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Screening of phosphate solubilizing bacteria from sugarcane plant rhizosphere as biofertilizer agent for sorghum growth (*Sorghum bicolor*)

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Abstract. *Prisillia RMA, Susilowati A, Solichatun. 2021. Screening of phosphate solubilizing bacteria from sugarcane plant rhizosphere as biofertilizer agent for sorghum growth (*Sorghum bicolor*). Asian J Trop Biotechnol 18: 37-45.* Sorghum plants (*Sorghum bicolor* L.) development in Indonesia is still behind due to limited application in a few locations. Sorghum is a crop that can be grown on marginal terrain. The scarcity of nutrients in marginal land necessitates the addition of nutrients. Biofertilizer is a biological fertilizer that comprises microorganisms that act as nutrient suppliers and promote plant growth in the soil. This research included soil sampling, prospective testing, and screening to identify a biofertilizer solubilizing bacterium candidate. Bacterial screening entails bacterial identification, bacterial growth measurement, and a media pH test. The biofertilizer application experiment used a completely randomized design (CRD) with two factors: the first component was the addition of phosphate solubilizing bacteria, which was divided into two levels, without bacteria, and with bacteria. The second factor was variations in the NPK (Nitrogen, Phosphorus, Potassium) fertilizer concentrations of 0, 25, 50, and 100%, which correspond to 0; 0.625; 1.25, and 2.5 g NPK / plant, respectively. The vegetative phase of sorghum growth was observed for 50 days, and height, the number of leaves, leaves color, root length, fresh weight, and dry weight of sorghum plants were determined using ANOVA and DMRT test levels of 5%. Seven isolates of phosphate solubilizing bacteria were obtained from this research. Two possibly superior isolates were produced as biofertilizers: BPF 5 and 12 I. Compared to control treatments, adding BPF 5 and 12 I isolate in combination with varied NPK fertilizer concentrations significantly altered sorghum growth parameters, such as plant height, root length, fresh weight, and dry weight. The addition of BPF 12 I isolates at 0% and 25% fertilizer concentrations results in sorghum growth.

Keywords: Biofertilizer, phosphate solubilizing bacteria, sorghum plant growth

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) production and development in Indonesia fall behind rice, corn, and other cereals due to sorghum's limited use as a food crop. Sorghum has excellent commercial development potential in Indonesia, owing to favorable agro-ecological conditions and the availability of sufficient acreage (Tuwu et al. 2012). Sorghum has many cultivated varieties, such as grain genotypes, fodder, fiber, sugar, and dual-purpose genotypes (Arbab and Dagash 2017). Sorghum can also be fermented to produce beer. Additionally, sorghum stalks and seeds can be utilized in the sugar business, and sorghum seeds can be converted to bioethanol. Biomass from sorghum harvesting can be utilized as a source material for biogas production (Sumarno et al., 2013). Sorghum growth and cultivation remain limited in some areas of Indonesia, most notably in Java, South Sulawesi, Southeast Sulawesi, West Nusa Tenggara (NTB), and East Nusa Tenggara (NTT), both as a source of indigenous food items and animal feed. Sorghum's tolerance for drought and waterlogging is one of its properties. Sorghum can be planted on a marginal, marsh, or acidic land (Anas 2007).

Because marginal lands have a limited supply of nutrients, they require additional nutrients such as fertilization to boost crop output. Excessive use of chemical fertilizers can harm the ecology of agricultural land, resulting in the loss of indigenous microbes and disrupting the soil's nutrient balance (Yuliar 2006). The use of an excessive dose of chemical fertilizer can be mitigated by using a biofertilizer that reduces the quantity and eliminates the need for chemical fertilizers (Sudiarti 2017). Biofertilizer is a biological fertilizer made up of microorganisms that encourage development by increasing the nutritional requirements of plants that act as a source of nutrients in the soil. They are readily absorbed by plants and have been shown to improve plant productivity (Nugraha et al., 2014). Numerous soil microbes in the rhizosphere, including non-symbiotic nitrogen-fixing bacteria, symbiotic nitrogen-fixing bacteria, and phosphate-solubilizing bacteria, can be employed as biofertilizers (Setyorini et al. 2006).

The rhizosphere is the zone between the root surface and the soil influenced by plant roots' exudation and microorganisms' interaction. The exudate produced by plant roots results in a greater bacterial population in the rhizosphere than in other soil regions (Akbari et al., 2007). Bacteria that solubilize phosphate can convert it to dissolved phosphate, which plants can take via the release of organic acids. Phosphate solubilizing bacteria can dissolve P ions attached to soil cations such as Al, Fe, Ca, and Mg and convert them to a form suitable for natural plant absorption (Subowo et al. 2010). Biofertilizer research to accelerate the growth of sorghum plants is still

in its infancy, and its use requires development. The goal of this research is to produce phosphate-solubilizing bacteria from the rhizosphere for use as a biofertilizer and a suitable concentration of chemical fertilizer for use in conjunction with the biofertilizer to accelerate the growth of sorghum plants in a controlled environment.

