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Journal of Agricultural Science
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Alteration of the Rhizosphere Bacteria Community Respond Differently to Plant Growth Promoting Rhizobacteria in Peanut Soil's Poteran Island

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ARTICLE INFO

Keywords:
Bacteria community
Biofertilizer
Plant soil's response
Poteran rhizosphere

Article History:
Received: September 28, 2022
Accepted: September 1, 2024

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) has been detailed to affect soil microbial exercises or community composition. There is a lack of information on the degree to which PGPR as a biofertilizer concurrently influences the action, estimate, and composition of the soil microbial community. This research investigated the impacts of the timing and frequency of PGPR application as biofertilizers on the rhizosphere bacteria community in arable soil. We compared four treatments in an experimental field site, namely: one-time treatment of PGPR at the beginning of planting (P1), twice treatment of PGPR (P2), 15-days of planting (DP), and 30-DP (P3); and without treatment of PGPR (WP). A total rhizosphere bacteria community fingerprint was surveyed using ribosomal intergenic spacer analysis (RISA) using a culture-dependent and culture-independent approach. The rhizosphere bacteria community was surveyed during 80 DP. The unweighted pair-group method with arithmetic mean (UPGMA) clustering showed that the rhizosphere bacteria community in three-frequency applications of PGPR was more abundant than without PGPR application and one or two PGPR applications. This study revealed that the rhizosphere bacteria community was increased in soil with PGPR application, especially in P3, three dosages of PGPR application. Increasing the rhizosphere bacteria community could strongly influence the clay foam soil nutrient.

INTRODUCTION

Poteran Island is a little island in East Java, Indonesia, and one of the clusters of Sumenep Rule. This island is located southeast of Madura Island. Its zone is 49.8 km^2, generally agrarian, and arrives without a waterway. The flow paths of surface water on this island are very short. These are causing the potential capacity of water within the ground to get smaller due to restricted revive. In Poteran, the typical temperature ranges from 27ºC to 29.6ºC, with a humidity of 70% and 86%. Air pressure is 1000 mbar with wind speed range of 5.556 km/h to 16.668 km/h (Tatas et al., 2015).

Soil is a critical part of fruitful horticulture and is the first source of the supplements that are utilized to develop crops (Anka, 2021). Poteran Island's soil surface is overwhelmingly a clay loam based on the United States Department of Agriculture (USDA) classification, with an inexact rate of 34% sand, 29% silt, and 37% clay. This condition is a primary concern for agricultural growth. Clay loam is composed mainly of clay rather than other types of minerals or rock. A loam is named for soil, a mixture of the soil type in the most significant proportions. The normal clay soil has exceptionally few particles

ISSN: 0126-0537

Cite this as: Ekawati, I., Wati, H. D., Koentjoro, M. P., Sudarwati, H., Isdiantoni, Prasetyo, E. N. (2024). Alteration of the rhizosphere bacteria community respond differently to plant growth promoting rhizobacteria in peanut soil's poteran island. *AGRIVITA Journal of Agricultural Science, 46*(3), 590-601. http://doi.org/10.17503/agrivita.v46i3.3947

and contains an incredible bargain of clay, which tends to be overwhelming since they are so thick (Kumari & Mohan, 2021). This condition causes swells to retain water when it's very wet. When conditions are dry, the clay solidifies, shrinks, and forms thick clouds and cracks on the soil surface. Therefore, this soil type can be difficult for farmers since it can stunt plant growth.

Dębska et al. (2016), Demir (2020), and South et al. (2021) reported that clay loam can be improved to be a very good growing medium by application of bio-fertilizer, which consists of plant growth-promoting rhizobacteria (PGPR). PGPR were included *Azotobacter chroococum*, *Azospirillum lipoferum*, *Bacillus polymyxa*, *Azotobacter vinelandii*, *Rhizobium leguminosarum*, *Pseudomonas* spp., *Pseudomonas fluorescens*, *Caballeronia*, *Pseudarthrobacter*, *Raoultella*, *Pseudomonas*, *Curtobacterium*, *Herbaspirillum*, *Pantoea*, *Ochrobactrum*, *Proteus vulgaris* and *Microbacterium* (Bhattacharyya et al., 2018; Iqbal et al., 2012; South et al., 2021). Applying PGPR and organic matter over time could reduce the effect of clay loam and increase soil organic matter status and carbon sequestration. The physical and chemical properties of clay loam cause applied nutrients to become less available. Consequently, frequent PGPR fertilization is required (Sedri et al., 2022). In addition, PGPR can increase plant performance by accelerating plant-microbe interactions (Pereira et al., 2020).

Maintenance of a bacteria abundance and community composition, especially in the rhizosphere, have a crucial effect on plant growth and agricultural sustainability. PGPR can increase plant performance by accelerating plant-microbe interactions (Piromyou et al., 2013). Fracchia et al. (2011) reported that the presence of *P. fluorescens* 92 did not affect the resident soil and biosolid eubacterial population. Fomina & Skorochod (2020) and Li et al. (2020) demonstrated that community composition analysis in the clay loam soil, consisting of 32% clay, 36% silt, and 32% sand, correlated consistently with soil moisture, total carbon, and total nitrogen content. However, how PGPR amendments modify the composition of soil microbial communities in clay foam has not been clarified.

