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Exploration and Identification of Potential Soil Microorganisms from The Pepper Rhizosphere

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Abstract. Pepper is one of the plantation products with high economic value. Cultivation businesses are generally carried out independently by the community using conventional methods. The limited availability of subsidized fertilizers is one of the obstacles faced by farmers, while the price of non-subsidized fertilizers is quite high. This is certainly a burden for farmers considering pepper is a type of plant that requires large amounts of nutrients. The utilization of soil microorganism services as biofertilizers is an alternative to replace the use of inorganic fertilizers. The purpose of this study was to identify the types of beneficial soil microbes in the roots of pepper plants, especially non-symbiotic nitrogen fixing bacteria and phosphate solubilizing bacteria, as well as phosphate solubilizing fungi. The isolation of bacteria was carried out by the spread method on agar media, while the isolation of fungi was carried out by the pour method. The density of microorganisms was calculated by the Total Plate Count (TPC) method. Total soil microorganisms were quite low, i.e., 8.8 x 10^5 - 3.1 x 10^7 colonies/gram of soil. Bacterial characterization was carried out by observing the macroscopic morphology of the colonies, Gram staining and endospores, and catalase and motility tests. Fungal characterization was carried out by observing macroscopic and microscopic morphology, which included hyphae, spore-producing, and spore-forming. Exploration of potential soil microorganisms carried out on pepper rhizosphere resulted in 3 groups of potential microorganisms, namely nitrogen-fixing bacteria, phosphate solubilizing bacteria and phosphate solubilizing fungi. The types of nitrogen-fixing bacteria obtained were from the genera Rhizobium, Bradyrhizobium, Acetobacter, Azotobacter, Azospirillum, and Clostridium. Phosphate solubilizing bacteria obtained were Azotobacter, Bacillus, Pseudomonas, and Thiobacillus. In contrast, the types of phosphate solubilizing fungi that have been isolated are Penicillum, Aspergillus, and Trichoderma. All isolates have the potential to be used as biofertilizers, but further tests are needed to determine which isolates have the best ability to provide optimal effects on plants.

INTRODUCTION

Pepper is one of the mainstays of export that is important for world trade. This spice contributes to foreign exchange for Indonesia. Pepper is the 4th largest foreign exchange earner for plantation products after palm oil (CPO), rubber, and coffee [1]. In addition, the various benefits and distinctive flavors of pepper make people use it as a mixture of cooking spices. Pepper is also one of the raw materials for the fast-food industry, cosmetics, and medicines, especially herbal medicine [2]. Even developed countries such as France use black pepper as an ingredient in making perfume [3]. Pepper has a great influence on the economic growth of a region, especially in

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East Luwu, South Sulawesi, Indonesia. Around 99.6% of pepper cultivation is carried out by the community independently. Thus, it has the potential to provide employment and increase income [2].

Pepper cultivation which is still managed traditionally, faces several problems that reduce the quality of production. According to [2], the obstacles that are often experienced by pepper farmers are the difficulty of obtaining superior seeds and fertilizers. Pepper plants require nutrients in large enough quantities. Thus they are able to grow and produce fruit optimally. According to [4], leaf tissue must contain at least 3.10% N, 0.16% P2O5, 3.40% K_2O, 1.66% CaO, and 0.44% MgO nutrients in order to grow healthy. Generally, farmers use inorganic fertilizers to meet the nutrient needs of pepper plants. However, the limitation of subsidized fertilizers is an obstacle for farmers. The price of pepper growth-promoting non-subsidized fertilizers is also high, which adds to the burden on farmers [1]. In addition, the use of chemical fertilizers on agricultural land and plantations in the long term can reduce the quality of the soil itself. The purpose of this study was to identify the types of beneficial soil microbes in the roots of pepper plants, especially non-symbiotic nitrogen fixing bacteria and phosphate solubilizing bacteria, as well as phosphate solubilizing fungi.