MATERIALS AND METHODS

Research site

This research was carried out from June to December 2019 at the Environmental Microbiology Laboratory, Center for Biological Research of the Indonesian Institute of Sciences (LIPI), located at Jl. Raya Jakarta-Bogor km. 46, Cibinong Science Center, Cibinong (16911), Bogor, West Java, Indonesia.

Materials

The soil samples were taken from the root zone of sugarcane plants from sugarcane estates in Tasik Madu, Karanganyar. Sorghum seeds were employed in this investigation (*Sorghum bicolor* l.) Seeds were obtained from the south Sulawesi cereal crops research institute. The nutrient agar (NA) media employed in this investigation contained 5 g peptone, 3 g beef extract, 20 g agar, and 1,000 mL sterile distilled water. The lysogeny broth (LB) media had 10 g polypeptone, 5 g yeast extract, 5 g sodium chloride, 10 g agar, and 1,000 mL of sterile distilled water. Selective media for phosphate solubilizing bacteria, namely Pikovskaya media (PK), consisted of dextrose 10 g, $Ca_3(PO_4)$ 2.5 g, yeast extract 0.5 g, $MnSO_4$ 0.0001 g, MgSO4 0.1 g, $FeSO_4$ 0.0001 g, $(NH_4)_2SO_4$ 0.25 g, KCL 0.2 g, agar 18 g, and sterile distilled water 1000 mL.

Experimental design

The biofertilizer application to sorghum plants was designed experimentally using a completely randomized design (CRD). The treatment entailed two components; the first was the administration of phosphate solubilizing bacteria, which came in two forms: without bacteria and with bacteria. The second factor is the change in NPK fertilizer concentration, which is divided into four levels: 0%, 25%, 50%, and 100%, which correspond to 0; 0.625; 1.25, and 2.5 g NPK/plant, respectively (Sudiarti 2013). The NPK fertilizer utilized in this study is Phonska NPK fertilizer, which PT Petrokimia Gresik manufactures. It contains 15% nitrogen (N), 15% phosphate (P_2O_5), and 15% potassium (K_2O). Three replications of each treatment were obtained, and the following treatment combinations were obtained: (i) The first treatment consisted of no bacteria (control) and a 0% NPK fertilizer concentration. (ii) The second treatment consisted of no bacteria (control) and a concentration of NPK fertilizer equal to 100%. (iii) The third treatment involved the administration of phosphate solubilizing bacteria and NPK fertilizer at doses of 0%, 25%, and 50%.

Soil sampling and isolation of rhizosphere bacteria

The rhizosphere was sampled by extracting soil from around the plant roots at a depth of 20 cm below the soil surface. Three random soil samples were gathered and composited. Bacteria were isolated from sugarcane plantation soil using a dilution procedure, which involved weighing 1 g of soil into a test tube containing 9 mL of sterile distilled water and vortexing for 15 minutes. The obtained suspension was diluted to a concentration of 10^{-5} dilution sessions (Amaria et al., 2013). The dilution results were multiplied by 100 and then grown in NA medium using the pour plate method and incubated at room temperature for 2 x 24 hours. Each bacterial colony growing on the NA medium was isolated using the streak plate method and purified until a single colony was produced. Each purified isolate was cultured in a slanted NA and LB medium.

Phosphate solubilizing bacteria potential test

The isolated bacteria were then cultured in PK using the spot method with sterile toothpicks. The edge of the petri dish was wrapped in plastic wrap and incubated at room temperature for 7 x 24 hours to avoid contamination. On pk media, the activity of phosphate solubilizing bacteria was assessed by the presence of a clear zone (Rao 1994). Observations determined the presence or absence of a clear zone. If the isolate produced a clear zone, it was chosen for further investigation, and the phosphate dissolution index was calculated using the following formula (Sharon et al., 2016):

$$\text{Phosphate Solubility Index (SI):} \frac{\text{Clear Zone Diameter} - \text{Colony Diameter}}{\text{Colony Diameter}}$$

Bacteria identification

Identification of bacteria was performed macroscopically and microscopically. Macroscopic observations were made by examining the morphology of the single colonies that formed following the application of the streak plate method. Single colony observations included colony shape, color, borders, and the height of bacterial colonies (Cappuccino and Sherman 1998). Microscopic observations were made by examining the Gram staining data. Microscopic investigations of the form of bacterial cells were also made. Purple staining indicates that the bacteria are Gram-positive, whereas red staining indicates Gram-negative bacteria (Fitri and Yasmin 2011).

Preparation of liquid Pikovskaya media containing phosphate solubilizing bacteria

Phosphate solubilizing bacteria isolates were rejuvenated on NA media for 1 x 24 hours, then grown on LB media with the same Optical density (OD) value as measured using a spectrophotometer with a wavelength of 600 nm. Then it was cultured into 50 mL of liquid PK. Each Erlenmeyer containing phosphate solubilizing bacteria was shaken using a shaker incubator at 120 rpm for seven days (Rahayu et al. 2014).