Therefore, this research aims to investigate the long-term impacts of PGPR inoculants as a bio-fertilizer in the composition of the clay foam rhizosphere bacteria community. This study investigates how to enhance soil health using partner crops, cover crops, catch crops, and zero-till and imported organic resources. The composition of the overall rhizosphere bacteria community was assessed by ribosomal intergenic spacer region analysis (RISA). In this study, two strategies were used for RISA, namely, culture-dependent and independent approaches. As previously study Joko et al. (2012), this study confirms that RISA is a significant tool for monitoring the rhizosphere bacteria communities based on the detection of the variation in size of the intergenic transcribed spacer (ITS) region between the bacterial 16S and 23S rRNA genes, namely intergenic spacer (IGS) region. This region is amplified by the Polymerase Chain Reaction (PCR) process (Pereira et al., 2020).

The objective of this consideration was to investigate the impacts of timing and frequency of PGPR application as biofertilizers on rhizosphere bacteria community in arable soil.

MATERIALS AND METHODS

Experimental Site, Plots, and Design

The study was conducted from January to July 2019 in the field with lowland dry land in Talango village, Talango district, Sumenep regency, East Java, Indonesia. Subtropical monsoons with abundant sunshine and rainwater control the climate of the experimental site. The annual average temperature is 29ºC, and the highest and lowest temperatures are 37.8 and 26.7ºC. The soil in the experimental site, which is classified as Mediterranean soil type (locally "red soil") contains 1.51% organic matter, CEC 0.219 cmol/kg soil, N-total content 0.12%, P-Olsen content 3.16 mg/kg, and Potassium 0.0018 mol/kg soil. The site has a dry climate with less than 3 consecutive wet months.

In the study, the application of PGPR was conducted using different methods, which included both single and combined applications. The experimental design involved 16 plots, each measuring 8 meters by 8 meters, where peanuts were planted in a corn-peanut-cassava intercropping system. These plots were divided into four groups to which different PGPR treatments were applied. The PGPR used in the experiment consisted of *Pseudomonas fluorescens* and *Bacillus polymyxa*, sourced from the Laboratory of Plant Disease and Pest Management in Pamekasan

District, Indonesia. Four PGPR treatment groups were established, with four replicates, as presented in Table 1. These treatments were applied based on a randomized complete block design (RCBD) with four replications. The application of P1, P2, P3, and WP treatments was randomized within each replication. The study also involved measuring the pH of the soil at each site. Additionally, the geographical location of the study area and the schematic representation of the experimental field design are presented in Fig. 1. Overall, the study utilized both single (P1) and combined (P2 and P3) applications of PGPR, along with untreated controls (WP), to investigate their effects on the growth of peanuts in an intercropping system.

