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Submission date: 01-Nov-2021 06:22AM (UTC+0100)

Submission ID: 1689717180

File name: Gusmiaty.pdf (698.56K)

Word count: 3529

Character count: 18693

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To cite this article: Gusmiaty *et al* 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **270** 012018

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Gibberellin And IAA Production by Rhizobacteria From Various Private Forest

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Abstract. Rhizobacterium is a bacterium that colonizes plant roots, known to produce gibberellin and Indol Acetic Acid (IAA) to spur plant growth. This study aims to isolate rhizobacteria from various stands of community forest and to know the ability of rhizobacterial isolates to produce gibberellin and IAA. Research methods included rhizosphere soil sampling, bacterial isolation, gibberellin content test, and IAA. Results of rhizobacterial isolation were obtained by 35 isolates having a gibberellin production capability of about 1,959–4,322 mg l⁻¹ and IAA about 0.389–2.370 mg l⁻¹. The highest gibberellin production was obtained on the isolates of BB1, BB2 and BB3 while the highest IAA was in JS9 and JB4 isolates.

1. Introduction

Gibberellin (GA) and Indole Acetic Acid (IAA) are secondary metabolites, important products in biotechnology that have high economic value and as natural plant growth hormones [1][2]. Gibberellin plays a role in breaking flowering dormancy, increasing flowering initiation, increasing stunted plants, spurring germination processes [3–5] while IAA plays a role in stimulating cell extension, regulating apical dominance, and stimulate the formation of lateral and adventive roots [6].

Rhizobacteria are known as free-living bacteria and actively colonize rhizosphere or around plant roots. Rhizobacteria have the potential to increase plant growth as it is capable of producing plant growth hormones, such as indole acetic acid, gibberellic acid, cytokines and ethylene, siderophores production, antibiotics and able to dissolve phosphate [7][8][9]. Bacteria capable of producing gibberellins, namely: *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Burkholderia*, and *Bacillus* [10][11]. Some rhizobacteria are also capable of producing IAA such as *Rhizobium* sp., *Bacillus* sp., *Pseudomonas* sp., *Burkholderia* sp., *Escherichia* sp., *Micrococcus* sp., *Staphylococcus* and *Bradyrhizobium japonicum* [12].

The source of rhizobacterial diversity can be obtained from the community forest. The types of plantation forests in South Sulawesi are superior local crops such as Uru stands in North Toraja Regency, ebony [13] and teak in Barru regency, bitty in Bulukumba Regency, red jaboron in Sidrap regency, mahogany in Takalar regency, have the prospect to be developed. These local seed types have not yet been explored biodiversity, especially gibberellin-producing rhizobacteria and IAA. In this relation,



research on the isolation of gibberellin and IAA-producing rizobacteria from various community forests is required.

2. Material and Method ¹³

The research was conducted at the Biotechnology and Tree Breeding Laboratory and Integrated Laboratory of Forestry Faculty of Hasanuddin University, Makassar

2.1. Soil Sampling

The soil is taken in the vicinity of the rhizosphere of some superior local species of Sulawesi such as Uru, ebony, teak, bitti, red jabol and mahogany. The sampling of soil from the bottom of the crop stands by determining the 10 trees that will be used as the sampling site for each local seeded species, then take the soil samples in the rooting section with a soil depth of 0 cm -25 cm with a distance of $\frac{3}{4}$ from the crown. The soil was taken randomly with a hoe around the root of the plant at four points, then the soil was composited and taken 1 kg of soil. The soil is then put into a plastic bag and labeled with the type of plant, the location of the collection, the date of collection, and the name of the sampler.

2.2. Isolation of Bacteria

Isolation of bacteria is done by multilevel dilution techniques. A total of 1 g of soil samples were suspended into a test tube containing 10 ml of distilled water and then homogenized using a vortex. The resulting suspension is diluted to dilution level of 10^{-4} by taking 1 ml of suspension and fed into a reaction tube containing 9 ml of distilled water. For dilution levels, 10^{-3} and 10^{-4} taken 1 ml then bred on NA media. Each dilution level is done as many as 3 replications. The growth of the bacteria in the cup will appear \pm 1 day after isolation.

2.3. Purification Process of Bacterial

The bacteria that grows at the dilution stage is purified on a new NA medium then stored in an incubator room.

2.4. Test of Gibberellin Content ¹⁶

Testing of Gibberellin was measured using the standard method of Borrow Isolates were grown on Nutrient Broth medium and incubated at room temperature for 7 days. After that centrifuged for 10 minutes at a speed of 8000 rpm. The culture was put into a 15 ml reaction tube and added 2 ml of zinc acetate solution. Subsequently, a 2 ml potassium ferrocyanide solution was added and centrifuged for 10 min at 8000 rpm. A total of 5 ml of supernatant was added 5 ml of 30% hydrochloric acid and incubated at 27°C for 75 minutes. The measurement of absorbance used a spectrophotometer at 254 nm while the concentration of Gibberellin was determined after comparing with the standard curve of Gibberelin.