Measurement of growth of phosphate solubilizing bacteria

OD measurement of phosphate solubilizing bacteria taken from liquid P.K. media. Then the bacterial culture in the medium was taken at 0.5 mL diluted with 7 mL of sterile distilled water and then put into a cuvette and measured using a spectrophotometer with a wavelength of 600 nm (Purnomo et al. 2017). With three replications, OD analysis was carried out every 1 x 24 hours from day 0 to day 7.

Media pH test

Measurement of changes in pH was carried out by taking 2 mL of the bacterial culture from liquid PK media in a test tube and measuring it using a pH meter. Analysis of changes in the pH of the media for each bacterial isolate was carried out every 1 x 24 hours starting from day 0 to day 7 with two replications.

Bacterial growth standard curves

The standard curve for bacterial growth was constructed by determining the population of live bacteria using the pour plate method and measuring the optical density of the bacteria using a spectrophotometer (Hadioetomo 1993). The optical density was determined directly on bacterial cultures grown in an LB medium using a spectrophotometer set to a wavelength of 600 nm. Inoculum is extracted from the rejuvenated stock culture and placed one time utilizing the ose into 50 mL of LB medium, then shaken. Bacterial culture from shaken LB media is added in a volume of up to 1 mL to 9 mL of physiological salt. Several dilution sessions and NA media growth using the pour plate method were performed (Nurhayati et al., 2007). Measurements were taken every two hours for 24 hours, from the 0th to the 24th hour. The number of bacteria growing on NA media was determined as follows using the TPC formula (Sukmawati and Hardianti 2018):

$$\frac{cfu}{ml} = \frac{(Number\ of\ Colonies)\left(\frac{1}{Sample Volume}\right)}{Dilution\ Factor}$$

Making biofertilizer

A 2% molasses solution was prepared by heating 14 mL molasses in 686 mL water to a boil and allowing it to cool before adding each bacterial culture. The molasses included 10% cultures (70 mL of bacterial culture from LB media in 630 2% molasses) (Sudiarti 2013). Bacterial cultures were added to molasses at a concentration of 2% with the same OD value. OD absorbance is determined using a spectrophotometer with a wavelength of 600 nm. Total Plate Count (TPC) was determined in bacterial cultures using a series of dilutions up to 10^{-5} and incubation at 37°C for 24 hours. TPC was determined using the cfu/mL unit (Sudiarti 2017) with three replications.

Preparation of sorghum seeds

Sorghum seeds are chosen for their uniform shape and lack of physical damage. Seeds were sterilized by washing with distilled water and 1% sodium hypochlorite to remove fungus infection and then immersed in hot water at 60°C for 24 hours to aid in germination. Seeds that swell and settle due to water absorption are used as research items.

Planting of sorghum seeds

Sorghum seeds are planted in ultisol soil. The soil was sterilized first in an autoclave at 121°C and 1 atm pressure for 20 minutes and then in an oven (Karti and Setiadi 2011). After baking, the soil was placed in polybags of the same size and weight as 300 g, and the pH of the soil was determined using a soil tester. Each polybag contained three sorghum seeds and was watered with 50 mL of sterile distilled water. Sorghum is grown for 13 days after planting and then treated with NPK fertilizer at various concentrations, namely 0%, 25%, 50%, and 100%, followed by biofertilizer in the form of molasses solution containing inoculated phosphate solubilizing bacteria and without inoculation bacteria (control) at a biofertilizer concentration of 15 mL/plant (Sudiarti 2013).

Observation of sorghum growth

For 50 days, observations of sorghum growth in the vegetative phase were made (Aqil and Bunyamin 2013). They were determined every three days by measuring the sorghum plant's height with a ruler and counting the leaves produced. Then, during the harvesting process, leaf color was determined using the Munsell color chart book's standards, root length was determined using a ruler, and the wet and dry weight of the complete sorghum plant was determined using a scale.

Analyses of data

This research gathered both qualitative and quantitative data. The Analysis of Variance (ANOVA) test was used to assess quantitative data on sorghum development to identify the effect of phosphate solubilizing bacteria and fluctuations in the content of NPK fertilizer at various concentrations. If a substantial difference exists, it will be further tested using Duncan's Multiple Range Test (DMRT) with a 5% test level. Simultaneously, descriptive analysis is used to analyze the qualitative data.

RESULTS AND DISCUSSION

Isolation and characterization of phosphate solubilizing bacteria

Bacterial isolates and purification from soil samples collected from sugarcane farms in Tasikmadu, Karanganyar resulted in the isolation and purification of 19 bacterial isolates, 15 of which were bacteria and four of which were actinomycetes. The isolated bacteria were then examined for their capacity to dissolve phosphate using Pikovskaya selective media containing $Ca_3(PO_4)_2$ (tricalcium phosphate). Five bacteria and two actinomycetes isolated exhibited a distinct zone, suggesting their ability to dissolve phosphate. The transparent zone generated by organic acids is composed of dissolved p. Organic acids can bind to the calcium ions in $Ca_3(PO_4)_2$ and liberate H_2PO_4, resulting in a clear colored zone (Widawati and Suliasih 2005).