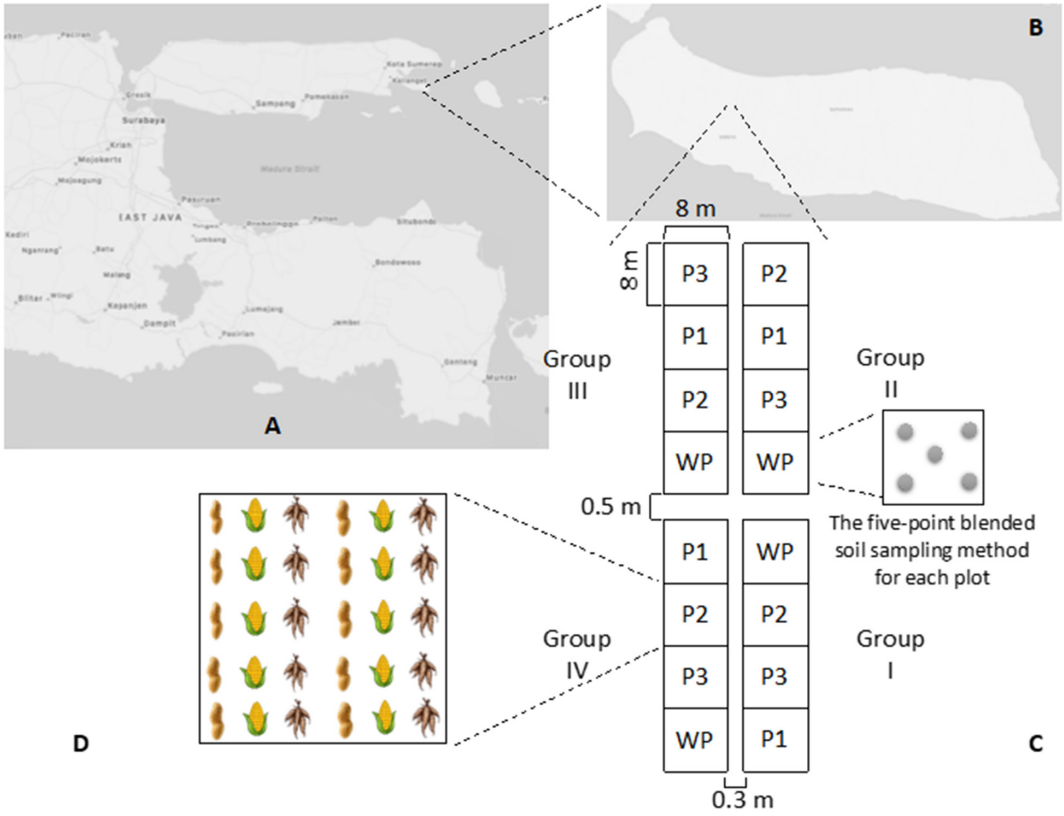

Fig. 1. Map of location of the studied area in East Java (A); Poteran Island, Indonesia (B); Experimental plot layout (C) consist of 16 plots which are divided into 4 groups. Each plot was by 8 x 8 meter. WP: without PGPR application, P1: PGPR addition (once time), P2: PGPR addition (twice at beginning of plant and 15 days of planting), and P3: PGPR addition (three time, first at beginning of plant, second time at 15 days of planting, and third time at 30 days of planting). Soil was collected from root in 20 cm far from the center of plant stem. (D) A plot arrangement within the study

Table 1. The concentration or doses of PGPR were applied in the study

Treatment	Application Frequency	PGPR Type
WP (Control)	Not applicable	None
P1	Once	*Pseudomonas fluorescens, Bacillus polymyxa*
P2	Twice (at planting, 15 days)	*Pseudomonas fluorescens, Bacillus polymyxa*
P3	Thrice (at planting, 15 days, 30 days)	*Pseudomonas fluorescens, Bacillus polymyxa*

Soil Sampling and Sample Collection

Soil was collected from the surface area around the peanut roots when the plants were 80 days old at a depth of approximately 10-15 cm. Five samples were obtained from each replicate plot using corers, with each sample weighing approximately 25 grams and reaching a depth of 0–5 centimeters. These samples were subsequently placed into Sterile Medi Pouch OneMed® to maintain their integrity. After collection, the samples were pooled together to reduce within-plot variation. They were then transported to the research facility and stored at 4ºC for later processing. The soil samples were utilized for both culture-dependent and independent analyses.

Rhizosphere Bacteria Community by Culture-Independent Approach and DNA Extraction

One gram manifold soil sample was subjected to direct lysis using liquid nitrogen, followed by procedures using the cetyl trimethyl ammonium bromide (CTAB) method. All soil samples were measured with a minimum of duplicates. Then, the sample that had been ground was transferred to a sterile tube. In each sample, as much as 1 g soil was mixed with 1 ml of lysis buffer (0.2 MTris-HCl pH 8.0; 0.02 M Na_2EDTA pH 8.0; 5 M NaCl; 10% SDS; 10% CTAB (Sigma Aldrich, Cat. 219374)) in centrifuge tubes and incubated at 56°C for 1 hour. Every 10 minutes, centrifuge tubes were stirred. The supernatant fluid was centrifugated at 10,000 rpm for 15 minutes. Further, the supernatant was displaced into a new sterile tube and added with Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v) followed by centrifugation at 10,000 rpm. The supernatant was transferred to a new tube and added with PCI (25:24:1, v/v), followed by centrifugation at 10,000 rpm. The aqueous lining of PCI was precipitated using 1/10 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of ethanol. The pellet was then recovered by centrifugation at 10,000 rpm and dried. Twenty microliters of TE buffer were used to dissolve the pellet.

RISA was amplified in a final volume of 25 µl of total DNA by using primers S926f (5' -CTYAAAKGAATTGACGG-3') and L189r (5' -TACTGAGATGYTTMAR-TTC-3'). Primer was annealed to positions 910 to 926 of the 16S rRNA gene and positions 189 to 207 of the 23S rRNA gene (*Escherichia coli* numbering), respectively. The reaction mixture contained 1 µl of the purified genomic DNA, 1 µl of each primer, and 12.5 µl GoTaq® Green master mix (Promega, USA) in an add up to the volume of 25 µl. The PCR temperature cycling condition consisted of an initial denaturing step at 95°C for 2 minutes, followed by 30 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 7°C and a final extension step at 72°C for 2 minutes (MyCycler, Bio-Rad) and then cooled and held at 4°C. Samples were run on a 2% agarose gel recolored with Ethidium Bromide (EtBr) (Thermofisher, Cat. 17896) and visualized under a UV light for the presence of amplified products. According to Winand et al. (2020), the variability of the IGS length was present in the resulting PCR.

Rhizosphere Bacteria Community by Culture-Dependent Approach

For the culture-dependent approach, two solid culture media were evaluated: nutrient agar (NA) (Merck, Germany) and Soil Extract Agar (SEA) Media. NA is a common culture media of low—or high-nutrient substances. SEA is media customarily designed to isolate bacteria from soil. The soil samples were cultures with a minimum of duplicates.

The SEA media was prepared by autoclaving 2,200 g of air-dried soil in 1.1 l of deionized water at 121°C for 15 minutes. Suspended soil was left to settle, and the top part was centrifuged at 4,000 rpm for 10 minutes at room temperature. The supernatant was filtered through Whatman™ paper No. 42 (Sigma Aldrich, Cat. WHA1442042). This process produces approximately 1 l of liquid and 1 l of the supernatant (TSE). SEA media was supplemented with 0.55 g K_2HPO_4, 0.11 g dextrose, and 1.8% agar (1 l of liquid soil extract with a final pH of 6.8).

