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THE POTENTIAL OF POTATO CULTIVATION *(Solanum tuberosum L.)* WITH THE APPLICATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND TRICHO POWDER COMMERCIAL ON MEDIUM LAND

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ABSTRACT

The market demand for potatoes is very high, and their cultivation can affect the environmental balance, so innovation in potato yield is needed. Medium land is an area with an elevation of 300-700mdpl. Crop yields in medium land are not optimal and more susceptible to disease than highlands. The role of microorganisms in PGPR and Tricho Powder can maximize crop yields and help reduce disease intensity. This study aims to determine the effect of different concentrations of commercial PGPR in increasing the yield of potato cultivation, the effect of different concentrations of Tricho Powder in suppressing the intensity of disease attacks, and find out one species in PGPR. This research was conducted with RAL (6 treatments with 4 replications). The treatments included P0 (Farmers' Habits), P1 (PGPR 20mL), P2 (PGPR 40 mL), P3 (Tricho Powder 80gram/10Liter), P4 (Tricho Powder 150gram/10L), and P5 (PGPR 20mL+Tricho Powder) 80gr/10L). The data was then analyzed by ANOVA. If there is significance, a follow-up test is carried out, which is determined based on the KK value. The results showed that differences in PGPR concentrations positively affected potato yields with the combination treatment of PGPR 20mL/10L+Tricho Powder 80 gr/10L (P5), which was the best treatment producing the highest mean in each parameter. In the same treatment, disease intensity was reduced by 37.55% compared to the control treatment. Based on its ability to produce IAA from 5 isolates, only 1 isolate (IS 5) showed positive results and was continued to molecular tests.

Keywords: Potato cultivation, PGPR, Trichoderma powder, Bacillus sp.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the plants belonging to the horticultural group and has high potential and nutritional content. The increase in demand related to potato productivity certainly impacts increasing land for potato cultivation. According to data from the Ministry of Agriculture, in the period, there was an increase in potato cultivation area by 6.41% in Central Java Province. Data from the Central Statistics Agency support this. In 2020, the harvest area of potato cultivation in Central Java Province reached 17,212 ha. So far, potatoes are cultivated in the highlands. Still, land expansion to increase potato productivity will be difficult considering the Government Regulation of the Republic of Indonesia No. 26 of 2008 concerning the National Spatial Plan, which regulates land use with a particular slope and elevation (State Gazette of the Republic of Indonesia No. 48 of 2008). The expansion of land in the highlands for potato cultivation will increase erosion, impacting environmental damage. Therefore, one alternative that can be done is to look for prospects and potentials in the medium plains, as the development of potato production areas aims to avoid increasingly severe environmental damage because the expansion of planting areas in the highlands is increasingly limited (Asgar, 2013). Medium land is an elevation (altitude) of 400-700 meters above sea level.

The transfer of potato cultivation from highlands to medium plains (480 masl) can cause other problems in potato productivity yield; It has been identified in the research of Basuki et al. (2009) that one of the main problems farmers face is low productivity. Low potato productivity due to the non-fulfillment of some ideal growing conditions for potatoes in medium plains. Efforts can be made by enriching nutrients that can stimulate potato growth to be more optimal, namely through the application of *Plant Growth Promoting Rhizobacteria* (PGPR). According to Tuhuteru et al. (2019), PGPR has a role in increasing both plant growth and production, which is thought to have something to do with PGPR's ability to synthesize growth hormones.

The application of PGPR in increasing crop production relies on the biological agent bacteria in it. These bacteria can spur significant growth through direct or indirect mechanisms. Ahmad and Kibret (2014) state that the tools are directly related to nitrogen fixation, phosphate dissolution, production of siderophores, phytohormones, and *1-aminocyclopropane-1-carboxylate deaminase*, while indirect mechanisms are associated with the production of antibiotics, hydrogen cyanide (HCN), and siderophores, competition of the growing environment, and the induction of systemic resistance. Several studies related to the application of PGPR to plants have been carried out, such as the study of Onikawijaya (2015) reported that variations in PGPR concentration were able to increase lettuce growth, research Syamsiah & Rayani (2014) also said that PGPR was able to increase plant height, number of fruits, and fresh weight of chili

plants, research Yazdani et al. (2009) PGPR can increase the growth and yield of corn seeds. Cahyani et al. (2018) also revealed that adding PGPR applications can increase potato growth and productivity. In addition to low productivity, according to Hamdani (2009), cultivating potato plants in medium plains experiences constraints against high temperatures, so disease attacks are increasing. Using biological agents to help suppress disease attacks is an alternative. Application of *Trichoderma sp.* applied to help fight the presence of pathogens. In their research, Nurahmi et al. (2012) mentioned that *Trichoderma sp.* They are included in delicious mushrooms. *Trichoderma sp.* is a saprophytic soil microorganism that will naturally attack pathogenic fungi to benefit plants. In its publication, Sudantha et al. (2011) also said that the activity of *Trichoderma sp.* Soil is a competitor both in space and nutrients and as a mycoparasite that can suppress the activity of soil infectious pathogens.

