ISOLATION AND CHARACTERIZATION OF NITROGEN-FIXING AND GA3 PRODUCING BACTERIA FROM RICE RIZHOSPHERE OF RAINFED RICE FIELD IN SOUTH SULAWESI, INDONESIA

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ABSTRACT

Nitrogen fixing bacteria can fix nitrogen and produce hormones. The surrounding environment strongly influences the ability of a bacterial strain to survive and produce metabolites. The same type of bacteria from different places does not necessarily have the same potential. This study aims to obtain and determine the characteristics of rhizosphere bacteria originating from rainfed rice fields in South Sulawesi, which can fix nitrogen and produce the hormone gibberellin (GA3). This study was conducted by taking samples from the rhizosphere of rice plants in three South Sulawesi districts in rainfed rice fields. The results showed 12 isolates that we can grow on nitrogen-free media with various morphological and biochemical characters and can fix nitrogen and produce GA3. NG4.1 isolate from Gowa produced the highest nitrogen concentration of 0.157%. Isolate NG7.1 from Gowa produced the highest GA3 hormone, 3,936 mg L^{-1}. Character profiles and isolates' physiological abilities need to be the basis for developing biofertilizers and biostimulants to develop environmentally-friendly rice cultivation.

Keywords: Nitrogen fixation; bacteria; GA3; isolate; rainfed rice fields.

INTRODUCTION

Microorganisms in the soil function as nutrient supply agents, remodel organic matter and organic mineralization, stimulate plant growth, become biological agents to control pests and plant diseases, and influence soil's physical and chemical properties [1] and degradation of pollutants [2]. Nitrogen-fixing microorganisms that live freely in the Rhizosphere have been reported in several conditions to be very beneficial to plants as a stimulant for plant growth [3]. Nitrogen fixation bacteria are microorganisms with the potential as biofertilizer agents because of their ability to provide nitrogen for plants [4,5]. Besides that, it also has the potential as a biostimulant agent because of its ability to produce hormones [6].

Nearly 80% of the total gas in the atmosphere consists of nitrogen [7]. However, nitrogen in free N_2 in the atmosphere cannot directly absorb by higher plants. Although N_2 enters plant cells together with CO_2 via stomata, the existing enzymes can only reduce CO_2 so that N_2 comes out again as soon as it enters [8]. Plants absorb nitrogen from the environment in ammonium compounds (NH_4^+); this element can obtain from the soil with nitrogen-fixing bacteria [9].

However, further studies are still needed to determine bacteria's ability as biofertilizers and biostimulant agents from various lowland agro-ecosystem conditions, exceptionally marginal rainfed lowland land in South Sulawesi, which is the center of rice production. The surrounding environment greatly influences bacterial strains' ability to survive and produce metabolites [10]. Next, [11] mentioning environmental factors such as soil properties, organic matter in the soil, cultivation techniques, fertilization, and pesticide application that affect bacterial strain. The same type of bacteria from different places does not necessarily have the same potential [12]. This study aims to obtain and determine the characteristics of rhizosphere bacteria originating from rainfed rice fields in South Sulawesi, which can fix nitrogen and produce the hormone GA3 to potential as a biofertilizer and biostimulant agent for the development of environmentally friendly and sustainable rice cultivation. Biochemical character profiles and physiological abilities of the isolates are the basis for developing biofertilizers and biostimulants.

MATERIALS AND METHODS

Determination of Location and Sampling

Samples were taken from three rice-producing districts in South Sulawesi, namely Gowa, Pangkep, and Barru Regencies, with rainfed rice fields agro-ecosystem conditions. The age of the rice at the time of sampling ranged from 25-40 days after planting. The soil taken is a rice root area with a depth of 0-20 cm at five points for each location. Samples from each point were then put into a sterile envelope, made a composite, then taken to the laboratory using a cool box for further analysis [13,14].

Isolation and Morphological Characterization of Bacterial Isolates

Nitrogen-fixing bacteria were isolated using nitrogen-free media to select bacteria that could fix nitrogen according to the method used by [15,16],. A total of 10 g of soil was put into 90 ml of sterile distilled water, and a series of 10^{-1} to 10^{-6} dilutions were made. The suspension was homogenized in a rotary shaker at a speed of 150 rpm for 30 minutes. The suspension was taken from a 10^{-3} to 10^{-6} dilution of 0.1 ml and spread on a solid-free Burk's N medium with a composition of sucrose, 20.0 g; K_2HPO_4, 0.64 g; KH_2PO_4, 0.16 g; $MgSO_4.7H_2O$, 0.20 g; NaCl, 0.20 g; $CaSO_4.2H_2O$, 0.05 ; $Na_2MoO_4.2H_2O$, (0.05%) 5.0 ml; $FeSO_4.7H_2O$, (0.3%) 5.0 ml; agar 21 g and aquadest 1000 ml) then incubated for 24 hours at room temperature. The single colonies that had grown on Burk's media were then cultured on NA media through a zigzag scratch method and then purified three times. Pure colonies that grew with different morphological appearances were then characterized morphologically.