Rhizobacteria were isolated by suspending up to 5 g soil collected from each sample in 45 ml sterile phosphate-buffered saline (PBS) and then agitated for 30 minutes at 28°C on a rotary shaker (250 rpm). The cultural conditions were selected based on their capacity to support the growth of the most abundant bacteria communities. To maintain cultural bacteria, an aliquot (100 µl) of each of four gradient dilutions (10^{-3}, 10^{-4}, $10^{-5,}$ and 10^{-6}) was incubated on Nutrient Broth (NB) (Sigma Aldrich, Cat. 70149) in the dark for 5 days at room temperature.

Next, 100 µl of each dilution of four soil samples by each of the two media was dispersed

onto four NA and SEA plates. Each medium was cultivated at 37°C for 2 weeks. After incubation, all bacterial colonies were numbered, and agent colony numbers from each medium and genotype were picked based on the colony's abundance and characteristic morphological criteria. Chosen colonies were purified by triple serial colony isolation within the same medium. Colonies in each Petri dish were scraped in Phosphate Buffer Saline (PBS) for DNA extraction.

DNA was extracted from 24 bacteria in Petri using the Promega Wizard® Genomic DNA Purification Kit (Promega, Germany). DNA quality and integrity were performed through electrophoresis on 0.8% agarose gel. The DNA samples were subjected to RISA analysis based on the bacterial community using a culture-independent approach.

Ribosomal Intergenic Spacer Analysis (RISA)

PCR products from DNA amplification were run in 2% agarose gel. Electrophoresis was carried out at 50V for 2 hours 1X TAE buffer (48.5 g/l tris; 11.4 ml/l glacial acetic acid; 20 ml/l 0.5 M EDTA (pH 8.0). The size of the product was estimated using 100 bp molecular weight marker φX174-Hinc II digest (Takara Bio). Gel was stained with Ethidium Bromide, viewed with an ultraviolet transilluminator, and recorded with a camera. Consequently, bands were identified from advanced pictures of the gel utilizing TI UV Transilluminator Avegene.

Data Analysis

To determine the effects of the addition of different PGPR in bacterial composition and community-level, data of independent and dependent-culture approach were subjected to analysis of relationship by clustering of correlation coefficients, namely unweighted pair group method with arithmetic means (UPGMA) (Tsai et al., 2019).

RESULTS AND DISCUSSION

This study investigated bacterial communities in the rhizosphere and employed a culture-independent and culture-dependent approach. The rhizosphere is a soil zone influenced by roots and a sufficient environment for diverse bacterial populations. The culture-independent approach was used to investigate bacteria that were unable to grow in culture. Hence, we compared and evaluated both methods to monitor the effect of timing and frequency of PGPR application as biofertilizers.

Rhizosphere Bacteria Community by Culture-Independent Approach

In the culture-independent approach, total community DNA was directly extracted from soil samples at the small-scale level. The DNA obtained was processed in PCR using a simplified ribosomal intergenic spacer analysis (RISA) technique. The amplified products were initially checked by electrophoresis in 1.5% agarose gels. Fig. 2 shows the banding profiles of the rhizosphere bacteria community obtained by RISA from the culture-independent approach. Each of the RISA band's patterns was expected to have a rhizosphere bacteria community. Many DNA bands depended on the rhizosphere bacteria community in the soil samples. The thick bands were expected to represent dominant community bacterial members. Conversely, the thin bands were expected to represent less dominant community bacterial members. In Fig. 2, the size of the DNA bands in agarose gels is different. This indicates that the spacers within the intergenic spacer (IGS) region exhibit significantly more heterogeneity in length and nucleotide sequence than the flanking 16S and 23S ribosomal genes. This fragment length is in the range of 100-1000 bp.

Effects dosage and frequency of PGRP application as biofertilizers on total bacterial communities in soil were analyzed by the total band that appeared in agarose gel and were estimated for the rhizosphere bacteria communities using the UPGMA algorithm and shown in Fig. 3. The clustering analysis appeared in two main groups: the first group consists of the inoculum and a sample collected 24 h afterward, whereas the second cluster contains the remaining samples. The second group was differentiated depending on the approach used. The most elevated bootstrap values were supposed for the tree obtained by the RISA method, while the most reduced values were for the tree built utilizing RISA.