MATERIAL AND METHODS

Collecting Soil Sampel

Soil samples were collected from several pepper plantations in the Sorowako sub district, near nickel mine (Sumasang, Salonsa and Popatea) with monoculture and polyculture cropping patterns. The polyculture cropping pattern applied is to plant pepper with plantation crops (cocoa, durian or rambutan) or forestry (gamal, kapok, or breadfruit). Sampling of the soil in the rhizosphere of pepper plantations was carried out diagonally at 5 points (T) for each plant. The sampling distance is 30-100 cm from the base of the stem with a depth of 5 -15 cm as much as 100-200 grams per point.

Isolation of Soil Microorganisms

The isolation of nitrogen fixing bacteria, phosphate solubilizing bacteria and phosphate solubilizing fungi was carried out by weighing 1 gram of soil sample and dissolving it in physiological NaCl solution. Samples at a dilution level of 10-2-10-5 were then inoculated on YEMA media for nitrogen-fixing bacteria and pikovskaya media for phosphate solubilizing bacteria and fungi. Density of bacteria and fungi was obtained by counting the number of bacterial and fungal colonies that grew on the selective media using the Total Plate Count (TPC) method using a colony counter. Furthermore, bacterial and fungal colonies that showed colony morphological characteristics according to Bergey's manual of determinative bacteriology [5] were purified for stock

Identification of Soil Microorganisms

Nitrogen fixing Bacteria and Phosphate Solubilizing Bacteria

Isolates of nitrogen fixing bacteria from stock were grown on YEMA media to which Congo red solution had been added. This is done to distinguish the types of nitrogen-fixing bacteria based on their ability to absorb the indicator solution. Further characterization was carried out by growing the isolates on YEMA media to which Bromtymol blue solution had been added. Inoculation of the isolates on the media was aimed to determine the nature and growth rate of the nitrogen-fixing bacteria that had been obtained. Physiological tests were carried out in the form of Gram staining, endospore staining, catalase test, and motility test. The same physiological tests were also carried out on isolates of phosphate solubilizing bacteria.

Phosphate Solubilizing Fungi

The purified phosphate solubilizing fungus was then made a single isolate. Colony diameter and clear zone formed on media containing phosphate were measured after 3 days of incubation to determine the phosphate solubilization ability of the fungus. Initial identification of phosphate solubilizing fungi was carried out by observing the colony morphology, including the color of the surface and reverse of the colony, the surface texture of the

colony and the presence of concentric and radial lines formed. Further identification by observing microscopic morphology, such as the shape and size of spores, spore producers and hyphae.

RESULTS AND DISCUSSION

Density of Potential Soil Microorganisms in Pepper Rhizosphere

The rhizosphere is a soil zone that is still influenced by plant root activity and is an ideal habitat that can support the growth of microorganisms [6]. The rhizosphere contains many nutrients derived from plant root exudates. This causes the population of rhizosphere microorganisms to be more numerous and diverse compared to the population of microorganisms in non-rhizosphere soils [7]. Bacteria that colonize plant roots are called rhizobacteria. According to [8], rhizobacteria play a role in nutrient cycles (such as N, P and C), soil formation, support plant growth and as biological controllers against plant diseases. Rhizobacteria are commonly known as Plant Growth Promoting Rhizobacteria (PGPR). PGPR consists of nitrogen fixing bacteria and phosphate solubilizing bacteria that provide direct and indirect benefits to plants.

TABLE 1. The density of potential soil microorganism in pepper rhizosphere.