Macroscopical and microscopical examinations were used to identify phosphate solubilizing bacteria. Macroscopic identification was accomplished by observing colony structure, edge, elevation, and color (Cappucino 1998), whereas microscopic observations were accomplished through Gram staining. Table 1 summarizes the observations of morphological characteristics and phosphate solubilizing bacterial cells.

Observation of phosphate solubilizing bacteria obtained bacteria with a variety of morphological characteristics. The acquired bacterial isolates were almost entirely circular colonies with flat (entire) colonies, with only one undulating. Three isolates were milky white, one was yellowish-white, one was brownish-white, and one was orange-yellow. Six isolates were Gram-positive in *Bacillus* and *Coccus* cells, while one isolate was Gram-negative in *Coccus* cells. Suryanto and Munir (2006) discovered that spherical colonies contained more bacteria than white colonies. Dewi (2008) found several Gram-positive bacteria in the form of *Coccus* and *Bacillus* cells. Gram staining is used to characterize bacterial cells and distinguish between Gram-positive and Gram-negative bacteria. It is purple in Gram-positive bacteria because of crystal violet iodine, which is retained even when treated with an alcohol bleach solution. In Gram-negative, on the other hand, it is red because crystal violet dissolves in an alcohol bleach solution, imparting a red hue to the safranin solution (Lay 1994).

Two isolates of actinomycetes, BPF 5 and BPF 15, were macroscopically examined for colony morphological characteristics, including substrate hyphae color, aerial hyphae color, and reverse pigment (Listiana et al., 2018). Microscopic investigations of hyphae structure using lactophenol staining. Septate (septate) hyphae were detected, and nonseptate hyphae (Nuryadi et al. 2016). The colonies of BPF 5 isolates were strongly connected to the media, with white substrate hyphae, gray aerial hyphae, brownish-yellow reverse pigment, and a non-insulated hyphae structure. By contrast, BPF 15 isolates were adherent to the media via white substrate hyphae. The aerial hyphae are gray, the reverse pigment is greenish-yellow, and the hyphae are septate (septate). Figure 1 shows the morphological characteristics of actinomycetes isolates.

Actinomycetes are prokaryotic organisms that include Gram-positive bacteria and are frequently found in various soil types (Akbari et al., 2017). Actinomycetes can be identified from other bacteria when examined in colonies in solid media. Actinomycetes colonies are distinct in that they are hard due to their growth on agar media, whereas other bacterial colonies are soft. Actinomycetes are characterized by their circular shape, flat edges, and uneven, starchy surfaces. The floury surface comprises many hyphae (Sulistyani and Akbar 2014). Actinomycetes are a kind of bacteria that generate hyphae (mycelium) composed of a substrate and aerial hyphae. Aerial hyphae develop from the mycelium substrate and quickly blanket the colony, like cotton or flour (Kumalasari et al., 2012). Hyphae that sprout straight from the substrate are called substrate hyphae. The reverse pigment is a color that develops at the bottom or base of an actinomycetes culture (Listiana et al., 2018). Additionally, actinomycetes can degrade phosphate, promoting plant growth and productivity (Chang et al. 1986).

Phosphate dissolution in Pikovskaya media

When cultivated on Pikovskaya agar media, bacteria can dissolve phosphate, as evidenced by forming a clear zone around the colony. The phosphate solubilization index (SI) is calculated as the ratio of the clear zone diameter formed by phosphate solubilizing bacteria to the diameter of their colonies. The dissolving index quantifies a bacterium's ability to qualitatively release phosphate, and the higher the dissolution index value, the greater the bacterium's ability to release phosphate (Elfiati et al., 2016). Table 2 contains the phosphate solubility index.

Table 1. Morphological characters and phosphate solubilizing bacterial cells

Isolate	Characterization					
	Colony morphology				Gram	Cell shape
	Shape	Edge	Elevation	Color		
BPF 5	Circular	Entire	Convex	Gray milky white with gray spots in the middle	+	Bacilli
BPF 6	Circular	Entire	Flate	Yellow-orange	+	Cocci
BPF 7	Circular	Entire	Raised	Brownish white	+	Cocci
BPF 12 I	Circular	Entire	Raised	Yellowish white (transparent)	-	Cocci
BPF 12 II	Irregular	Undulate	Flate	Milky white	+	Cocci
BPF 13	Circular	Entire	Raised	Orange white	+	Cocci
BPF 15	Circular	Entire	Flate	Milky white	+	Cocci

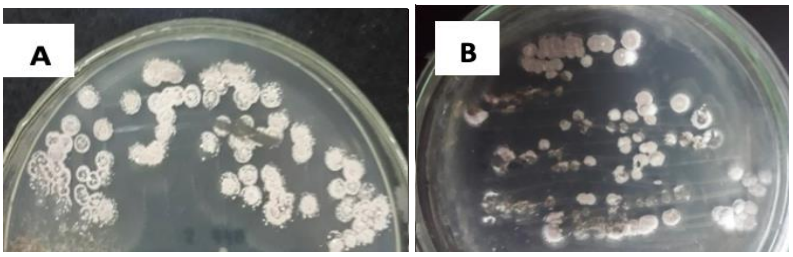

Figure 1. Observation of morphological characters at 100 x magnification (A) isolate BPF 5 and (B) isolate BPF 15