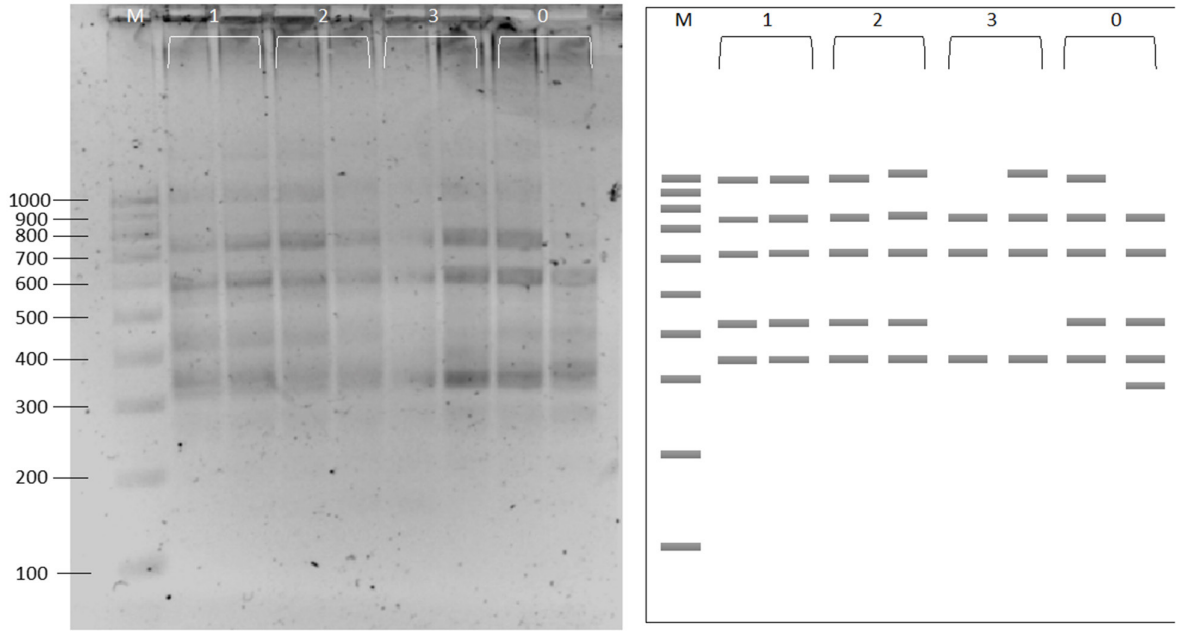

Fig. 2. RISA band profiles of fragments of 16SrDNA amplified using the total genomic DNA extracted from soil samples. The codes above in each lane indicate the names of samples, namely Lane 1, Once time PGPR Application (P1); Lane 2, Two-time PGPR Application (P2); Lane 3, Three-time PGPR Application (P3); Lane 0, Without PGPR (WP); Lane M, 100 bp molecular weight marker φX174-Hinc II digest (Takara Bio); B. Representative gel showing Ribosomal Intergenic Spacer Analysis (RISA) profiles of the amplified ITS gene from 300-1057 bp

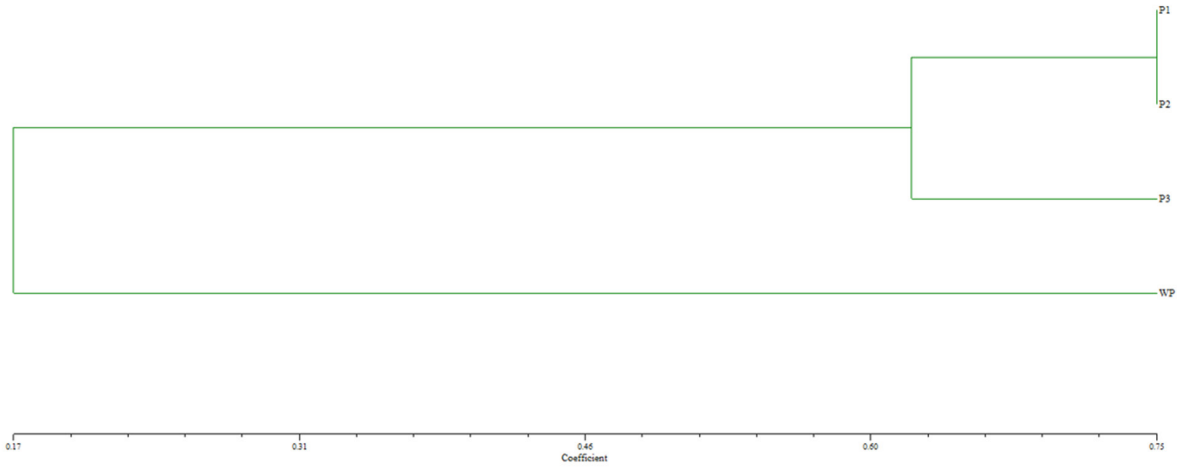

Fig. 3. UPGMA cluster analysis of bacterial RISA patterns of the total genomic DNA extracted from soil samples (culture-independent approach)

Rhizosphere Bacteria Community by Culture-Dependent Approach

In the culture-dependent approach, rhizosphere bacterial community composition was conducted to reveal the culturable bacterial diversity. Table 2 shows the rhizosphere bacterial populations in NA and SEA medium. Bacterial colonies that grew on each medium were estimated for the bacterial population as unit CFU (colony forming units) ranged from 23.97 to 38.05 x 10^6 for NA and 22.10 to 39.86 x 10^5 for SEA medium. Results for cultures revealed that the application of PGPR increased the total number of rhizobacteria. Results also showed that three-time PGPR application enhanced the growth of rhizobacteria. PGPR applications in more than single inoculations stimulated and increased the number of rhizosphere bacterial populations.