Location	The density of soil microorganism			Total of Soil Microorganism (colony/gram)
	Nitrogen-Fixing Bacteria (cfu/gram)	Phosphate Solubilizing Bacteria (cfu/gram)	Phosphate Solubilizing Fungi (colony/gram)	
Petea/monoculture	$1{,}5 \times 10^7$	$8{,}8 \times 10^3$	$2{,}4 \times 10^3$	$1{,}5 \times 10^7$
Petea/polyculture	$9{,}4 \times 10^6$	$3{,}4 \times 10^3$	$1{,}1 \times 10^4$	$9{,}4 \times 10^6$
Sumasang 1/monoculture	$3{,}1 \times 10^7$	$2{,}3 \times 10^3$	$4{,}2 \times 10^3$	$3{,}1 \times 10^7$
Sumasang 3/monoculture	$2{,}7 \times 10^7$	$3{,}0 \times 10^3$	$3{,}0 \times 10^2$	$2{,}7 \times 10^7$
Sumasang 3/polyculture	$8{,}8 \times 10^5$	$2{,}7 \times 10^3$	$1{,}5 \times 10^3$	$8{,}8 \times 10^5$
Salonsa/polyculture	$6{,}2 \times 10^6$	$7{,}7 \times 10^3$	$5{,}0 \times 10^2$	$6{,}2 \times 10^6$

PGPR will quickly form colonies around the rhizosphere when it gets nutrients and is supported by suitable soil and environmental conditions for its growth. Isolation of PGPR from pepper rhizosphere indicated the presence of nitrogen fixing and phosphate solubilizing bacteria that colonize pepper roots. Density of nitrogen fixing and phosphate solubilizing bacteria can be seen in Table 1. Besides PGPR, phosphate solubilizing fungi are also found in pepper rhizosphere. Various potential soil microorganisms that live around the roots will provide benefits for plants and soil fertility.

The total soil microorganism density in the observation plot was 8.8×10^5 - 3.1×10^7 colony/gram soil. The population of soil microorganisms which is quite low indicates that the soil in the pepper plantations is less fertile. The potential microorganisms that make up fertile agricultural soil contain more than 100 million ($>10^8$) microorganisms in one gram of soil [9].

The lowest population of nitrogen fixing bacteria was produced in the Poly Sumasang 3 plot (8.8×10^5 cfu/gram) and the highest in the Mono Sumasang 1 plot (3.1×10^7 cfu/gram). Different from nitrogen fixing bacteria, the lowest density of phosphate solubilizing bacteria was in the Mono Sumasang 1 plot (2.3×10^3 cfu/gram) and the highest was in the Mono Petea plot (8.8×10^3 cfu/gram). The lowest density of phosphate solubilizing fungi was found in the Mono Sumasang 3 plot (3.0×10^2 colony/gram) and the highest in Poly Petea (1.1×10^4 colony/gram). The difference in the density of nitrogen-fixing bacteria in several observation plots is caused by several factors, such as the metabolic activity of plant roots and soil conditions which include soil physical and chemical properties [10].

Plant roots excrete metabolites through the roots called exudates. Generally, exudates consist of metabolites such as sugars, glycosides, nucleotide compounds and their bases, amino acids, organic acids, enzymes and vitamins [9]. These metabolites are utilized by soil microorganisms as nutrients so that they can survive. The composition of exudate compounds produced by plants determines the condition of soil microorganisms in the rhizosphere [11]. Added by [9] that different root metabolic activities in several types of plants cause the composition of exudate to be different so that it affects the population and types of bacteria in the root area.

Identification of Soil Microorganisms Colonizing Pepper Rhizosphere

Nitrogen Fixing Bacteria

Nitrogen is an essential macronutrient needed by plants because it the main constituent of amino acids and is an integral part of chlorophyll [12]. The need for N elements is met, causing the leaves to turn dark green. Photosynthesis that takes place properly will stimulate plant growth, especially in stems, branches, leaves, and fruit formation. Nitrogen is one of the main constituent elements of the atmosphere. The amount is very abundant, about 80% of the total gas in the atmosphere [13]. The atmosphere contains about 386 x 1016 kg of nitrogen [14]. However, these elements are in a form that is not available to plants so that they cannot be used directly. Atmospheric nitrogen will enter the soil, physically, chemically, and biologically. Some soil microorganisms are able to fix atmospheric nitrogen and convert it to NH4+ and NO3-. Potential soil microorganisms capable of fixing nitrogen at 139 x 109 kg/year [15]. Nitrogen fixation by microorganisms can be done through symbiosis with plant roots or free living in the soil. Isolation of nitrogen-fixing bacteria from pepper rhizosphere showed various types of bacteria from the observation plot as shown in Table 2.