RESEARCH METHODS

Time and Place

This research was conducted in Gondowangi Village (480 masl), Sawangan District, Magelang Regency, Central Java Province, Biotechnology Laboratory, Faculty of Science and Mathematics, Diponegoro University, and Diponegoro University Integrated Laboratory. This research was conducted from October 2021 - May 2022.

Tools and Materials

The tools used are autoclaves, Petri dishes, test tubes, ose needles, microscopes, incubators, vortexes, micropipettes, magnetic stirrers, Arlen Meyers, centrifuges, PCR, Electrophoresis, Gel Doc, Heat Blocks, glass objects, silver plastic, hoes or soil processing tools, stirrers, fertilizer containers, measuring cups, electronic scales, meters, rulers, and name tags or markers, pH meters, mobile phones, stationery, and laptops. While the materials used in this study were Nutrient Agar powder, Potato Dextrose Agar powder, Triptic Soy Agar, Nutrient Broth, MyTag, Instagene Kit, Loading Dye, TAE Buffer, Agarose, Salkowski Solution, ddH2O, primer 1492R (GGTTACCTTG TTACGACTT), primer 27F(AGAGTTTTTGATYMTGGCTCAG), violet crystal solution, iodine solution, acetone solution, safranin solution, aquadest, 70% alcohol, immersion oil, soil media with pH 6, Commercial PGPR and Tricho Powder from the Central Java Biological Agent Laboratory, granola (G2) variant potato seeds, organic manure (crystal), rice husks, KCl fertilizer, Pearl fertilizer, Za fertilizer, TSP fertilizer, Phonska fertilizer, and water. This study was conducted with a Complete Randomized Design (RAL consisting of 6 treatments with 4 repeats. The treatment in this study included P0 (Farmer's Habit), P1 (PGPR 20mL), P2 (PGPR 40 mL), P3 (Tricho Powder 80gram/10Liter), P4 (Tricho Powder 150gram/10L), and P5 (PGPR 20mL+Tricho Powder 80gr/10L).

Potato cultivation

The growing medium consists of a soil mixture with a pH of 6 and essential fertilizer. Essential fertilizers consist of phonska (15kg), TSP (15kg), KCl (10kg), and Za

(10kg). After the planting medium is ready, it is covered using mulch and given a hole with a planting hole distance between seedlings, which is 60 x 60 cm, with 16 holes. After that, it is allowed to stand for a week. Planting potato seeds is carried out using seedling tubers placed in spots dug right in the middle with the position of the shoots facing up. PGPR for P1, P2, and P5 treatment is given on days 15, 25, and 35 HST. Tricho Powder for P3, P4, and P5 treatment was given on days 15, 25, and 35 HST.

The parameters observed include potato yields, including potato mass (grams) and number of tubers (fruit), and disease attacks measured in percentage of disease intensity. Crop yield measurement occurs when the plant has entered the harvest period. Disease intensity was observed on days 49, 56, and 63 HST, respectively. The data was analyzed using the fingerprint / ANOVA method at a 95% confidence level, then continued with further tests determined based on the KK (Coefficient of Diversity) value obtained.

Identification of One Type of Commercial PGPR Microorganisms

Characterization of Isolates

The commercial PGPR identification method aims to determine the most dominant and active types of bacteria in influencing plant growth; identification of PGPR bacteria is carried out through macroscopic and microscopic observations, followed by biochemical tests. The selected isolate results will be identified molecularly. First, the sample was isolated by dissolving 2mL of commercial PGPR into 100mL of aquadest, then stratified dilution to 10-4. Then grown on NA media and incubated at room temperature for 2-3 days. The isolates obtained are then purified in NA media and incubated at room temperature for 2-3 days. After the pure isolate grew, macroscopic observations were carried out and biochemical tests continued. Visible obsercommentsude colony shape, colony elevation, colony surface, colony size, colony diameter, and colony color. While biochemical tests in the form of gram staining and IAA qualitative tests.