Gram Reaction Test and Catalase Test for Selected Isolates

The selected isolates from the nitrogen fixation test results and GA3 production were then carried out by the Gram reaction test and catalase test.

The Gram test is carried out according to the method used by [15] by mixing a 1-loop of bacterial isolates using a 1-loop needle and smeared it on a glass that has been dripped with 10 µL of 3% KOH then stirred repeatedly and let stand 5 to 60 seconds then observed the formation of mucus. If the mucus was formed, the bacteria were grouped into Gram-negative (-), but if no mucus was formed, it was classified as Gram-positive (+).

The catalase test using a modification of the method was carried out [12], which aims to test the catalase enzyme-producing microbes' ability to degrade hydrogen peroxide. Catalase production can be proven by adding 1-loop of pure single colony culture smeared on a slide that has been given two drops of 3% H_2O_2. The positive reaction of the catalase test is characterized by the emergence of gas bubbles from free oxygen, while the negative reaction does not.

Test Nitrogen Fixation Ability

Bacteria that we're able to fix nitrogen qualitatively were tested for their ability to grow on Burk's N-free media, which refers to the method of [16]. Quantitatively, by determining the total N content of bacterial isolate cultures using the Kjeldahl method, nitrogen content was determined by measurement by distillation. Measurement by distillation, starting with all sample extracts transferred to a boiling flask, then prepared a container to accommodate the released NH_3. The container is an Erlenmeyer containing 10 ml of 1% boric acid plus 3 *Conway* indicator drops and connected to a distillation device. 10 ml of 40% NaOH was added to the boiling flask containing the sample extract and closed immediately. Then do the distillation until the volume of the container reaches 50 - 75 ml. Furthermore, nitrogen levels were measured.

Test the Ability of the Bacterial Isolate to Produce the GA3 Hormone

The potential of bacteria in producing the hormone gibberellin (GA3) was tested based on standard methods [17]. The test procedure was carried out by making 30 ml of sterile Nutrient Broth media which was put in a 150 ml sterile glass bottle. Planting pure bacterial isolate samples by inserting one loop of bacterial isolate samples using a loop needle into a glass bottle, then incubated in an incubator by adjusting the temperature at 37^{o}C for seven days. A total of 10 ml of culture filtrate was taken and centrifuged at 8000 rpm for 10 minutes. In the next step, 5 ml of the supernatant was transferred to a test tube and then added 30 % hydrochloric acid (HCl). The solution mixture was then incubated for 75 minutes at room temperature 28^{o}C. Previously, 5 ml of HCl was prepared to be used as a blank. After incubation, GA concentrations are compared with standard curves GA3 (Sigma-Aldrich) in the range 0.25 - 2.25 ppm. Qualitative measurement is to look at the color change that occurs in the solution to brownish-yellow [18].

RESULTS AND DISCUSSION

Isolation and Morphological Characterization Bacteria

The bacterial isolation on rice rhizosphere soil samples from 3 sampling locations using nitrogen-free media obtained 12 isolates. The 12 bacterial isolates with the potential as nitrogen fixation bacteria general have various colony morphological characteristics (Table 1). Bacterial isolates almost did not show the same morphological characters, although there were bacterial isolates that showed similar morphological characters but obtained from different soil samples, it was still assumed that these isolates were different isolates. This morphological character obtained is in accordance with some morphological data of nitrogen-fixing bacterial colonies that have been reported in previous studies by [14-19].

The results of the Gram test using 3% KOH show that 5 isolate belongs to the Gram-negative (-) bacterial group because it produces mucus. Meanwhile, 7 isolate was included in the Gram-positive (+) group of bacteria because it did not produce mucus at the time of the test. Non-pathogenic bacterial isolates, both Gram-positive and Gram-negative, have an essential role as biocontrol agents in controlling plant diseases [15]. The catalase reaction test showed that the all selected isolates reacted with a positive catalyst indicated by the appearance of gas bubbles when given 3% H_2O_2. Catalase-positive bacteria have the enzyme catalase, breaking down hydrogen peroxide into water and oxygen [20]. In respiration, bacteria produce various components, one of which is hydrogen peroxide which is a poison that can damage the bacterial metabolic system and cause bacterial death [21].