Table 2. Phosphate solubilization index for seven days of incubation

Isolate	Phosphate solubility index							SI Means
	Day -							
	1	2	3	4	5	6	7	
BPF 5	-1.00	-1.00	1.00	0.67	1.00	1.00	1.25	0.42
BPF 6	-1.00	2.00	2.00	1.00	1.00	0.67	0.67	0.90
BPF 7	0.50	0.67	1.00	1.33	1.00	0.60	0.50	0.80
BPF 12 I	2.00	2.50	3.00	2.00	2.67	2.25	2.50	2.42
BPF 12 II	1.50	2.00	2.50	1.67	2.00	2.33	2.67	2.10
BPF 13	0.50	0.67	0.50	0.75	0.60	0.80	0.67	0.64
BPF 15	-1.00	-1.00	0.50	0.50	1.00	1.50	1.00	0.36

The phosphate solubilization ability of 7 isolates capable of dissolving $Ca_3(PO_4)_2$ was indicated by forming a clear zone around the bacterial colonies. Observations were made for seven days, and various clear zones were created. The BPF isolate, which had the highest phosphate SI, was characterized by forming the largest clear zone, namely BPF 12 I of 2.42 with a clear zone of 1.4 cm. In contrast, the actinomycetes isolates were BPF 5 of 0.42 with a clear 0, 9 cm zone. It indicated that BPF 12 I and BPF 5 isolates had higher phosphate solubilization activity in releasing P bound to $Ca_3(PO_4)_2$ media, and organic acids produced by BPF isolates reacted with $Ca_3(PO_4)_2$ to form a chelate of Ca release higher P so that the application of BPF isolates as biofertilizer is based on the speed of BPF isolates in dissolving phosphate and the diameter of the clear zone which is expected to improve plants experiencing P deficiency. Superior phosphate solubilizing bacteria can produce the largest clear zone diameter compared to other bacterial colonies (Rahayu et al., 2014). Each isolate will create a phosphate SI that varies from one another. It is due to producing organic acids such as acetic acid, lactic acid, oxalic acid, malic acid, and citric acid produced by bacteria (Richardson 2001).

Growth of phosphate solubilizing bacteria on Pikovskaya media media

Bacterial growth can be measured by cell concentration or cell density. The bacterial growth curve can be divided into four phases: the lag phase (adaptation phase), log phase (exponential phase), stationary phase, and death phase. The results of measuring bacterial growth by looking at the absorbance value of OD can be seen in Figure 2. Based on Figure 2, the growth chart of phosphate solubilizing bacteria increased from day 0 to day 7. On day 0, BPF isolates experienced a lag phase (adaptation phase), where the time needed by BPF isolates to adapt to their new environment. From day 1 to day 3, BPF isolates experienced a log phase (exponential phase). BPF isolates experienced a cell division phase that would divide until the maximum number of cells was reached. From the 4th day to the 6th day, there was a stationary phase, in which the number of dead cells was the same as the number of living cells (constant growth), while on the 7th day, there was an increase in the growth of all BPF isolates and had not yet experienced the death phase. The growth medium used is rich in P, so bacteria take a long time in the metabolic process to dissolve P.

One way to determine the quality of a biofertilizer is the number of bacteria. The calculation results of the number of bacterial colonies showed that the number of phosphate solubilizing bacterial colonies was 1010 CFU/mL. It is in accordance with the quality standard of the biofertilizer, which must at least reach a concentration of 107 CFU/mL (Simanungkalit et al. 2006). BPF isolates that had the highest average number of bacterial colonies during the incubation period were BPF 6 of 86.66 x 1010 CFU/mL and 12 I of 81.69 x 1010 CFU/mL, while actinomycetes isolates were BPF 15 of 103.83 x 1010 CFU/mL and BPF 5 of 98.09 x 1010 CFU/mL so that they can be used as biofertilizer candidates. The application of biofertilizers with a high number of bacterial cell densities can compete with microorganisms found in the soil so that they can dominate around plant roots (Santoso 2000).

Changes in pH in Pikovskaya media media

The decrease in pH is one of the causes of the dissolution of Ca-phosphate into orthophosphate ions. The mechanism of phosphate dissolution results in changes in pH due to the synthesis of organic compounds released into the media (Widawati et al. 2008). Changes in the pH of the media during the seven-day incubation period can be seen in Figure 3.