Table 2. Rhizobacteria population in the rhizosphere on NA and Soil Extract Agar with different frequency of PGPR application

Treatment	Nutrient Agar (CFU/g)	Soil Extract Agar (CFU/g)
WP (Control)	23.97 x $10^{6\ a}$	22.10 x $10^{5\ a}$
P1	30.49 x $10^{6\ b}$	26.60 x $10^{5\ ab}$
P2	34.98 x $10^{6\ bc}$	26.60 x $10^{5\ ab}$
P3	38.05 x 10^6	39.86 x 10^{5c}

Remarks: Numbers followed by the same letter set inside column are not essentially distinctive at P >0.05 level

In Table 2, even though the WP treatment received no PGPR application, it has some bacterial diversity. This research implies that various environmental conditions and microbial communities influence the soil microbiota. A correlation study demonstrated bacterial diversity in the WP treatment was related to soil pH, moisture level, and organic matter concentration. These associations show the complex interaction between soil features and microbial diversity, implying that farming practices and soil characteristics play critical roles in determining microbial community structure. In this study, the farming conditions on Poteran Island were characterized by a dry environment with no more than three consecutive wet months. This meteorological situation most likely affected soil moisture levels and nutrient availability.

The bacterial isolates were used for further study. To identify differences in PGPR application in the rhizosphere bacteria community, this study used rapid estimation by a RISA. Fig. 4 shows the banding profiles of the bacterial community obtained by settling the ribosomal intergenic spacer region in 2% agarose gel. The total band numbers differ depending on the bacterial culture media. The numbers of lanes show the RISA's fragments in the range between 79 and 1000 were observed. Several DNA fragments and thick DNA bands were present in the SEA medium rather than the NA medium. Each band represents a member of the rhizosphere bacteria community. In addition, each line contains a number of DNA bands clustered by the UPGMA algorithm, as shown in Fig. 5

Clustering analysis based on IGS showed two main groups (Fig. 5A) from NA culture; the first group was comprised of soil without PGRP (WP), and the second group was soils with PGPR application (P1, P2, P3). In the second group, the three-time PGPR application (P3) has the highest bootstrap values out of clusters P1 and P2 (significant correlation 0.68). The highest bootstrap was observed for the tree obtained by the RISA method, while the least for the tree built utilizing RISA. This dendrogram indicated that the closeness of bacterial community concerning the PGPR application dosage is one (P1) and two PGPR applications (P2).

UPGMA analysis from SEA media (Fig. 5B) shows two groups with significant correlation (0.7): P1 as an outlier and the second group consisting of P2, P3, and WP. P2 and P3 have a coefficient similarity of 0.75. This revealed that the bacterial community in P2 and P3 is similar.

A low diversity was observed in the culture-dependent and culture-independent approach within soil without PGPR application (WP), and differences were observed in the dosage of PGPR application (P1, P2, P3). It is generally believed that PGPR application in soil was positively correlated with the composition of rhizosphere bacteria communities (Pereira et al., 2020). In this work, PGPR was applied to clay foam soil, and at the end of the experiment, rhizosphere bacteria communities were available. This indicated that PGPR could adhere to soil and be involved in the rhizosphere.