Symbiotic nitrogen-fixing bacteria are bacteria that are only able to fix free nitrogen in the atmosphere when symbiotic to plant roots. Rhizobia bacteria are prokaryotic species capable of fixing free nitrogen and converting it into a form available to plants. Root nodules are able to return more nitrogen to the soil than other nitrogen-fixing microorganisms. The rhizobia are able to fix atmospheric nitrogen about 65% (89 x 109 kg) of the total microbiological tethering done every year [14].

TABLE 2. Nitrogen-fixing from pepper rhizosphere.

Location	Number of Isolates	Genus of Bacteria
Petea/monoculture	9	*Rhizobium* (1)
		Bradyrhizobium (1)
		Acetobacter (4)
		Azotobacter (2)
		Clostridium (1)
Petea/polyculture	8	*Rhizobium* (2)
		Acetobacter (3)
		Clostridium (3)
Sumasang 1/monoculture	10	*Rhizobium* (1)
		Bradyrhizobium (3)
		Acetobacter (4)
		Azotobacter (2)
Sumasang 3/monoculture	9	*Acetobacter* (4)
		Azotobacter (1)
		Azospirillum (2)
		Clostridium (2)
Sumasang 3/polyculture	9	*Rhizobium* (3)
		Bradyrhizobium (1)
		Acetobacter (1)
		Azotobacter (3)
		Azospirillum (1)
Salonsa/polyculture	8	*Bradyrhizobium* (1)
		Azotobacter (5)
		Azospirillum (1)
		Clostridium (2)

The rhizobia bacteria obtained from the pepper rhizosphere were from the genera Rhizobium and Bradyrhizobium. The growth of Rhizobium bacterial colonies on YEMA media showed characteristics such as circular, flat edges (entire), raised and convex elevations (convex), milky white to brownish white, and smooth to slimy surfaces. Physiological tests carried out showed that the isolates had Gram negative characteristics with the shape of rod cells (bacilli), did not have endospores, did not or slightly absorb the red color on YEMA media with

Congo red indicator solution added, acidic on YEMA media with bromthymol blue added (marked by media changes to yellow color) and has a fast growth of about 3-5 days. Chemical tests showed that Rhizobium was catalase positive and motile on semi-agar media. Similar results were revealed by [16] that Rhizobium isolated from the rhizosphere of edamame soybean in Jember district showed a round colony shape, flat edges, convex elevation, and milky white color. The cells are rod-shaped and generally growth begins after 24 hours of incubation.

Bradyrhizobium bacterial colonies have similar characteristics to Rhizobium. A distinctive feature of Bradyrhizobium is that when grown on YEMA media with congo red added, the center of the colony will slightly absorb the indicator solution so that it is pink while the edges of the colony are white. In addition, this type of bacteria is alkaline when grown on media with the addition of Bromtymol Blue (marked by the color of the medium which remains blue) and slow growth for about 5-7 days. Bradyrhizobium is a type of slow growing rhizobia that grows on YEMA media after 8-10 days of incubation [17].

Non-symbiotic nitrogen-fixing bacteria are microorganisms that are able to convert free nitrogen molecules into ammonium without the help of other organisms [18]. The bacterial isolates obtained from the observation plots had characters that lead to the types of Acetobacter, Azotobacter, Azospirillum, and Clostridium. Isolates with characters leading to Acetobacter were obligate aerobes, oval-shaped cells to short rods, did not have endospores, and were motile although some species were found to be non-motile.