According to Figure 3, the pH in the media decreased starting on day 0, which was caused by heating during sterilization, which disrupted the Ca-phosphate bond, resulting in the formation of dissolved phosphate (Rahayu et al. 2014), where the initial pH before sterilization was Neutral pH is 7. After seven days of incubation, the pH of the media was changed from neutral (pH 7) to acid (pH 5 - 4). According to Figure 3, the BPF 12 I isolation had the most significant drop in medium pH (4.14). In contrast, the actinomycetes isolate BPF 5 had the most significant decrease in media pH (4.45). It indicates that BPF 12 I and BPF 5 isolates exhibited the highest phosphate solubilization activity by producing high levels of organic acids, which resulted in a significant decrease in the pH of the media, implying that the use of phosphate solubilizing bacterial biofertilizers can increase the availability of P nutrients by liberating bound phosphate ions into available forms that plants can absorb, thereby improving soil fertility in areas with phosphate deficiency.

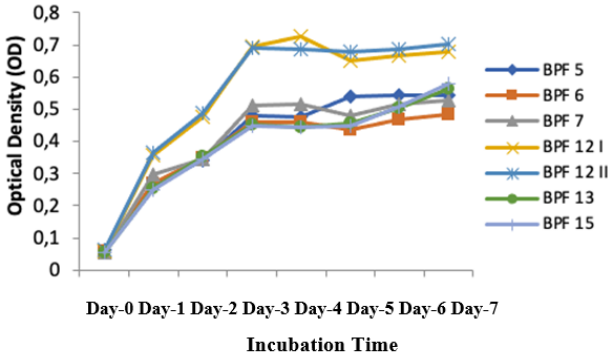

Figure 2. Phosphate solubilizing bacteria growth

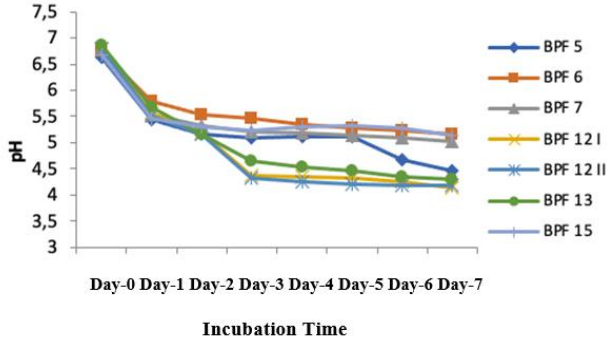

Figure 3. Changes in pH of P.K. media on BPF isolates

The pH of the media decreased during the incubation period due to the activity of BPF isolates that created organic acids as a mechanism for dissolving phosphate. Organic acids react with phosphate-binding elements such as Al_3^+, Fe_3^+, and Ca_2^+ to generate stable organic chelates in plant-utilizable forms (Firdausi et al., 2016). Bacterial organic acids result from oxidative respiration or fermentation of organic carbon sources to liberate phosphate ions bound to media containing Ca_3PO_4 and convert them to H_2PO_4- ions via the exchange of acid anions with phosphate anions or the formation of stable organic chelates (Elfiati 2005).

Sorghum plant measurement

Measurement of plant height and number of leaves was carried out when the plant was 13 days old with a height of 10 cm and the number of leaves in four pieces (Figure 4). The application of various concentrations of NPK fertilizer combined with biofertilizer in the form of BPF 12 I and BPF 5 isolates with an absorbance value of 0.557 OD and a colony number of 4.7 x 108 cfu/mL. Measurement of plant height and number of leaves was carried out periodically when the plants were 13, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, and 50 days after planting. Measurements of root length, wet weight, dry weight, and leaf color were measured during the harvesting process for plants aged 50 days after planting. Figure 4 demonstrates that the BPF 12 I treatment with 0% and 25% NPK fertilizer concentrations produced significantly higher plant height, number of leaves, root length, wet weight, and dry weight than other treatments. The results from the BPF treatment with a 50% NPK fertilizer content were less than optimum. 0% and 100% resulted in the most diminutive average plant height, several leaves, root length, wet weight, and dry weight of plants, indicating that a low concentration of NPK fertilizer mixed with BPF 12 I could improve plant growth.

Because the significance value for the ANOVA test was 0.00 (0.05), it was determined that the application of various concentrations of NPK fertilizer and biofertilizer had a significant effect on the height, root length, wet weight, and dry weight of sorghum plants. Thus, the DMRT test was continued. Because the significance value of 0.77 (0.05) indicated that the administration of various NPK fertilizers and biofertilizers quantities had no significant influence on the number of leaves, the DMRT test was discontinued, and the Tukey test was used. (BNJ) level 5% to determine the effect of the treatments, which are shown in Table 3.

According to Table 3, the BPF 12 I treatment and NPK fertilizer concentrations of 0% and 25% had significantly distinct impacts on plant height, root length, wet weight, and dry weight, but not on the BPF 5 fertilizer concentration of 0% NPK. The number of leaves produced by treatments with varied concentrations of NPK fertilizer mixed with BPF 5 and 12 I was not substantially different from the control treatment.

Table 3. Growth of sorghum after treatment with 0%, 25%, 50%, and 100% concentration of NPK fertilizer and biofertilizer in the form of BPF 5 and BPF 12 I isolates.