Nitrogen Fixation Ability

The quantitative nitrogen fixation ability test carried out by the Kjeldahl method was directly proportional to the qualitative testing of nitrogen-fixing bacteria using free Burk's N media. All bacterial isolates were qualitatively able to fix nitrogen with their ability to grow on nitrogen-free media. Quantitatively, all isolates were able to produce nitrogen in varying amounts (Figure 2). NG4.1 isolate from Gowa had the highest ability to fix nitrogen at 0.157%, while NP1.1 isolate from Pangkep produced the lowest nitrogen at 0.136%. This difference is thought to be due to different types of bacteria, so that their ability to fix nitrogen is also different.

Table 1. The results of the morphological analysis of 12 nitrogen fixation bacterial isolates from the rice rhizosphere of rainfed lowland rice in South Sulawesi

Isolate	Colony morphology				Reaction (+/-)	
code	Form	Edge	Elevation	Color	Gram	Catalase
NG1.1	Irregular	Lobate	Raised	Cream	-	+
NG4.1	Irregular	Entire	Umbonate	Cream	-	+
NG.5.1	Irregular	Serrate	Raised	Beige	-	+
NG7.1	Irregular	Lobate	Raised	Beige	+	+
NP1.1	Rhizoid	Filamentous	Umbonate	White	+	+
NP7.1	Rhizoid	Filamentous	Raised	Beige	-	+
NP11.1	Irregular	Lobatus	Umbonate	Cream	+	+
NP12.1	Round	Entire	Umbonate	Cream	-	+
NB1.1	Irregular	Serrate	Raised	Cream	+	+
NB10.1	Rhizoid	Filamentous	Umbonate	Yellowish	+	+
NB12.1	Irregular	Serrate	Raised	Cream	-	+
NB13.1	Rhizoid	Filamentous	Raised	White	-	+

Note: NG: Gowa isolate, NP: Pangkep isolate, NB: Barru isolate

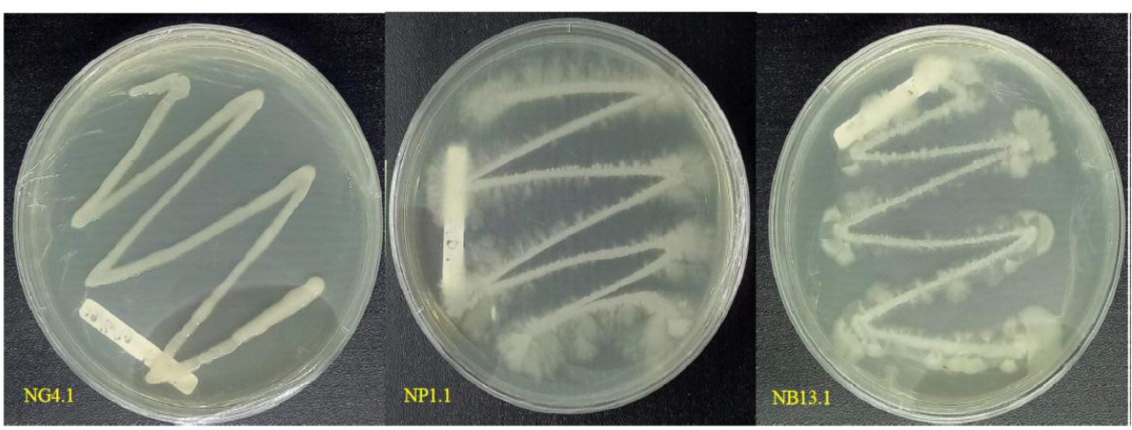

Fig. 1. Pure nitrogen fixation bacterial isolates isolated from rice rhizosphere from several rainfed rice fields locations in South Sulawesi

