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Effectiveness of Rhizosphere Azotobacter Bacteria in Promoting Rice Growth and Yield in a Greenhouse

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Abstract.
Azotobacter is a nitrogen-fixing bacteria. The growth and yield of rice (*Oryza sativa* L) in a greenhouse were studied to examine physiological characterization and efficacy of *Azotobacter* isolated from the rhizosphere and soil. The goal was to isolate *Azotobacter* that could be used as a biological fertilizer. There were 21 *Azotobacter* isolated, with 17 capable of nitrogen-fixing activity, 16 capable of protease activity, six capable of IAA activity, and 13 capable of phosphate dissolution. Each treatment was carried out three times in the experiment, which was completely randomized. At 105 days, the seedlings were harvested. The height of the plants, the number of leaves, the dry weight of the straw and grain, and the number of panicles and seeds were all evaluated. The results showed that rice yields were higher when 1 KZ was isolated from the turi plant (*Sesbania grandiflora*) and 15 KZ was isolated from the akasia plant (*Acasia mangium*).

Keywords: *Azotobacter*, Rice Growth, Plant Growth Promoting Rhizobacteria

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1. Introduction

East Kalimantan is the largest province in Indonesia, which has abundant natural resources, including mining for gold, coal, oil and natural gas, and forest products that have not been optimally utilized. The forests in this area have a high biodiversity value. There have been many efforts to explore biological resources, but for the types of microbes, especially soil microbes, not much information has been disclosed, so it is necessary to explore the types of microbes mentioned above, especially the types of soil bacteria.

To find out and increase the level of soil fertility and land productivity in the East Kalimantan region, it is necessary to support data and information about soil bacteria that can support soil fertility in the area, rhizobacteria found in the rhizosphere of plants, namely the thin layer of soil covering the root surface. It Will have a positive

effect on plant growth, and there are several genera of rhizobacteria reported as Plant Growth Promoting Rhizobacteria (PGPR), PGPR is a group of bacteria commonly used as biological fertilizer, because they are able to support plant growth, increasing agriculture yields by means of inoculation such as Azotobacter is highly recommended because it is an environmental technology. Azotobacter bacteria are soil bacteria whose life is not in symbiosis with legume plants, known as non-symbiotic nitrogen-fixing bacteria, which are free-living in the soil and root areas. These bacteria are also commonly referred to as free-living bacteria in the soil. This bacterium has advantages over other non-symbiotic atmospheric N-fixing bacteria because it can synthesize hormones such as IAA. Synthesis of IAA in these bacteria through the indole pyruvic acid pathway. IAA secreted by bacteria stimulates root growth directly by stimulating cell elongation or division [1].

These bacteria also have the role of fixing free nitrogen, then associating with plant roots, which can supply growth hormones through the production of growth-regulating substances such as auxins, gibberellins, and cytokines that stimulate growth so that they will spur plant growth [2], besides that Azotobacter bacteria are also known as rhizobacterial species as N_2 diazotroph fixing agents, which can convert dinitrogen to ammonium through electron reduction and dinitrogen gas protonation [3], Besides N-fixing, Azotobacter is also capable of producing siderophores, antifungal compounds and growth-regulating activities such as hormones and vitamins [4].

Azotobacter is found in the rhizosphere with numbers that vary widely from zero to one thousand per gram of soil. The type of plant and fertilization influences this bacterial population; besides soil type, soil pH reaction, moisture, environmental factors are also very influential [5]. In addition, soil microorganisms closely related to roots play an important role in stimulating plant growth [6], and their effects can be mechanically mediated directly or indirectly. Growth support mechanisms whose direct effects are most often associated with the provision of biological nitrogen, dissolving minerals such as phosphate, production of siderophores, and production of plant growth hormones such as auxins, gibberellins, and cytokines, while indirect mechanisms include suppression of pathogens by producing antibiotics, hydrogen lytic enzymes cyanide and catalase [7]. Giving Azotobacterin, in general, increases soil fertility and increases the availability of nutrients, especially soil nitrogen; giving Azotobacter 30 g / kg of soil has been able to provide optimal total Nitrogen of soil [8].

To determine the existence of the Azotobacter bacteria mentioned above, an inventory and isolation of the Azotobacter bacteria were carried out. They then carried out a physiological characterization test and an effectiveness test on rice plants' growth and

yield, hoping that a potential Azotobacter bacterial isolate would be obtained, which could be developed as a biological fertilizer to increase soil fertility and rice yields.