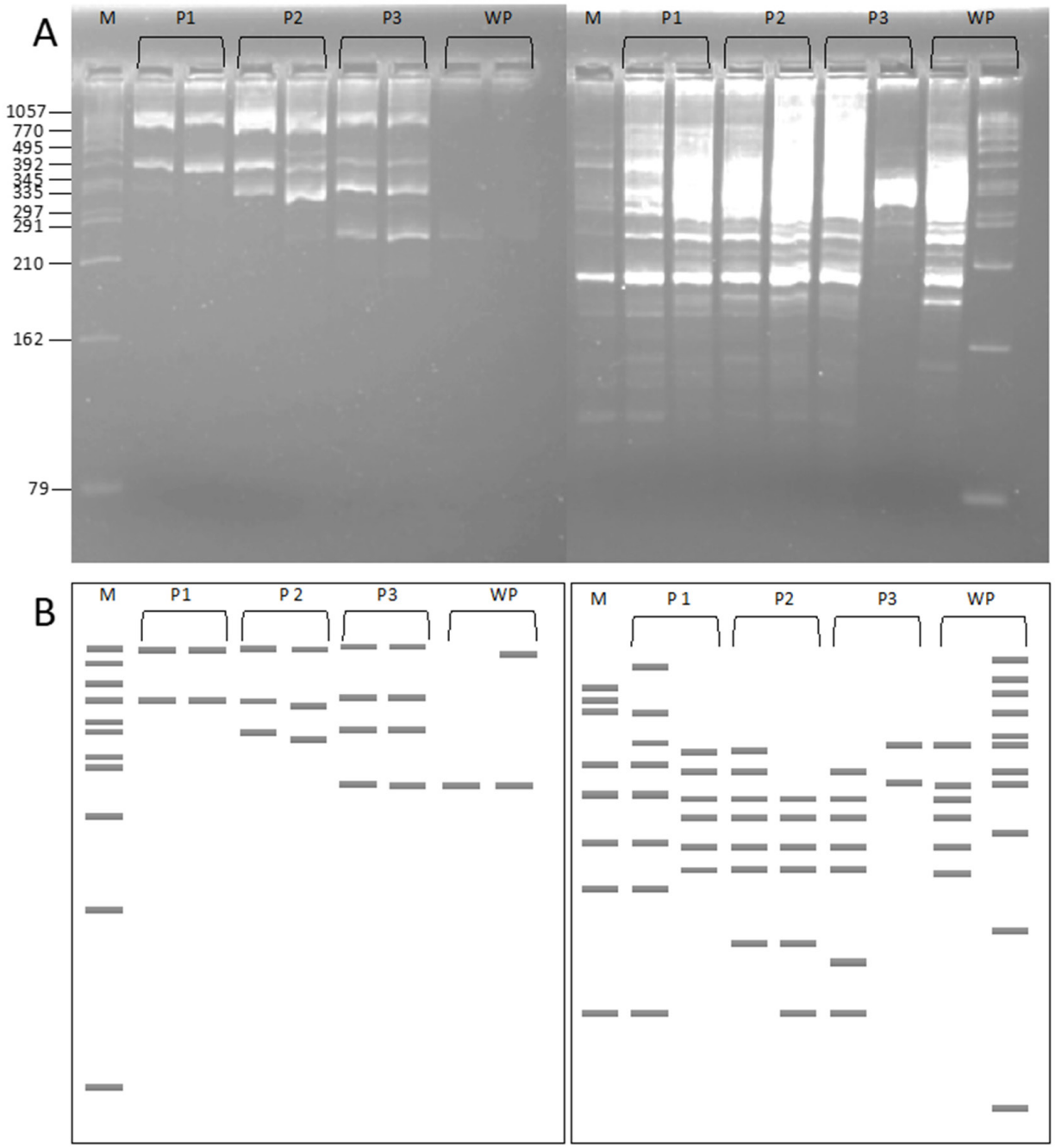

Fig. 4. The PCR product were running in 2% agarose gel. DNA source from (A) NA and SEA (B) medium. Lane M, 100 bp molecular weight marker φX174-Hinc II digest (Takara Bio); Lane 1, Once time PGPR Application (P1); Lane 2, Two-time PGPR Application (P2); Lane 3, Three-time PGPR Application (P3); Lane 0, Without PGPR (WP). A. The banding profiles of the bacteria community in agarose gel. B. Representative gel showing Ribosomal Intergenic Spacer Analysis (RISA) profiles of the amplified ITS gene from 79-1057 bp

In the culture-dependent approach, variance analysis of bacteria culture in NA and SEA cultures showed that the application of PGPR in soils planted by intercropped peanuts with corn and cassava significantly affected the total bacterial population in the rhizosphere. PGPR application at planting, 15 days of planting, and 30 days of planting (P3) increased the rhizosphere bacteria population, both grown on nutrient agar (NA) and soil extract agar (SEA) media. The application of PGPR three times (P3) showed the highest bacterial population in both the NA and SEA media (Table 2). It revealed that PGPR could adapt to dryland habitats planted with food crop intercropping systems. The PGPR in the rhizosphere influences affect in physical, chemical, and biological soil (Bertola et al., 2021). Mohammad (2015), Sedri et al. (2022) and Fang et al., (2022) reported that the number of soil bacteria positively correlated with organic matter, total nitrogen content, soil moisture content, and soil pH. Therefore, this study infers that PGPR would help provide nutrients for plants or bacterial communities in the rhizosphere.