Molecular Identification

Selected isolates (producing IAA hormone) were grown on 4 mL of NB (*Nutrient Broth*) media for 24 hours at room temperature. Bacterial identification begins with isolating DNA using methods from the InstaGene kit. A colony of 100 ìL of pure isolate was centrifuged for 1 minute at 13,000 rpm at 4°C. This process is carried out 4 times until the pellets obtained are sufficient. The supernatant was removed while pellets were added with an InstaGene kit of 200 ìL, then the solution was incubated using a heat block at 56ºC for 30 minutes and vortex at 100 rpm for 10 seconds. Then heat at 100ºC for 10 minutes. The final stage of DNA extraction is to separate DNA (supernatants) from other components (pellets) by centrifuge at 13,000 rpm for 3 minutes. They continued the process of PCR analysis, electrophoresis, and sequence amplification. Isolates that have successfully amplified their 16S rRNA gene can be seen to be related to other prokaryotes in the database based on their 16S-rRNA gene

sequences. The sequence is carried out in the 1st BASE Laboratory, and the partial sequence data obtained will go through 21 edits using the Bioedit program. After receiving contig data on nucleotide sequences based on amplification with universal primers, the homology will be compared with other prokaryotes in the database at Gene Bank. Then proceed to create a phylogenetic tree using the MEGA 10 program.

RESULTS AND DISCUSSION

Yields

In this study, potato harvesting was carried out when the plants were 70 HST; according to Gunadi et al. (2014), potatoes were ready to be harvested when the plants turned yellow and died. The potato harvest obtained was then analyzed by ANOVA followed by the BNT Test (Smallest Real Difference) presented in Table 1. Based on the results of the BNT test, treatment with the application of PGPR and Tricho Powder showed a significant effect on all potato yield parameters compared to treatment without PGPR and Tricho Powder.

This research was conducted on 480 masl lands with sunlight intensity, high enough temperature, and less loose ground. This affects the growth and yield of potato crops. High temperatures will impact tuber formation due to the imbalance of photo-synthesis results used for tuber formation. The photosynthesis results will be divided into its use as energy to carry out faster respiration, high temperatures, and its use for tuber formation. The P0 (Farmer's Habit) treatment gave minor results across all parameter components in this experiment. This is because potatoes, without the help of biological agents, are less able to survive in extreme conditions and under stress-prone environments that impact the growth and yield of potato crops. While the treatment of P0 (Farmer's Habits), P1 (PGPR 20mL), P2 (PGPR 40 mL), P3 (Tricho Powder 80 grams/10Liter), P4 (Tricho Powder 150gram/ 10L), and P5 (PGPR 20mL+ Tricho Powder 80gr/10L) gives high enough results for each parameter. This proves that the application of PGPR and Tricho Powder plays a role in increasing crop yields in the median land; supported by previous studies that did not

Table 1 BNT test results average yield

Treatment	Number of Tubers (tuber)	Mass Tuber (gr)
P0 (Farmer's Habit)	1.75a	34.5a
P1 (PGPR 20ml)	4b	93b
P2 (PGPR 40ml)	4.5b	94.75b
P3 (Tricho powder 80g/10L	4.5b	94b
P4 (Tricho powder 150g/10L)	4.5b	94.75b
P5 (PGPR 20ml + Tricho powder 80g/10L	5b	113.75b

Description: Numbers followed by different letters showed a marked difference in effect between treatments based on BNT follow-up tests.

use the application of PGPR and Tricho Powder, the results in this study are superior. Previous research by Sa'diyyah et al. (2017) conducted on median land with an altitude of 480 meters above sea level without the application of biological agents showed low yields when compared to products in treatment using PGPR and Tricho Powder applications (P1, P2, P3, P4, and P5). Similar results are also seen in the research of Sofiari et al. (2014) conducted on land with an altitude of 550 meters above sea level using the same potato varieties, namely granola without PGPR application and Tricho Powder, resulting in low yields as well. The behavior of P5 (PGPR 20mL + Tricho Powder 80gr/10L) is the best because it is the highest result in this study across all parameters.

In this study, PGPR provides adequate nutrients for plant growth and helps plants survive in extreme environments. According to Basu et al. (2021), the mechanism of PGPR to increase plant growth is classified into 2, n: direct and indirect. In this study, PGPR played a direct role through its ability to provide additional nutrients. This is to the opinion of Gouda et al. (2018) that the natural mechanism of PGPR activity is to increase plant nutrition through the provision of phytonutrients such as nitrogen or minerals that cannot be mutated from the soil (P, K, Zn, Fe, and other essential mineral nutrients) and stimulate plant growth and development by regulating phytohormone levels (auxins, cytokinins, gibberellins, abscisic acid, and ethylene).

