>
JS 4			<i>Clostridium</i>
JS 5			<i>Streptomyces</i>

JS 7				<i>Bacillus</i>
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Table 5. Characterization of Rhizosfer bacteria from rejuvenation in Teak stands

Isolate Code	Front Figure		Back Figure	Genus [15]
BR 1				<i>Bacillus</i>
BR 2				<i>Bacillus</i>
BR 3				<i>Streptomyces</i>
BR 4				<i>Bacillus</i>
BR 5				<i>Coryneform</i>
BR 6				<i>Bacillus</i>
BR 7				<i>Clostridium</i>



3.2. IAA Content Test for *Rhizosfer Bacteria*

Indole Acetic Acid (IAA) is an auxin group that controls many critical physiological processes, including cell enlargement and division, tissue differentiation, and response to light [4]. IAA testing can be done by looking at two aspects, which are qualitative and quantitative. The qualitative aspect of IAA can be known by looking at the color change into pink of the bacterial collection after the addition of the salkowski solution, which incubated for 30 minutes. Another method is a quantitative method that can be seen from the IAA concentration, which measured by using a spectrophotometer.

3.3. Color Changes in IAA Test

The results of color changes observed in each isolate after 30 minutes incubated in a dark room, there was bacterial isolates supernatant which changed to pink, but the color change that occurred varied and depending on the level of color density, as in (Figure 2b) appeared not

bright, in (Figure 2c) began to appear a change in pink color. It was also found that the supernatant of bacterial isolates that changed color to pink was seen compared to control and (Figure 2d).

According to Dewi, et al. (2016) [18], isolates that are able to produce IAA qualitatively changes to pink because of the reaction between IAA and Fe to form complex compounds $[\text{Fe}_2(\text{OH})_2(\text{IA})_4]$, IAA is indole-3-acetate. Hormone production, which indicates the presence of IAA from the pink level of concentrations that are formed because of the indole ring [19]. The indole ring is formed after the isolated supernatant is reacted with the Salkowski reagent.

Salkowski is a coloring reagent that can be used to test indole compounds and their derivatives. Salkowski reagent oxidizes indole compounds and their derivatives. IAA is an example of a compound that has an indole group; its reaction with Salkowski will produce a pink color [19]. The brighter pink color indicates that the higher content of IAA produced by bacteria. The higher the concentration of Salkowski used, the greater the potential for color change. IAA produced by microbes from plant roots, especially in the rhizosphere, which has a metabolism pathway through the synthesis of L-tryptophan [5].

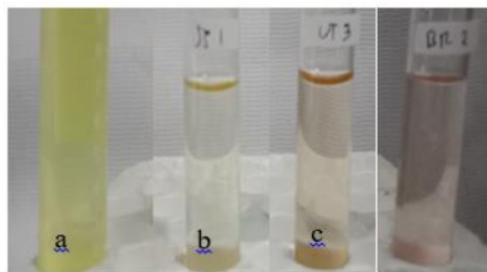


Figure 2. IAA test results of bacterial isolates on community forests stands based on differences in color obtained (a) Salkowski, (b) JS 1 Isolate, (c) UT 3 Isolate, (d) BR 2 Isolate

3.4. Measurement of IAA concentrations

Spectrophotometric test results show the relationship between IAA standard solution (x) and absorbance (y) by obtaining a regression equation $y = 0.018 X - 0.001$ from the results of IAA concentration measurements (Table 6) which show that bacterial isolates are able to produce IAA with varying concentrations. From rhizosphere bacterial isolates incubated for 30 minutes, the result showed that the highest IAA hormone concentration in BR 2 isolates was 5.22 ppm. This can also be seen from the change in color of the isolate into pink color. Isolates with the highest concentration was an isolate from genus *Bacillus*. The lowest IAA hormone production was found in BB 3 isolates from genus *Coryneform* and JS 1 with a concentration of 2.16 ppm from genus *Agrobacterium ralstonia*.