The Azotobacter isolates obtained had good growth on YEMA media, indicated by the growth of thick colonies. Colony shape is circular, elevation is raised, surface is smooth to slimy. Colonies are white, brownish white, yellow, yellow-brown, brown and orange. Gram staining showed Gram negative cells with various cell shapes, namely rods, short rods (almost oval) and round. The catalase test showed that the bacteria were aerobic characterized by the formation of air bubbles after the addition of H2O2 to the test preparation. Bacterial cells do not have endospores and are motile on semi-Agar medium. One of the Azotobacter isolates from the Poli Sumasang 3 plot formed a clear zone on YEMA media with congo red added. The clear zone formed from the fading of the red color in the media was an indicator of good nitrogen fixing ability by bacteria [19]. Azotobacter colonies grown on agar media had round colonies with intact edges, the surface of the colonies was convex and white [20]. Several types of Azotobacter have varied pigments, such as yellow and brown [21]. Azotobacter are pleomorphic, rod-shaped cells with rounded ends, short to round rods and can produce mucus in the form of exopolysaccharides [22].

Some bacterial isolates have characteristics such as the type of Clostridium based on Bergey's manual of determinative bacteriology [5]. Gram staining showed that Clostridium bacteria were gram-positive with rod-shaped cells, catalase positive, lacked endospores and were motile. Clostridium is a Gram-positive bacterium, is aerotolerant and motile [5]. Azospirillum obtained from Mono Sumasang 3 and Poli Salonsa plots had irregular colonies with undulate and filamentous edges, Gram negative, bacillus, catalase positive and motile. This is in accordance with Rasool et al. (2015) that the characteristics of Azospirillum are round or irregular shaped colonies, Gram negative, bacilli with a size of 3-5 m, motile with a single flagellum.

Nitrogen-fixing bacteria are also called diazotrophs because they are able to utilize atmospheric N as a source of N for their metabolism [23]. In addition to fixing nitrogen, bacteria are also able to produce growth hormone Indole Acetic Acid (IAA) [24]. Utilization of nitrogen fixing bacteria as biofertilizer can increase farmers' production and income, costs are relatively cheaper due to the ability of bacteria to multiply, and soil quality is maintained.

Phosphate Solubilizing Bacteria

Phosphate is the second essential macro element that plays an important role in plant metabolism after nitrogen. Phosphate in the soil cannot be used directly by plants. Generally, the available phosphate in the soil is less or equal to 0.01% of the total P, most of which is in the bound form [7]. The binding of phosphate causes the application of large amounts of phosphate fertilizer to be inefficient because only 15-20% can be absorbed by plants [7]. Utilization of phosphate solubilizing microorganisms is an alternative solution to overcome the low availability of phosphate in the soil. Isolation of phosphate solubilizing bacteria from pepper rhizosphere showed a low bacterial density as shown in Table 1.

The density of phosphate solubilizing bacteria in the observation plot ranged from 2.3 x 103 - 8.8 x 103 cfu/gram. Meanwhile, according to [7], the population of phosphate solubilizing bacteria can reach 12 x 106 cfu/gram soil. The viability of microorganisms is influenced by the availability of organic matter in the soil that can affect their life activities. The bacterial isolates obtained had characteristics that lead to the genus Azotobacter, Bacillus, Pseudomonas, and Thiobacillus (can be seen in Table 3).

TABLE 3. Phosphate solubilizing bacteria in pepper rhizosphere.