2.5. Test of Indole Acetic Acid (IAA) Content ³

The Indole Acetic Acid bacteria test was performed by the modified [14]. Isolates were grown on 100 mL of liquid Nutrient Broth medium added with 1.0 mM L-tryptophan and incubated at room temperature in dark conditions for 5 days. A 1.5 mL bacterial culture was centrifuged for 10 minutes at a speed of 8000 rpm. 1 mL the supernatant was added with 4 mL of Salkowski reagent (150 mL H₂SO₄, 250 mL sterile aquades and 7.5 mL FeCl₃·6H₂O 0.5 M) and the solution mixture was incubated in the dark room for 24 hours at room temperature under dark conditions. Measurement of absorbance of IAA isolates used spectrophotometer at 520 nm wavelength. The concentration of IAA was determined after comparing it with the IAA standard curve.

3. Results and discussion

3.1. Isolation of Rhizobacteria from some of the Community Forests Stands

Results of isolation and purification of rhizosphere bacteria from 6 community forest stand obtained 35 isolates. Most rhizobacterial isolates were obtained from teak stands of 11 isolates while in mahogany stands were found to be at least 2 isolates (Figure 1).

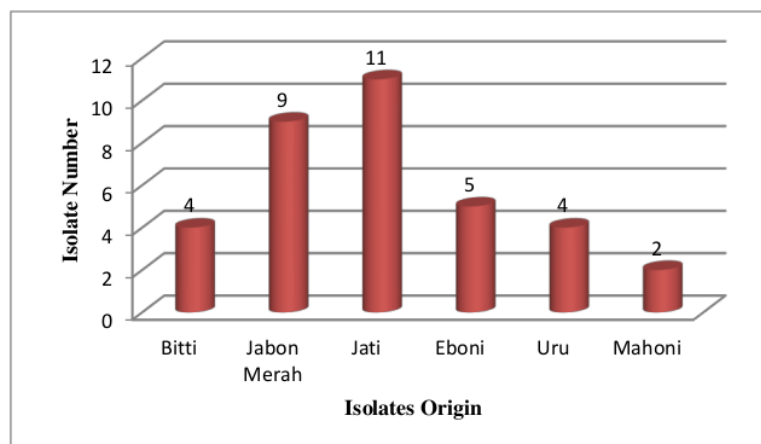


Figure 1. Result of Isolation and Purification of Isolate Rhizobacteria from various Community Forests Stands

Based on the isolation results indicate that the number of isolates obtained is different between the stands of the community forest. Differences in the number of isolates obtained due to different rhizosphere environments between one stand and the other stands. Rhizosphere shows the maximal activity of microbes with the confined environment consisting of many essential micro and macronutrient. Root exudates act as the nutrient source and are responsible for the difference in microbial population between surrounding and rhizosphere [15]. The rhizosphere environment is rich in energy sources from organic compounds released by plant roots (root exudates) and habitats for different types of microbes to thrive. The presence of plant rhizosphere bacteria depends on the root exudates. The root exudate becomes the determinant of diversity and population of microorganisms [16]. Each plant releases root exudates with different compositions so that it also acts as a microbial selector promotes the development of certain microbes and inhibits the development of other microbes. Root exudate composition issued by each plant may vary in components of sugar, amino acids, and organic acids [17].

The beneficial association occurring between plant and microbes in rhizosphere determines the health of plant and fertility of soil [18][19]. Many of these mutualistic organisms can act as biofertilizers, increasing the efficiency of nutrient absorption by the plant and producing substances that promote growth. It is estimated that biofertilizers could reduce the use of common fertilizers by 50 % with no yield losses [20][21].

3.2. IAA Production Capability Test Isolate Rhizobakteri from Various Community Forests

Results of analysis of gibberellin and IAA content in 35 rhizobacterial isolates showed that all rhizobacterial isolates could produce gibberellin and IAA. The other studies that 10 bacterial isolates

were successfully isolated as IAA producer from rhizosphere soil among which 5 were selected based on IAA production ability [22]. Rhizobacterial isolates produce gibberellin and IAA at different concentrations. This is indicated by the large production of gibberellin and IAA produced by each rhizobacterial isolates (Table 1).