One of the factors for the formation and development of tubers is the acceptable content of P and N elements in the soil. This study shows that the earth is less loose, supported by yields in P0 treatment (Farmer habits) which are very small. One of the abilities PGPR possesses is its ability to dissolve phosphate and nitrogen fixation. This ability can provide P and N elements that were not enough in the soil to be available and able to be used by plants for the formation and growth of tubers. Arora and Prakash (2019) stated that using the genus Bacillus sp., identified as content in PGPR products as phosphate solvent microbes, can increase crop production, one of which is rising tuber weight. In addition to the role of PGPR, Tricho Powder also takes a role in helping to provide adequate nutrients in the soil, especially element N. In Nurmansyah's research (2020), Trichoderma sp. acts as a biological agent that can remodel coarse compounds and provide N nutrients that encourage the formation of fruits and seeds. The study also proved that treatment with the addition of Trichoderma sp gave the highest results on the average mass of onion bulbs. Therefore, the treatment using PGPR and Tricho Powder (P1, P2, P3, P4, and P5) showed significantly different results from the non-application treatment across parameters, especially in the diameter and mass of tubers due to the ability of PGPR and Tricho Powder to provide additional elements.

In addition to providing nutrients, the direct mechanism of PGPR is the production of

phytohormones or growth substances (auxins, cytokinins, and gibberellins) and the induction of systemic resistance (Singh, 2018). These resulting growing substances or phytohormones are necessary for the initiation of tubers. In his research, Sembiring (2020) stated that one factor that encourages the formation of tubers is the presence of growth substance hormones in plants. Bacillus sp. has been identified as one of the components of PGPR products used, Nookaraju et al. (2017) said that the strain of Bacillus sp. can produce the hormone IAA (Indole-3-Acetic Acid), which has a role in helping plant growth in the vegetative phase including the formation and initiation of tubers. So the highest number of tubers was obtained in the treatment with the application of PGPR and Tricho Powder. The highest and best results were shown in the P5 treatment. Namely, the average number of tubers reached 5 per p.lant. Although the number of tubers produced in this study was higher than similar studies in the median land when compared to Fatchullah's (2016) research which carried out cultivation on land with an altitude of 1250 meters above sea level, it was still relatively low. The highest yield of the average number of tubers in this study was five tubers per plant, while in Fatchullah's research (2016), the lowest output of the average number of tubers reached 14.03 per plant. Mailangkay et al. (2012) revealed that the height of planting land can affect the number of harvested tubers. In addition to hormones and growing substances, tuber formation can also be affected by depth at the time of planting, seedling tuber size, land/soil moisture, nutrients, and temperature. In this case, temperature plays a vital role in tuber formation, which impacts the number of tubers. During the study, the temperature in the Sawangan area was relatively high and not ideal for tuber formation. The optimum temperature for the initiation process of tuber formation is 15°C-20°C; higher temperatures will delay until it inhibits tuber formation (Struik, 2007). This was also conveyed by Hancock et al. (2017) that high temperatures will suppress the expression of protein tuberization signals, named StSP6A, so that tuber formation becomes inhibited.

Disease intensity

In this study, the disease that attacked plants was Fusarium wilt, caused by Fusarium solani sp. Symptoms experienced by potato plants in the form of spots on the leaves; some leaves roll up, and some plants experience loss in the phase close to harvest. Observations related to disease attacks in this study were carried out when plants were 49 HST, 56 HST, and 63 HST. Based on the words in Figure 2. The frequency of disease increases with the age of plants, with the highest frequency found in the treatment of P0 (Farmer Habits), P1 (PGPR 20mL), and P4 (Tricho Powder 150gr/10L) by 75%. It can also be concluded that in the treatment of P0, P1, and P4, at least 1 plant is not attacked by disease from a total of 4 plant repeats.

Disease intensity was also observed when plants aged 49 HST, 56 HST, and 63 HST were calculated using the formula of disease attack intensity. The results showed that the power of disease attacks in this study

Table 2 Frequency of Potato Plant Disease at 49 , 56, and 63 Days After Planting (DAP)

Treatment	Frequency of disease (%)		
	49 DAP	56 DAP	63 DAP
P0 (Farmer's Habit)	75	75	75
P1 (PGPR 20ml)	25	75	75
P2 (PGPR 40ml)	0	50	75
P3 (Tricho powder 80g/10L	0	50	50
P4 (Tricho powder 150g/10L)	25	50	75
P5 (PGPR 20ml + Tricho powder 80g/10L	0	50	50

fluctuated. The highest disease intensity in the P0 treatment reached 56.25%, and P1 reached 31.25%. According to Halterman et al. (2008), the power of the disease <40% is classified as resistant to disease attacks, so the treatment of P1, P2, P3, P4, and P5 indicates that plants can still withstand disease attacks. Furthermore, the data obtained were further tested in the form of the BNT Test, and it was accepted that there was a significant influence between the addition of PGPR and Tricho Powder products compared to without the addition of effects. But it is known that each treatment between the acquisition of biological agents in the product does not show different results. Efforts are made to overcome Furaium wilt disease by utilizing Tricho Powder containing *Trichoderma sp.*