Treatment	Plant height (cm)	Number of leaves (strands)	Root length (cm)	Wet weight (g)	Dry weight (g)
Control 0%	$24.56^{a} \pm 0.10$	$4.98^{a} \pm 0.42$	$17.67^{a} \pm 2.10$	$36.66^{a} \pm 1.42$	$16.48^{a} \pm 1.13$
Control 100%	$25.71^{ab} \pm 0.64$	$5.21^{a} \pm 0.46$	$20.60^{b} \pm 2.02$	$40.78^{b} \pm 0.57$	$20.49^{b} \pm 1.73$
BPF 5 0%	$28.48^{e} \pm 0.51$	$5.37^{a} \pm 0.58$	$23.88^{cd} \pm 0.96$	$51.86^{de} \pm 1.89$	$27.65^{de} \pm 0.72$
BPF 5 25%	$27.97^{de} \pm 0.43$	$5.45^{a} \pm 0.39$	$23.40^{c} \pm 0.70$	$49.08^{cd} \pm 1.99$	$25.77^{cd} \pm 0.67$
BPF 5 50%	$26.66^{bc} \pm 0.46$	$5.30^{a} \pm 0.46$	$22.34^{bc} \pm 0.83$	$46.59^{c} \pm 2.09$	$24.08^{c} \pm 0.54$
BPF 12 I 0%	$30.00^{e} \pm 0.88$	$5.60^{a} \pm 0.49$	$25.88^{e} \pm 0.60$	$55.35^{f} \pm 1.48$	$30.72^{f} \pm 1.62$
BPF 12 I 25%	$28.95^{e} \pm 0.27$	$5.54^{a} \pm 0.51$	$24.59^{cd} \pm 0.87$	$52.97^{ef} \pm 2.34$	$28.00^{e} \pm 1.29$
BPF 12 I 50%	$27.11^{cd} \pm 0.46$	$5.30^{a} \pm 0.48$	$23.09^{c} \pm 0.59$	$49.01^{cd} \pm 2.79$	$25.40^{c} \pm 0.70$

Note: The same letter in the same column shows no significant difference based on the DMRT test and BNJ test at = 5%