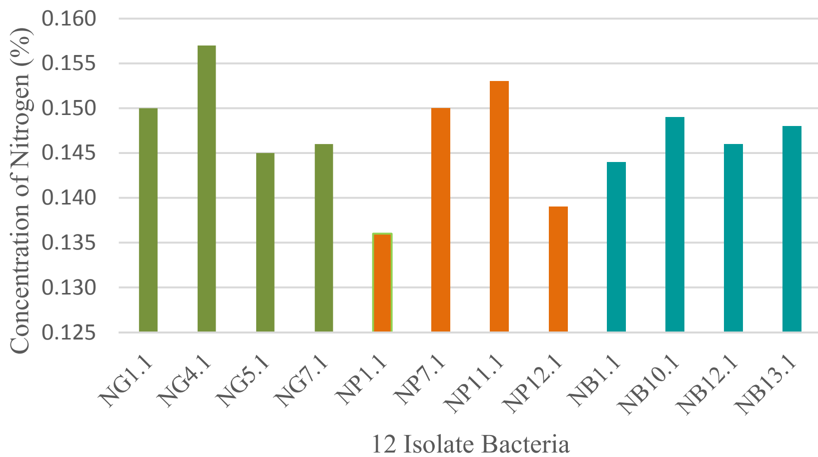

Fig. 2. Quantitative analysis of nitrogen fixation ability by bacterial isolates by distillation

Nitrogen-fixing bacteria can fix nitrogen from the atmosphere because this type of bacteria has a specific enzyme in the cell known as nitrogenase composed of two mutually supporting components, namely Fe protein and Mo-Fe protein [22]. Non-symbiotic nitrogen-fixing bacteria in the rhizosphere of gramineae plants, such as *Azotobacter paspali* and *Beijerinckia* spp, are one of a group of aerobic bacteria that colonize the root surface. On average, isolates from rainfed rice fields have sufficient nitrogen-fixing ability [1]. The possibility of this is due to rainfed rice fields, which are planted only once a year during the rainy season, which also affects less intensive chemical input as in irrigated rice fields so that it affects the diversity of activity of N_2-binding bacteria. Intensive agricultural practices that make use of extensive irrigation, with increased use of agrochemicals and heavy machinery, can lead to degradation [23], which affects the biological life of the soil [24].

The Ability to Produce the GA3 Hormone

Analysis of the levels of GA3 20 bacterial isolates, both qualitatively and quantitatively, showed that all bacterial isolates could produce GA3 even though the concentration differences were not too significant. Qualitatively, it can be seen that the color change in the bacterial suspension becomes brownish-yellow compared to the control (Fig. 3).

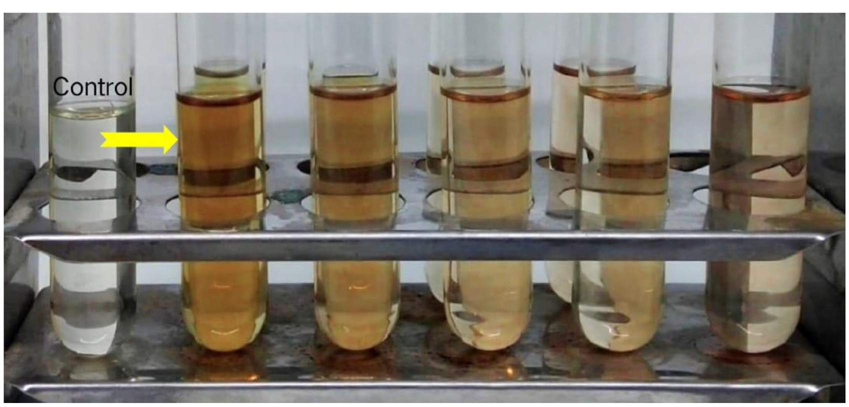

Fig. 3. Qualitative analysis of GA3 production by bacteria with a brownish color change that occurs in the supernatant

NG7.1 isolate from Gowa produced the highest GA3 valued at 3,936 mg L^{-1}, while NP12.1 isolate from Pangkep produced the lowest GA3 valued at 3,232 mg L^{-1}. The ability of rhizobacterial isolates to produce gibberellins was not the same, this was influenced by biochemical characteristics and environmental factors [12-18]. In rainfed rice fields which not flooded all year round, aerobic bacteria can grow well. Apart from agro-ecosystems, cultivation techniques also significantly influence a bacterial isolate's type and ability [25].

CONCLUSION

The 12 bacterial isolates isolated from the roots of rainfed lowland rice had various morphological characters and physiological abilities. All of the isolates could fix nitrogen and produce GA3 hormone. The highest nitrogen fixation ability found in isolated NG4.1 with a concentration of 0.157% and isolated NG7.1 produced the highest GA3 is 3,936 mg L^{-1}. The morphological character profile and physiological abilities of these bacterial isolates can be the basis for developing biofertilizers and biostimulants to develop environmentally-friendly rice cultivation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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