Trichoderma sp. It is a fungus that can naturally attack pathogenic fungi, benefiting plants. Some studies related to the use of Trichoderma sp. as a biocontrol agent have been widely done, for example, in the research of Wattimury et al. (2021) in tomato cultivation and able to reduce disease intensity by 33.3%, Fatima et al. (2015) in tomato and potato cultivation able to inhibit disease intensity by 86%, and Al-Mugharabi (2008) in potato plants reduced disease intensity by 61%. In this study, using Tricho Powder at doses of 80 gr, 150 gr, and a combination of PGPR and Tricho Powder reduced the average disease intensity by 37.5% and 43.75%, respectively. The best results are obtained by applying Tricho Powder at a dose of 150gr/10L. The decrease in the intensity of this disease indicates the application of both Tricho Powder and PGPR pathogenic disease attacks caused by Fusarium solani sp. Trichoderma sp. contained in the product can suppress pathogenicity in

Table 3 Potato Disease Intensity at 49 DAP, 56 DAP, and 63 DAP

Treatment	Disease intensity (%)			Total (%)	Aver age (%)	Descrip-tion
	49 DAP	56 DAP	63DAP			
P0 (Farmer's Habit)	31.25	43.75	56.25	131.25	43.8	Vulnerable
P1 (PGPR 20ml)	6.25	18.75	31.25	56.25	18.8	resistant
P2 (PGPR 40ml)	0	12.5	18.75	31.25	10.4	resistant
P3 (Tricho powder 80g/10L	0	12.5	18.75	31.25	10.4	resistant
P4 (Tricho powder 150g/10L)	6.25	12.5	18.75	37.5	12.5	resistant
P5 (PGPR 20ml + Tricho powder 80g/10L	0	12.5	12.5	25	8.3	resistant

Table 5 Disease Intensity (IP) BNT Test

Treatment	AverageIP (Data Transformation)
P0 (Farmer's Habit)	43.8b
P1 (PGPR 20ml)	18.8a
P2 (PGPR 40ml)	10.4a
P3 (Tricho powder 80g/10L	10.4a
P4 (Tricho powder 150g/10L)	12.5a
P5 (PGPR 20ml + Tricho powder 80g/10L	8.3a

Description: Numbers followed by different letters showed a marked difference in effect between treatments based on BNT follow-up tests.

several ways, such as antibiotics, nutrient competition, inactivation of pathogenic enzymes, induction of plant resistance, and microparasites. A possible mechanism that occurs is Trichoderma sp. induces systemic plant resistance. There are two ways Trichoderma sp. in generating opposition to plants, namely by producing pathogen-related (PR) proteins directly and phytoalexin compounds as a result of pathogenic micro-organisms attack and other ways of making cellulase enzyme components consisting of exoglucanase enzymes (-1.4glycohydrolase), and cellobiose (-glucosidase) where these two enzymes can damage the cell walls of pathogenic fungi directly. In addition to the induction of plant resistance, the role of Trichoderma sp. Suppressing pathogenicity is by reducing or inhibiting the mycelium growth of pathogenic fungi. Gomez and Rojas (2021) state that Trichoderma sp. is a fungus that is antagonistic and can suppress mycelium growth by fighting over the nutrients the host plant provides.

Identification

The results of isolation from PGPR products with a dilution level of 10-4 grown on NA (Nutrient Agar) media obtained 5 isolates of bacteria that have different macroscopic morphology of, bacteria can be seen in Figure 1. Visible observations include colony shape, colony surface, colony periphery, colony color, colony diameter, and colony size. Identification of PGPR bacteria is then continued through gram staining. Isolates