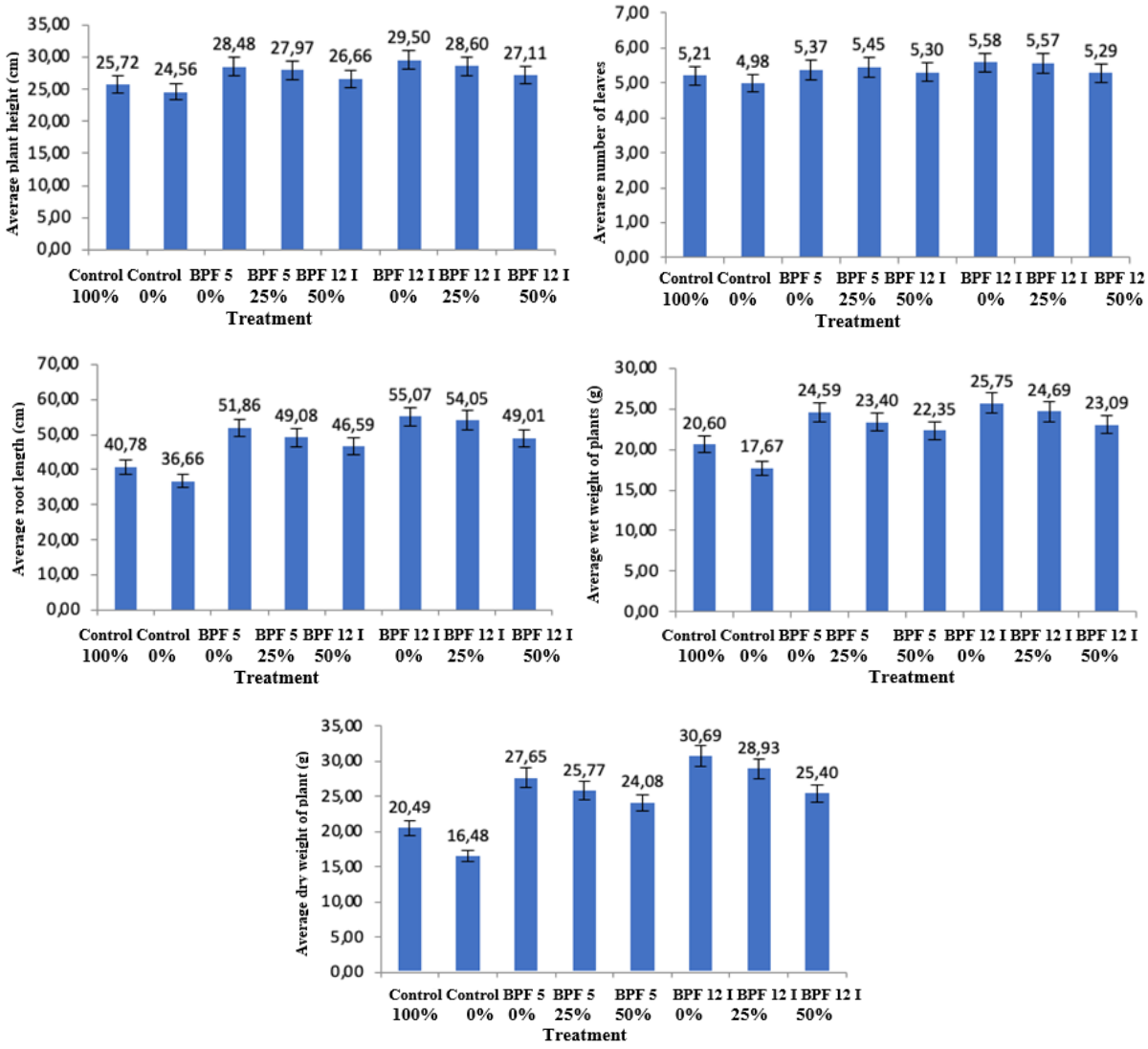

Figure 4. Sorghum plant growth 50 days after planting (DAP)

Because measurements are taken every three days until the plant reaches 50 days following planting, the data acquired are not statistically significant between treatments. Nonetheless, NPK fertilizer and biofertilizer administration can provide the nutrients necessary for plant growth. According to these findings, BPF 12 I treatment with 0% and 25% NPK fertilizer boosted plant growth compared to 50% NPK fertilizer and control treatment. Biofertilizers can help reduce the amount of NPK fertilizer used because both fertilizers can provide and complement the nutrients required by plants. As a result, crop yields are more optimal, and soil fertility is improved, providing long-term benefits and promoting sustainable agriculture (Pangaribuan et al. 2017).

Using 50% and 100%, NPK fertilizers resulted in less optimal sorghum growth. High concentrations of NPK fertilizers cause poisoning in plants, inhibiting chemical reactions such as metabolism, photosynthesis, and respiration, thereby impairing plant growth and productivity (Rahmatika and Sasmito 2017). When large doses of NPK fertilizer are applied to young seedlings, the plants will not thrive because they cannot synthesize the fertilizer (Pasaribu et al., 2018).

Leaf color was observed when the plant reached its maximal vegetative phase, approximately 50-60 days following planting. Leaf color was observed using the Munsell color chart to assess the chlorophyll content of plants. Table 4 summarizes the results of the leaf color analysis.

The findings of the leaf color observation using the Munsell color chart indicated that the level of greenish hue in the leaves varied according to the concentration of NPK fertilizer mixed with biofertilizer. According to Table 4, the BPF combination treatment with NPK fertilizer concentrations of 0%, 25%, 50%, and 100% showed darker green leaf color, namely 5 GY 6/6 and 5 6/8, compared to the 0% control treatment with 2.5 GY 6/10 and 5 GY 7/8. It is possible because the availability of nutrients such as N, P, and K affects the leaf's green hue. The darker the green color of the plant, the more nutrients it absorbs, which increases the plant's dry weight and yield (Nugroho 2015).

Table 4. Observation of sorghum leaf color based on Munsell Color Chart

Treatment	Fertilizer concentration	Leaf color	Color on Munsell
Control	100%	5 GY 6/6	
Control	100%		
Control	100%		
Control	0%	2.5 GY 6/10	
Control	0%	5 GY 7/8	
Control	0%		
BPF 5	0%	5 GY 7/8	
BPF 5	0%		
BPF 5	0%	5 GY 6/6	
BPF 5	25%	5 GY 6/8	
BPF 5	25%		
BPF 5	25%	5 GY 6/6	
BPF 5	50%	5 GY 6/8	
BPF 5	50%		
BPF 5	50%		
BPF 12 I	0%	5 GY 6/8	
BPF 12 I	0%		
BPF 12 I	0%	5 GY 6/6	
BPF 12 I	25%	5 GY 6/6	
BPF 12 I	25%		
BPF 12 I	25%		
BPF 12 I	50%	5 GY 6/6	
BPF 12 I	50%	5 GY 6/8	
BPF 12 I	50%		

The aging process is accelerated in the control treatment. Simultaneously, the addition of NPK and biological fertilizers results in a lighter and darker coloration of the leaves since the microbes included in biological fertilizers can offer nutrients during the vegetative period (Suryadi et al., 2019). The usage of NPK fertilizers containing nitrogen affects the color of the leaves as well. If these nutrients are in short supply, the leaves will become yellow or yellowish-green, impairing the photosynthetic process.

To conclude, seven isolates of bacteria with potential as phosphate solubilizing bacteria were recovered from the rhizosphere area of sugarcane. Five isolates of bacteria and two isolates of actinomycetes were obtained, with two isolates having outstanding potential, namely isolates BPF 12 I and BPF 5. The addition of phosphate-solubilizing bacteria from the Sugarcane rhizosphere to an NPK fertilizer substantially affected the growth of sorghum (*S. bicolor*) growth, particularly on parameters such as plant height and root length leaf color, wet plant weight, and dry plant weight. The combination of BPF 12 I isolate with fertilizer concentrations of 0% and 25% significantly affected and resulted in improved sorghum growth.

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