In this study, bacteria were isolated from the soil and root areas of various plants on Jensen's media (specific media for Azotobacter bacteria). The bacterial isolates obtained were carried out for physiological characterization, and tested and tested for effectiveness on the growth and yield of rice plants, with the hope that potential Azotobacter bacteria isolates will be obtained, which can be develped as biological fertilizer to increase soil fertility and rice yields

2. Materials and Methods

2.1. Isolation of Azotobacter bacteria

Soil samples collected from Balikpapan and Samarinda, East Kalimantan. The media used is for Azotobacter bacteria using Azotobacter media (Jensen's media [9]. Isolation was carried out by inserting 1 gram of soil sample into a small test tube filled with aquadest, then vortexing, making a series of dilutions by pipetting 1 ml of solution into 9 ml of distilled water in a test tube and so on until a series of 10-1 dilutions was obtained. 10-7, 0.1 ml pipette was poured into a petridish containing solid Jensen's media, leveled with a spatula, then incubated at room temperature (27-30^0 C) for 3-7 days, observed for growth every day until colored colonies appeared. White. Counting the number of colonies was carried out using the plate count method [10]. The growing colonies were transferred in a petridish containing Jensen,s medium.

2.2. Purification and Characterization of Azotobacter bacteria

Purification of the isolates was carried out by taking the sample colonies with a loop. The sample was put in sterile 5 mL aquadest and mixed with a vortex. About 0.1 mL of the resultant suspension was poured into a Petri dish containing Ashby's media, leveled with a spatula, and incubated at room temperature (27-30˚ C) for 3-7 days. Isolated single colonies were grown in a slanted medium in a test tube (as a pure culture).

2.3. Nitrogen-fixing activity test

The nitrogen fixing activity test was carried out qualitatively using semi-solid media NFB (Nitrogen Free Medium). Bacterial isolates were grown in the semi solid NFB media in

a small test tube, incubated at room temperature (27^0_30^0 C) for 3-7 days. A positive result was characterized by the formation of a white ring on the surface of the media indicating that these isolates were able to fixing nitrogen [11].

2.4. Test of protease production

The protease activity test was carried out qualitatively using the method proposed by Basha and Ulaganathan (2002)[12], isolates were inoculated in the center of a petridish dish that already contained protease media, then incubated for 1-4 days. A positive result was a clear zone around the colony, indicating that these isolates were able to produce a protease enzyme.

2.5. Test of IAA production

Qualitative test of IAA production activity was carried out by growing bacterial isolates to be tested on TSB media, consisting of 10 g peptone, 2.5 g NaCl, 22 g agar, and 1000 mL aquadest. Bacterial isolates to be tested were inoculated at the center of a petridishes which has been filled with the media mentioned above, then incubated at room temperature (28-30^0 C) for 2-5 days. Colonies that grow are then dripped with Salkowsky's solution of approximately 1 ml. Pink colonies indicated that the microbial isolate was able to produce IAA (positive) (Gravel. 2007) [13]

2.6. Phosphate solubilize activity test

Phosphate dissolving activity test was carried out qualitatively using Pycosvkaya media as proposed by (Gupta, 1994)[14]. The isolates to be tested were inoculated in the middle of a petridish dish that already contained Pycosvkaya media, then incubated at a temperature (28-30^0 C) for 2-7 days. A positive result was a colony that produces a halo zone, indicating that the bacteria can solubilize phosphate

2.7. The effectiveness test

The research was carried out in a greenhouse for Microbiology, Research Center for Biology, LIPI, using sterile soil media in 0.5 gallon plastic pots. A total of 1.5 kg of sterile soil was used as a growth medium. The strains been used which have high activity, there were 8 isolates were: 1 KZ, 2 KZ, 3 KZ, 6 KZ, 7 KZ, 15 KZ, 6 KZ and 20 KZ.

Azotobacter isolates were grown in large test tubes, incubated for 5 days, then added 25 ml of distilled water, and in the match sticks then transferred to a large test tube and vortexed. The rice seeds are first sown in a tub filled with soil, after 15 days, the rice seedlings are soaked in each treatment of Azotobacter isolate solution, after 1 hour they are planted in experimental pots, 3 plants are planted in each pot, after 1 week of age leaving 2 plants of uniform height. To maintain humidity (24%) watering was carried out every day using rain water. As a control plant without being inoculated and without fertilizing N (K1) and plants without being inoculated and added with N fertilizer equivalent to I00 kg/ha (K2). The design used was a completely randomized design with 3 replications for each treatment. Crops harvested at 105 days, the parameters observed: height of plants and number of leaves (age 2, 4, 6, 8, 10 and 12 weeks) , dry weight of straw, grain and panicle seeds, number of panicle and panicle seeds.