Isolate Codes	Macroscopic Characteristic						Gram Staining		Biochemic Test		
	Shape	Elevation	Margin	Surface	Colour	Size	Gram (+)	Gram (-)	Catalase test	Endospore staining	Motility test
BPSAL1	Circular	Raised	Entire	Smooth and slimmy	brownish white	Small		Short bacil	++	-	-
BPSAL2	Irreguler	Flat	Undulate	dry	white	Small	Bacil		++	-	-
BPSAL3	Circular	Convex	Entire	Smooth and slimmy	brownish white	Small		Short bacil	+++	-	-
BPSAL4	Circular	Convex	Entire	Smooth	brownish white	Pintpoint		Short bacil	++	-	+
BPSAL5	Circular	Convex	Entire	Smooth and slimmy	brownish white	Small		bacil	+	-	+
BPSAL6	Circular	Convex	Entire	Smooth	brownish white	Small		bacil	++	-	-
BMPET1	Circular	Flat	Entire	dry	Yellowish white	Small	Bacil		+	-	-
BMPET 2	Circular	Convex	Entire	Smooth	brownish white	Small	Bacil		-	-	-
BMPET3	Circular	Raised	Entire	Smooth	brownish white			Short bacil	++	-	-
BPPET1	Irreguler	Raised	Lobate	Smooth	brownish white	Moderate		Short bacil	+	-	-
BPPET2	Circular	Flat	Entire	dry	Light brown	Small		Short bacil	+	-	-
BPPET3	Circular	Raised	Entire	Smooth	brownish white	Small		Short bacil	+	-	+
BPPET4	Irreguler	Raised	Lobate	dry	Bone white	Moderate		Short bacil	+	-	-
BMSUM1.1	Circular	Raised	Entire	Smooth	white	Small		Basil pendek	++	-	-
BMSUM1.2	Irreguler	Flat	Lobate	Smooth	brownish white	Small		Short bacil	++	-	-
BMSUM1.3	Irreguler	Raised	Lobate	Smooth	brownish white	Small		Short bacil	+	-	-
BPSUM3.1	Circular	Raised	Entire	Smooth	yellow	Small		Short bacil	++	-	+
BPSUM3.2	Irreguler	Raised	Undulate	Smooth	brownish white	Small		Short bacil	++	-	-
BPSUM3.3	Irreguler	Raised	Lobate	Smooth and slimmy	white	Moderate		Bacil	++	-	+
BPSUM3.4	Circular	Raised	Entire	Smooth	brown	Small		Bacil	++	-	-
BPSUM3.5	Irreguler	Raised	Lobate	Smooth	Brownish yellow	Small		Short bacil	+	-	-
BMSUM3.1	Circular	Convex	Entire	Smooth	white	Small		Bacil	+	-	-
BMSUM3.2	Circular	Raised	Entire	Smooth	yellow	Small		Bacil	++	-	-
BMSUM3.3	Circular	Convex	Entire	Smooth and slimmy	brownish white	Small		Bacil	++	-	+
BMSUM3.4	Irreguler	Raised	Undulate	Smooth	brownish white	Small	Bacil		+	-	-
BMSUM3.5	Irreguler	Raised	Undulate	Smooth	brownish white	Moderate		Bacil	++	+	-
BMSUM3.6	Irreguler	Raised	Undulate	Smooth	Yellowish white	Moderate		Short bacil	++	-	+
BMSUM3.7	Circular	Raised	Entire	Smooth and slimmy	Yellowish white	Moderate		Coccus	-	-	-

Bacteria with isolate code PSAL4, PSAL5, PPET3, PSUM3.1, PSUM3.3, MSUM3.3, and MSUM3.6 have characters that point to the genus Azotobacter. The characterization of Azotobacter conducted by [25] showed that the cells were rod-shaped, Gram negative, and motile. Cell movement is aided by several peritrichous flagella [5]. However, some species of this genus were found to be non-motile [26]. Azotobacter are aerobic, characterized by a positive reaction during the catalase test, but can be facultative anaerobes when the solubility of oxygen in the growth medium decreases [10]. The genus Azotobacter is also capable of fixing nitrogen [5].