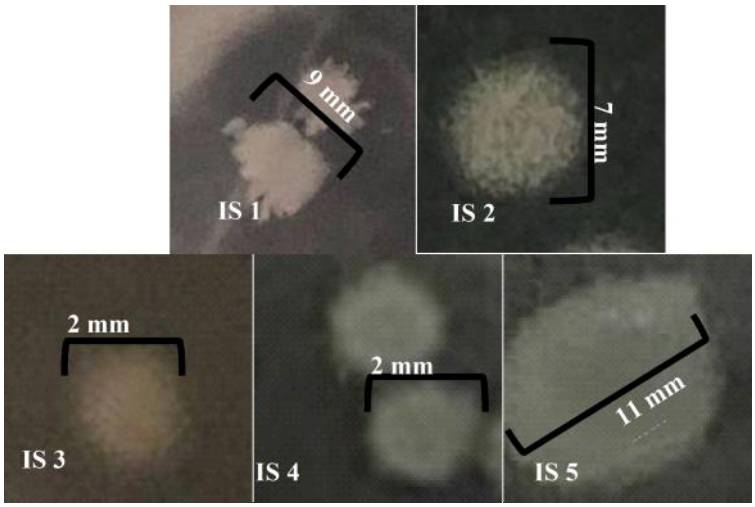

Figure 1 Results of macroscopic observations of PGPR isolated isolates

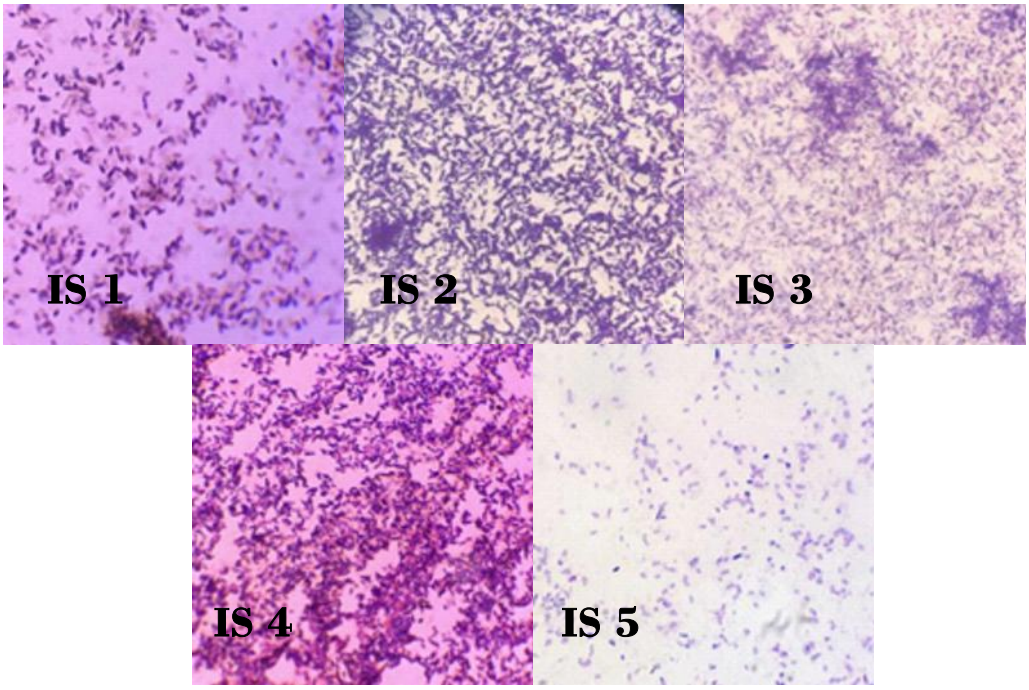

Figure 6 Gram staining results

Description: The staining of 5 isolates showed a positive gram result in bacilli observed at a magnification of 1000x.

were observed using a microscope with a magnification of 1000x (ocular lens 10x and objective lens 100x). Based on the gram staining results, the 5 bacterial isolates obtained showed similar results, namely gram-positive and bacilli-shaped. The results of isolate characterization can be seen in Table 5.

One of the characteristics of PGPR bacteria is their ability to produce IAA (Indole-3-Acetic Acid). The IAA qualitative test is carried out as one of the biochemical tests to determine isolates that can produce IAA. Based on the results that can be seen in Figure 3, only 1 isolate (IS 5) was obtained that gave a positive impact with a marked change in color to pink after dripping the Salkowski solution. According to Mohite (2013), this indicates the production of IAA produced by IS 5 due to the interaction between IAA and Fe to form a complex compound [Fe2 (OH) 2 (IA) 4]. Thus IS 5 can produce the hormone IAA. IS 5 was the

Table 5 Characterization Results of 5 Isolates from PGPR Isolation

Characteristic	IS 1	IS 2	IS 3	IS 4	IS 5
Colony Form	Irregular	Circular	Circular	Circular	Circular
Colony surface	Flat	Flat	Flat	Convex	Convex
Suburbs of the colony	Lobate	Lobate	Lobate	Smooth	Smooth
Colony color	Cream	Grayish-white	Cream	Milky White	Milky White
Colony Size	Moderate	Moderate	Small	Small	Moderate
Colony diameter	9 mm	7 mm	2 mm	2 mm	11 mm
Gram	+	+	+	+	+
Cell Shape	Bacil	Bacil	Bacil	Bacil	Bacil
Uji IAA	-	-	-	-	+