3. Results and Discussions

The results of the isolation of soil samples from East Kalimantan showed that the population of Azotobacter bacteria ranged from 11 - 49 x 10^5 CFU / g of soil, obtained 21 pure isolates, the highest population was obtained from the soil on the roots of the Turi plant (Sesbania grandiflora) (Table 1). When compared between the root area and soil, the bacterial population is higher than in the root area, and this is because Rhizobacteria are associative with plants, where these bacteria can take advantage of organic matter or root exudates released by plants through roots that are used as nutrients for bacteria so that the root area obtained a higher population. As stated by [15], the rhizosphere is the part of the soil with the highest metabolic activity, which is defined as a fraction of the volume of the soil which is directly affected by root growth and metabolism. Plants and microbes interact and stimulate each other caused by root exudates, while root exudates will affect the growth and activity of microorganisms in the rhizosphere, rhizoplanes, and surrounding soil [16].

In addition, plant root areas will be occupied by beneficial organisms that can utilize organic substrates from organic matter or plant exudates as a source of energy and nutrients. Several microbes play an essential role in healthy soil and determine soil quality [17]. The Azotobacter bacterial isolates obtained were 21 isolates, then tested their activity, testing on NFB media showed that 17 isolates were able to fix nitrogen (Table 2) which was qualitatively marked by the formation of a white ring on the surface of the media and a change in the color of the media from yellow to blue. This indicates that the isolate can fix nitrogen, Azotobacter is anaerobic, heterotrophic bacteria, and

its primary ability is to tether non-symbiotic N_2 [18]. In the protease medium, 16 isolates formed clear zones (Table 2). This indicated that these isolates were able to produce protease enzymes. Bacteria that we can produce protease enzymes showed that these isolates could hydrolyze proteins into simpler molecules, such as oligopeptides or acids—amino acids, where amino acids are needed by plants for their growth [19].

TABLE 1

Table 1. The population of Azotobacter bacteria from East Kalimantan

No	Code of isolates	Origin of soil/ plant root	Population (x 10^5 CFU/g)	Amount of isolat
1.	1 KZ	Root of Sesbania grandiflora	49	1
2.	2 KZ	Root of Manihot utilissima	31	1
3.	3 KZ	Soil lakeside	17	1
4.	4 KZ	Black soil coal	19	1
5.	5 KZ	Soil near the tofu waste	41	1
6.	6 KZ	Soil near the cassava chips waste	25	1
7.	7 KZ	Soil mangrove forest	14	1
8.	8 KZ	Soil mangrove forest	22	1
9.	9 KZ	Root of Brassica chinensis	36	1
10.	10 KZ	Root of Carica papaya	27	1
11.	11 KZ	Root of Lycopersicon esculentum	29	1
12.	12 KZ	Root of Capsicum frutescens	33	1
13.	13 KZ	Root of *Amaranthus* sp.	24	1
14.	14 KZ	Soil without plant	13	1
15.	15 KZ	Root of Acasia mangium	29	1
16.	16 KZ	Rock	12	1
17.	17 KZ	Peat soil without plant	11	1
18.	18 KZ	Peat soil *Acasia* sp.	34	1
19.	19 KZ	Peat soil *Albizia* sp.	47	1
20.	20 KZ	Peat soil and coal	20	1
21.	21 KZ	Soil mangrove forest	16	1

In TSB media, there were 6 isolates where the colony color changed from white to red, this indicated that the isolates were able to produce the IAA hormone (Table 2). The IAA hormone is a very important phyto hormone that functions as a regulator of plant development, the high IAA hormone will control many physiological processes including cell enlargement and division, besides being able to produce more lateral roots and root hairs, so that the roots are able to take nutrients from more soil which in turn will increase the growth and yield of a plant [20], and on the Pikovskaya's media, there

Table 2

Table 2. Physiological characterization test of Azotobacter isolates from East Kalimantan