The results obtained by this study are higher compared with the results of the study by Khairani (2009) [20], in which the highest IAA hormone was 0.93 ppm on bacterial isolates from the roots of corn plants. Meanwhile, IAA concentrations in that study were lower compared to [21]. Bacterial isolates from suren stand produced the highest IAA concentrations, which was 7.94 ppm. Based on a study by Lestari et al. (2007) [22], at the beginning of incubation, the source of nutrition is high; thus, IAA production is also high and continues to increase although it is not significant but consistent until the end of incubation.

Table 6. IAA Concentration Measurement Results obtained from Rhizosphere Bacteria Isolates in Tree Stands of Community Forest with 520 nm Wavelength.

No	Isolate Code	Absorbance Value ($\lambda = 520 \text{ nm}$)	IAA Concentration (ppm)
1	BR 2	0.093	5.22
2	BR 14	0.085	4.77
3	BB 2	0.07	3.94
4	BB 4	0.068	3.83
5	BB 5	0.065	3.66
6	JS 4	0.059	3.33
7	BR 5	0.059	3.33
8	BR 6	0.059	3.33
9	UT 3	0.058	3.27
10	BR 13	0.058	3.27
11	BR 1	0.056	3.16
12	BR 9	0.054	3.05
13	BR 11	0.054	3.05
14	BR 4	0.053	3
15	BR 3	0.051	2.88
16	BR 8	0.051	2.88
17	UT 2	0.05	2.83
18	BR 12	0.05	2.83
19	EB 2	0.05	2.83
20	BR 10	0.05	2.83
21	UT 1	0.049	2.77
22	JS 2	0.047	2.66
23	BR 7	0.047	2.66
24	JS 5	0.046	2.61
25	JS 7	0.043	2.44
26	BB 6	0.043	2.44
27	EB 3	0.04	2.27
28	BB 3	0.038	2.16
29	JS 1	0.038	2.16

Differences can influence different results between each IAA concentration of bacterial isolates in the concentration of tryptophan added to the media [23]. This difference is also due to the conditions of each sampling location, incubation time, and its ability to convert tryptophan contained in the media to IAA [20] and the standard solution used.

Based on the results of measurements of IAA concentrations of bacterial isolates, the highest IAA concentration values found in isolate BR 2 with genus *Bacillus*. These results are similar to the study by Widiyanti (2007) [24] that the *Bacillus sp.* bacteria are able to produce higher IAA concentration for 67.2 ppm if compared with *Pseudomonas* bacteria. Another study reported by Mukamto et al. (2015) [25] which tested the bacterium *Bacillus sp.* to determine the ability of these bacteria to produce IAA and also in dissolving phosphate in the soil. Asril (2017) [26] tested the bacteria to determine the potential of *Bacillus* bacteria in producing IAA and Istiqomah, et al. (2013) [27] studied about the ability of the bacterium *Bacillus subtilis* to dissolve phosphate and produce IAA hormone that can be applied as a composition of compound biological fertilizers like in chili peppers for gibberellin hormone [28].

From the test results, it can be seen that not all IAA concentrations of *Bacillus* bacteria have a high amount since the IAA concentration of BR 4 bacterial isolates was only three ppm. The difference between IAA concentrations of the two *Bacillus* bacterial isolates on the same tree

with BR 4 and BR 2 isolates code may be due to the different species of bacterial isolates. This is in line with Khairani (2009) that the concentration of IAA can be influenced by several factors, including the type of microbes.

4. Conclusion

Based on the results of the research, it can be concluded that:

1. Rejuvenation results of bacterial isolates obtained from 29 isolates included in 5 genera namely *Bacillus*, *Streptomyces*, *Coryneform*, *Clostridium*, *Agrobacterium ralstonia*
2. Rhizosphere bacterial isolates that were around the roots in community forest stands can produce IAA hormones. Bacterial isolate BR 2 (*Bacillus*) produced the highest IAA, while bacterial isolates BB 3 and JS 1 produced the lowest IAA.

3

Acknowledgements

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