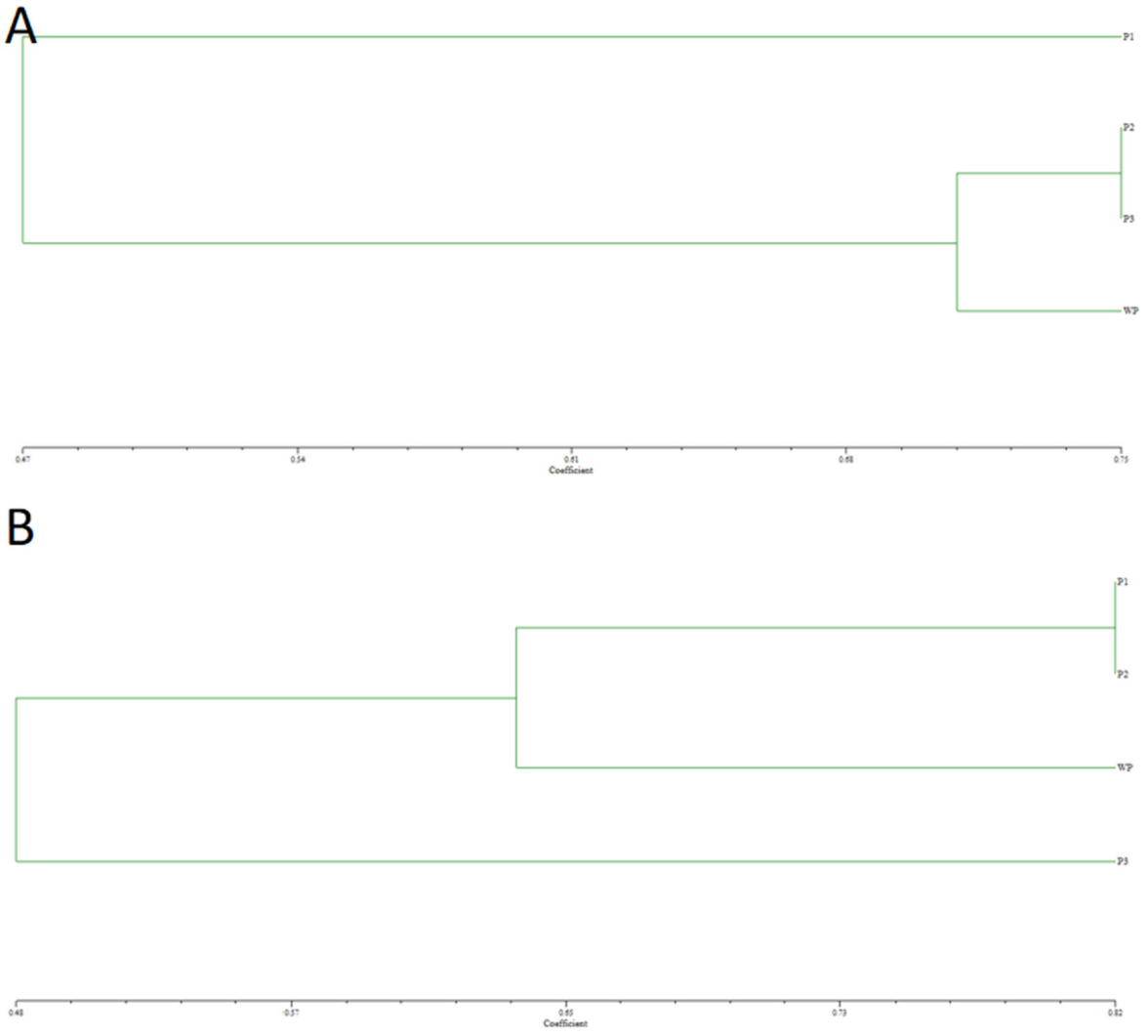

Fig. 5. A dendrogram was produced by unweighted pair group method with arithmetic averages method for the clustering of RISA banding patterns. The samples collected from culture-dependent approach, namely (A) NA and (B) SEA media. It is noticeable that some clusters had absolute or relative dominance of samples from the same location. P3 has 0.68-0.70 coefficient similarity with P1 and P2

This study planted the plants in intercropping systems, namely peanuts, corn, and cassava. Intercropping is an ancient and effective planting method of growing crops/animals' side by side that can increase yields and reduce pests and weeds. The internal mechanisms of the cassava/peanut cover crop system were elucidated by analyzing the physicochemical properties of rhizosphere soil and microbial communities. The cassava/peanut cover crop system was found to increase levels of DA101, Pyrimeria, and Pyrimeria, thereby increasing available soil N and improving soil quality (Tang et al., 2020).

The results are conceptually steady with the discoveries of Pagnani et al., (2020) and Correia et al., (2021) which demonstrated that the double application of PGPR positively affects the diversity of the rhizosphere bacterial community. These results are confirmed by Scanning Electron Microscope (SEM) imaging of roots. Bacteria are able to colonize and make biofilm on root surfaces.

In more recent studies, Renoud et al., (2022) stated the inoculation dosage of PGPR *Azospirillum lipoferum* CRT1 has affected the rhizosphere bacterial community in field maize. The dosage of inoculation is correlated with survival and the interaction of bacteria in the rhizosphere. Bacteria need time to colonize, survive, and associate with rhizosphere bacterial communities. The intercropping system also has roles in bacteria in root colonization (Faridvand et al., 2021). In this situation, rhizobacteria could help to increase nutrient uptake.

Additionally, this work describes the PGPR application that could be established in clay loam soil. The number of colonizing rhizobacteria tended to be higher in three applications of biofertilizer (P3). Rhizobacteria interactions with clay loam minerals, common mineral constituents of soil, are a fundamental part of soil formation and function processes (Fomina & Skorochod, 2020). All the plots had a comparable pH and a comparable substance of clay soil. Thus, it is obtained that bacterial diversity is found in the rhizosphere. The rhizosphere is distinct microenvironments that support bacterial diversity found in soils (Hemkemeyer et al., 2018).

CONCLUSION

This work describes the initial determination of rhizobacteria of peanuts from culture-dependent and culture-independent approaches from different dosages of PGPR application. The results obtained in this study showed that the bacterial community diversity present in soil without PGPR application is low. Three dosages of PGPR application have a high dominance of a few bacterial communities. Additionally, this result provides new insight into the rhizosphere profile, especially in peanuts with a corn-peanut-cassava intercropping system. The result suggests that the bacterial community is decently on the frequency of PGPR application.

ACKNOWLEDGEMENT

This work was financially supported by the Directorate of Research and Community Service, Directorate General of Research and Development, Ministry of Research, Technology and Higher Education. Thank are also extended to Muhamad Taufik Hidayat for figures preparations on the manuscript.

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