Table 1. Production of Gibberellin (GA) and Indole Acetic Acid (IAA) Rhizobacteria from various community forests

No.	Isolate Code	Isolate Origin	GA (mg ^l ⁻¹)	IAA (mg ^l ⁻¹)
1	BB1	Bitti	4,223	0,778
2	BB2	Bitti	4,322	0,685
3	BB3	Bitti	4,150	0,944
4	BB4	Bitti	3,838	1,000
5	JS1	Red Jabon	3,450	0,389
6	JS2	Red Jabon	3,601	0,481
7	JS3	Red Jabon	3,528	0,611
8	JS4	Red Jabon	3,089	0,852
9	JS5	Red Jabon	3,575	0,593
10	JS6	Red Jabon	3,261	0,759
11	JS7	Red Jabon	3,626	0,611
12	JS8	Red Jabon	3,383	0,426
13	JS9	Red Jabon	1,959	2,370
14	JB1	Teak	3,643	1,130
15	JB2	Teak	3,523	1,278
16	JB3	Teak	3,511	1,167
17	JB4	Teak	3,581	2,056
18	JB5	Teak	3,255	1,093
19	JB6	Teak	3,463	1,037
20	JB7	Teak	2,093	1,630
21	JB8	Teak	3,610	0,778
22	JB9	Teak	3,762	0,889
23	JB10	Teak	3,741	1,259
24	JB11	Teak	3,630	1,667
25	EB1	Eboni	2,480	1,241
26	EB2	Eboni	3,635	1,556
27	EB3	Eboni	3,782	1,648
28	EB4	Eboni	3,758	1,407
29	EB5	Eboni	3,618	1,259
30	UT1	Uru	3,256	0,963
31	UT2	Uru	2,980	1,241
32	UT3	Uru	3,556	0,685
33	UT4	Uru	3,672	1,519
34	MT1	Mahogany	3,325	1,333

35 MT2 Mahogany 3,732 1,907

The rhizobacterial isolate produced the lowest gibberellin 1,959 mgL⁻¹ in isolate JS9, the highest of 4,322 mg L⁻¹ in the BB2 isolate. The other study reported that many of the isolates produce gibberellic acid and the production of gibberellin was in the range of 7.50 g/ml to 93.93g/ml [23] Rhizobacteria can produce optimal gibberellin when affected by several factors such as isolate or strain species and isolate culture conditions [24]. The culture conditions of the isolates were influenced, among others: pH growth medium, temperature, incubation time, and incubation conditions moving or still, and dark or bright. Gibberellins are plant growth-promoting hormones, where it plays a role in seed germination [25], response to abiotic stress [26], stem elongation [27], flowering [28], and other physiological effects that occur in its interaction with other phytohormones [29]. Gibberellin can be obtained from plants, fungi, and bacteria [30].

The rhizobacterial isolates produced the lowest IAA on JS1 of 0.389 mgL⁻¹ and the highest IAA in JS9 isolates of 2.370 mg L⁻¹, lower than bacterial isolates from the rhizosphere of potato (5.816 mg l⁻¹)[31]. 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 and incubated for 24 hours at room temperature and dark conditions. The color change shown in all the tested isolates turns pink to the reaction result (Fig. 2).

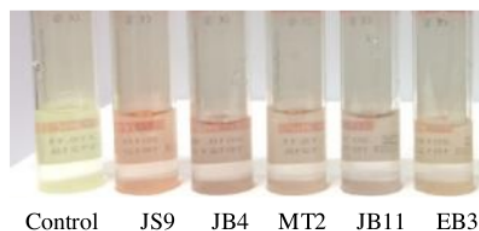


Figure 2. IAA Production of several Rhizobacterial Isolates from the Community Forest Stand

Various types of plant growth-promoting bacteria produce various types of phytohormone. Indole-3-Acetic Acid is a key hormone for many aspects of plant growth that can regulate many physiological processes [32][33]. Indole 3 acetic acid (IAA) can stimulate growth such as cell lengthening and cell division and differentiation [34].

The process of bacterial synthesis in generating IAA is stimulated by the presence of L-tryptophan. The addition of L-tryptophan into bacterial culture medium can increase the biosynthesis of IAA up to 2.7 times. L-tryptophan is an amino acid with an indole group that can function as a physiological precursor of IAA in plants and microorganisms because it contains active compounds that can trigger microbial growth. In soil microorganisms, IAA biosynthesis can be triggered by the presence of L-tryptophan derived from root exudates [35].

4. Conclusion

Results of rhizobacteria isolation from several community forests stand obtained 35 isolates having a gibberellin production capability of about 1,959-4,322 mgl⁻¹ and IAA of about 0.389-2.370 mg l⁻¹. The highest gibberellin production was obtained on the isolates of BB1, BB2, and BB3 while the highest IAA in isolates JS9 and JB4.

Acknowledgement

The author would like to thank Ristekdikti who has funded this research through Higher Education Applied Research Scheme based on the Decree of the Director General of Strengthening Research and Development of the Ministry of Research 2018.

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