Isolate number	Location	N-fix	Protease	IAA	P solubilization
1 KZ	Rengganis Village, South Balikpapan	+	+	+	-
2 KZ		+	+	-	+
3 KZ		+	+	+	+
4 KZ		-	+	-	+
5 KZ	Dam Village, South Balikpapan	+	-	+	-
6 KZ		+	+	+	+
7 KZ	Manggar Beach, East Balikpapan	+	+	+	+
8 KZ		+	-	-	+
9 KZ		+	+	-	-
10 KZ		+	-	+	-
11 KZ		-	+	-	+
12 KZ		+	-	-	+
13 KZ		+	+	-	-
14 KZ		-	+	-	-
15 KZ	Suharto hill area, KM 34	+	+	-	+
16 KZ	Rapak Dalam Village	+	+	-	+
17 KZ	Makroman Village, Sambutan District	+	+	-	-
18 KZ		+	+	-	-
19 KZ		-	+	-	+
20 KZ		+	+	-	+
21 KZ	Somber Village, Batu Ampar, Balikpapan	+	-	-	+

Note: +: has N- fixing activity, produces protease enzymes, produces IAA hormones, and can to dissolve phosphate -: no activity

were 13 isolates, a clear zone was formed around the colony (Table 2), this indicated that the isolate was able to dissolve phosphate. Isolates that are able to form a clear zone because they produce organic acids, these organic acids cause the dissolution of phosphate from the bound phosphate source into available forms, organic acids are able to bind with Ca ions from Ca3(PO4)2 and liberate H2PO4 to form clear or clear colored areas. , besides that it was also explained that organic acids produced by soil microbes release phosphate minerals that are bound to Al, Fe and Ca [21], the difference in isolates in dissolving phosphate is due to differences in physiological and biochemical characters, or the ability to dissolve phosphate from each isolate depending on the strain [22].

The results showed that 8 bacterial isolates were found where the eight isolates had been tested on rice plants in vivo and showed results that had a significant effect on the growth of rice plants such as plant height, indicating that at 2 and 10 weeks of age the highest values were found in plants inoculated with isolate 1 KZ. At four weeks of age, the highest values were found in plants inoculated with 20 KZ isolates. At the age of six weeks, the highest values were plants inoculated with 15 KZ isolates. At 8 and 12 weeks of age, the highest values were found in plants inoculated with 1 KZ and 15 KZ isolates (Figure 1).

The number of leaves parameter showed the highest value was found in the 3 KZ isolate treatment at 2 weeks of age. For the number of leaves at the age of two weeks the highest value was found in plants inoculated with 3 KZ isolate. For the age of 8 weeks the highest value was found in the treatment of isolates 15K, 6 and 12 weeks of age the highest value was found in the treatment of isolates 1KZ, then at the age of 10 weeks the treatment of isolates 1 KZ and 7 KZ showed the highest results (Figure 2).

The dry weight parameter of straw for the treatment with 15 KZ isolates, then for the dry weight of grain for the 1 KZ isolate treatment showed the highest results, the dry weight parameter of 100 seeds in the 1 KZ isolate treatment. , 2 KZ and 15 KZ show the highest values (Figure 3). The panicle number parameter for the 1KZ isolate treatment showed the highest yield. The highest seed panicle parameters were shown by the 15 KZ isolate treatment (Figure 4).

All the parameters observed showed that the plants inoculated with 1 KZ and 15 KZ isolates gave the best results for rice plants. Both isolates were effective isolates and had a good match for their host plants so that they could increase growth and yield. As stated by [23] that inoculation will have a significant effect on growth and yield if the isolate has effectiveness and compatibility with the host plant. Besides that, the isolate can compete with indigenous bacteria [24].

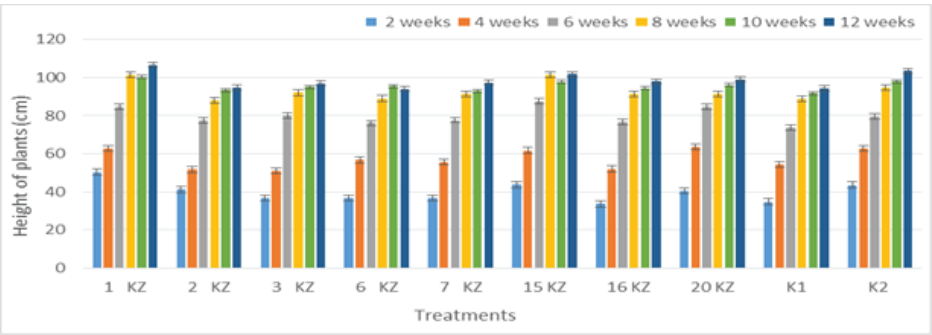

Figure 1: The average height of Rice plant with Azotobacter inoculation (cm).