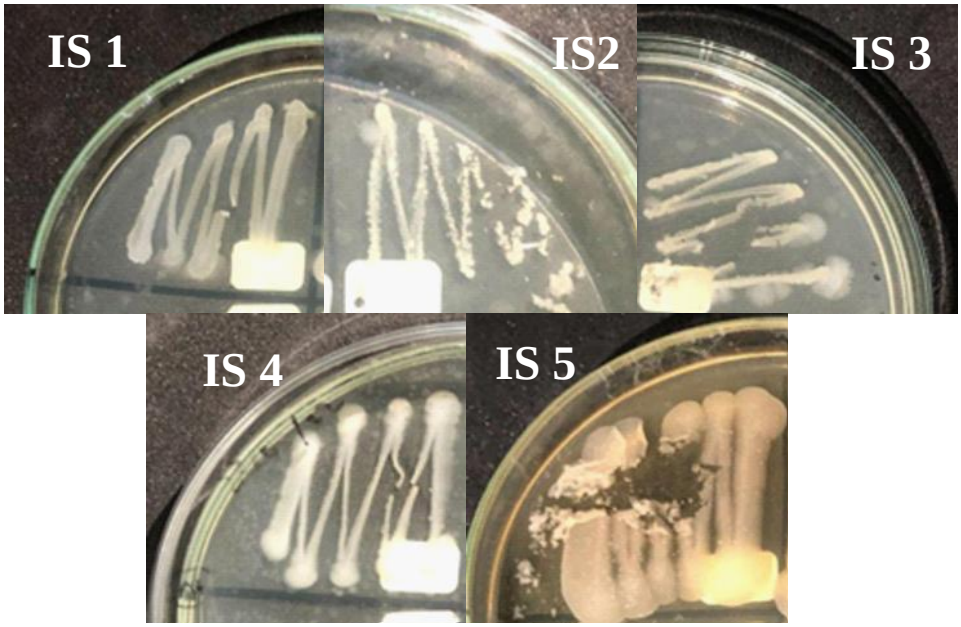

Figure 3 IAA Qualitative Test Results
Description: A positive result is characterized by a change in isolate color from white to pink after dripping Salkowski's solution. A positive impact is shown in IS 5.

only isolate that showed positive IAA test results, then continued molecular identification.

Based on molecular identification of consensus results obtained later in BLAST at NCBI, and results that are 5 is a genus of Bacillus sp can be seen in Figure 9. This is in line with the statement of Beneduzi et al. (2012) that the genus Bacillus is one of the bacterial genera identified as PGPR bacteria, added to the opinion of Kunar et al. (2011), which states that of several other PGPR bacterial genera, the genus Bacillus is the genus that is most often studied and used as PGPR because of its ability to act as biofertilizer. The BLAST results also showed that IS 5 resembles Bacillus velezensis and Bacillus subtillis. Bacillus velezensis strain FZB42 16S ribosomal RNA, a complete sequence, which is the highest result of BLAST, comes from the study of Chen et al. (2007) with samples of B. amyloliquefaciens FZB42, whose role is similar in this study is as PGPR.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Bacillus velezensis strain FZB42 16S ribosomal RNA, complete sequence	Bacillus velezensis	1029	1029	98%	0.0	84.01%	1550	NR_075005.2
Bacillus subtilis subsp. subtilis strain 168 16S ribosomal RNA, complete sequence	Bacillus subtilis subsp. subtilis	1029	1029	98%	0.0	84.01%	1550	NR_102783.2
Bacillus subtilis strain DSM 10 16S ribosomal RNA, partial sequence	Bacillus subtilis	1029	1029	98%	0.0	84.01%	1517	NR_027552.1
Bacillus subtilis strain JCM 1465 16S ribosomal RNA, partial sequence	Bacillus subtilis	1029	1029	98%	0.0	84.01%	1472	NR_113265.1
Bacillus subtilis strain SBMP4 16S ribosomal RNA, partial sequence	Bacillus subtilis	1029	1029	98%	0.0	84.01%	1463	NR_118383.1
Bacillus spizizenii strain NBRC 101239 16S ribosomal RNA, partial sequence	Bacillus spizizenii	1029	1029	98%	0.0	84.01%	1475	NR_112686.1
Bacillus subtilis strain NBRC 13719 16S ribosomal RNA, partial sequence	Bacillus subtilis	1029	1029	98%	0.0	84.01%	1475	NR_112629.1
Bacillus vallismortis strain NBRC 101236 16S ribosomal RNA, partial sequence	Bacillus vallismortis	1029	1029	98%	0.0	84.01%	1475	NR_113994.1
Bacillus vallismortis strain DSM 11031 16S ribosomal RNA, partial sequence	Bacillus vallismortis	1029	1029	98%	0.0	84.01%	1530	NR_024696.1
Bacillus velezensis strain CBMB205 16S ribosomal RNA, partial sequence	Bacillus velezensis	1029	1029	98%	0.0	84.01%	1445	NR_116240.1

Figure 4 BLAST consensus results on NCBI

After obtaining BLAST results, proceed with phylogenetic analysis using the MEGA X application to determine the closest kinship of isolate 5. The results of phylogenetic analysis (Figure 5) obtained IS 5 has the most immediate kinship with the species *Bacillus subtilis strain SBMP4*, characterized by having the shortest branch length compared to the species Bacillus sp. Others and bootstrap values reach 100%. According to Rosdiani et al. (2013), if the bootstrap value is between 70 to 100, the chance of changing the arrangement of branches in the cladogram formed is meager. Subari et al. (2021) also added that the phylogeny tree could be trusted if the bootstrap value reaches 90% and cannot be charged if the bootstrap value is 20%. Therefore, based on the results obtained by IS 5 with the Bacillus subtilis strain, SBMP4 shows a very close kinship supported by very high branch strength, so the possibility of branch change is very low.

Based on the results of BLAST and phylogenetic analysis, it can be concluded that IS 5 is a genus of Bacillus sp that has the closest relationship with *Bacillus subtilis and Bacillus amyloliquefaciens*. As PGPR, *Bacillus subtilis* plays a role in inducing disease resistance against the CMV virus (Zhender et al., 2000), increasing plant productivity (Gupta et al., 2017), systemic induction (Khabbaz et al., 2014), production of secondary metabolite compounds and antibiotics to control bacterial wilt (Singh et al., 2016), and has a mechanism as a

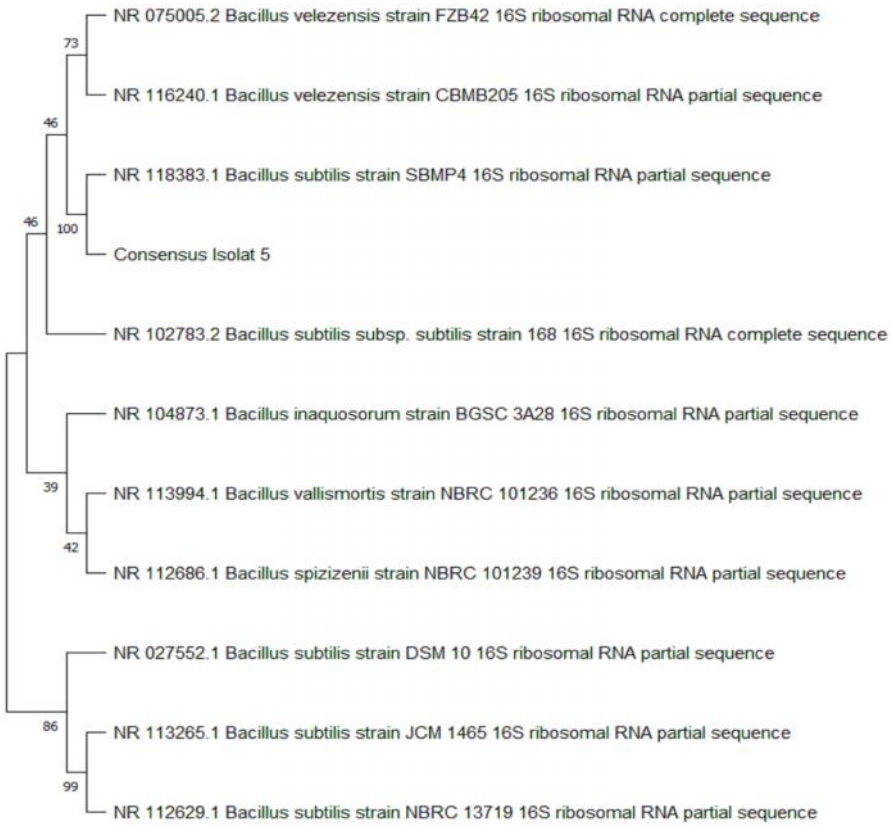

Figure 5 Phylogenetic Analysis Results of selected isolates IS 5

biocontrol to fight pathogenic compounds (Elbeshehy et al., 2016). Bacillus amyloliquefaciens as PGPR plays a role in mitigating resistance to toxic substances (Zafar-Ul-Hye et al., 2020), improving plant quality by preventing bacterial wilt and decay (Chowdhury et al., 2013), and increasing plant productivity (Zhender et al., 2000).

CONCLUSION

To obtain maximum results, potato cultivation in medium land can be done by combining PGPR products (20mL) with Tricho Powder (80gr/10L). In addition, applying differences in Tricho Powder concentrations can suppress disease attacks in potato cultivation on medium land by 37.5% in Fusarium wilt disease. The components that contain PGPR Commercial products are the genus Bacillus which is related to Bacillus velezensis and characterized by its ability to produce IAA compounds.

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