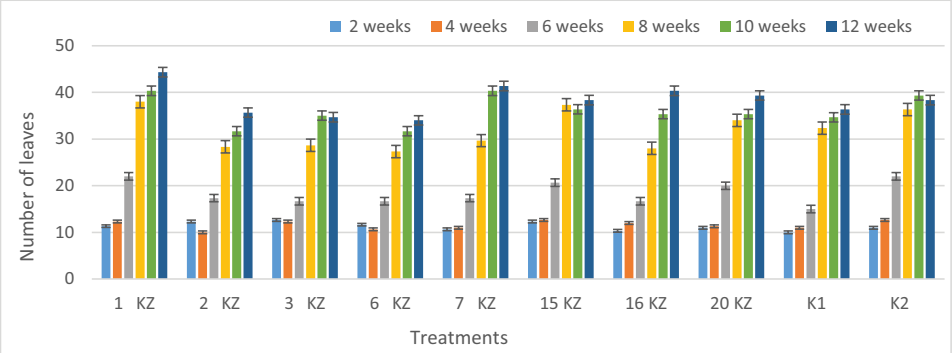

Figure 2: The average number of leaves Rice plant with Azotobacter inoculation.

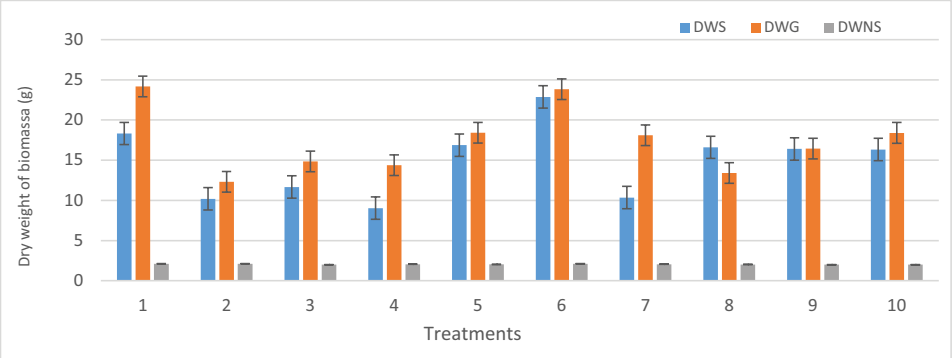

Figure 3: The average dry weight of straw (DWS), grain (DWG), and seeds panicle (DWNS) Rice plant with Azotobacter inoculation.

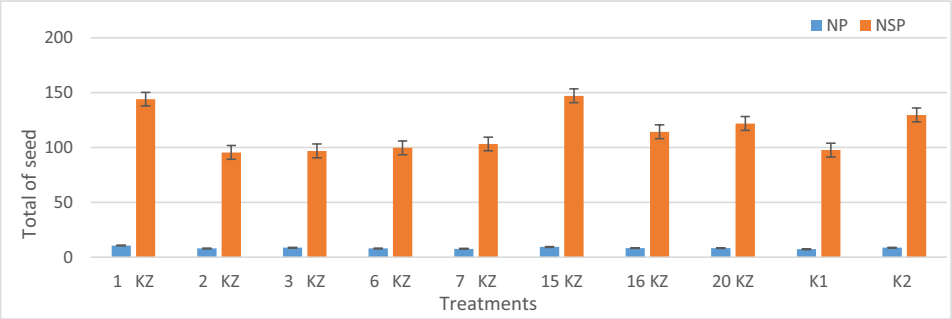

Figure 4: The average number of panicle (NP) and seeds panicle (NSP) Rice plant with Azotobacter inoculation.

4. Conclusion

The results showed that isolated of Azotobacter physiological characters studied had different characters. There were 21 Azotobacter isolated, 17 isolated capable of nitrogen-fixing activity, 16 isolated with protease activity, 6 isolated with IAA activity, and 13 isolated are able to dissolve phosphate. Testing the effectiveness of Azotobacter isolates

showed that 1 KZ isolated from Turi (Sesbania grandiflora) plant and 15 KZ isolated from Akasia (Acasia mangium) plant gave better yields of rice.

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