The characters of PSAL2, MPET1, MPET2 and MSUM3.4 isolates were similar to the Bacillus genus. Bacillus are aerobic which utilize oxygen as the last electron acceptor in the cell respiration chain, but some bacteria can be facultative and even anaerobic [27]. Bacillus can form endospores that can protect cells when environmental

conditions are unfavorable [10], this character is owned by isolates MSUM3.5. However, most of the isolates did not show the presence of endospores when treated with Malachite green dye. The walls of Bacillus spores were thin so they were not easy to stain [5].

Bacterial isolate codes that have similar characters to the genus Pseudomonas are PSAL6, MSUM3.1, MSUM3.2, MSUM3.7, and PSUM3.4. Pseudomonas has a straight or curved stem cell shape, 0.5-0.1µm – 1.5-4.0 m in size, does not form endospores, is Gram negative, and grows optimally at a temperature of 35°-37°C [27]. Pseudomonas produces greenish, bluish, violet, yellow or other color pigments, and is motile with a single polar flagellum although some species are not motile [5].

Character isolates PSAL1, PSAL3, MPET3, PPET1, PPET2, PPET4, MSUM1.1, MSUM1.2, MSUM1.3, PSUM3.2, and PSUM3.5 were similar to the genus Thiobacillus. Thiobacillus bacteria were Gram negative, short rod-shaped, yellow colonies on agar media, and did not have endospores [28]. Thiobacillus bacteria can move using a single flagellum, although there are also non-motile, aerobic, or facultative anaerobes when the medium contains nitrate [5].

Phosphate solubilizing bacteria are able to produce organic acids and phosphatase enzymes that can dissolve phosphate in the media [29]. Qualitative dissolution of phosphate can be seen by the formation of a clear zone (halozone) around the bacterial colonies on Pikovskaya media. The application of phosphate solubilizing bacteria as biological fertilizer has advantages such as being relatively energy efficient, not damaging the environment, helping to increase the solubility of bound P elements, producing growth regulators and antibiotic compounds that can prevent pathogens from infecting plant roots [30].

Phosphate Solubilizing Fungi

Phosphate solubilizing fungi is one of the phosphate solubilizing microorganisms in addition to phosphate solubilizing bacteria and actynomicetes. The population of phosphate solubilizing fungi in the soil only ranged from 2 x 10^4 - 10^6 colonies/gram soil, less than the population of phosphate solubilizing bacteria [7]. Isolation of phosphate solubilizing fungi in pepper rhizosphere showed varying densities and was still relatively low. The lowest population density was in the Mono Sumasang 3 plot (3.0 x 10^2 colony/gram soil) and the highest was in the Poly Petea plot (1.1 x 10^4 colony/gram soil). The types of phosphate solubilizing fungi that have been isolated are Penicillum, Aspergillus, and Trichoderma. The lowest diversity of phosphate solubilizing fungi was found in the Mono petea plot which has 1 genus of phosphate solubilizing fungi. The highest diversity was in the plots of Mono Sumasang 1 and Poli Salonsa which had 3 fungal genera. Land cultivated in monocultures may be deficient in a non-nutrient species. This is due to the limited variety of organic compounds in plant root exudates that return to the soil [31]. These conditions will have an impact on soil biological conditions, especially the presence and diversity of potential microorganisms that play an important role in maintaining soil sustainability [32].

Fungal isolates with JMPET1, JPPET1, JPPET2, JPPET3, JMSUM1.1, JMSUM1.4, JMSUM3.2, JMSUM3.4, JMSUM3.7, JPSUM3.2, JPSUM3.3, JPSAL2, JPSAL3, and JPSAL5 have characters that lead to genus Penicillum (Table 4). Penicillum has clear hyphae (hyaline) insulated, conidiospores resembling a broom with a metula consisting of 3-5 phyalids, single conidiophores (mononematus) or compound (synematous), and conidia spherical (globose) or semi-spherical (subglobose) [33]. Besides being able to dissolve phosphate, Penicillum is also able to protect plants from pathogen attacks [34].

Fungi with isolate codes JMSUM1.3, JMSUM3.1, JMSUM3.3, and JPSUM3.1 have characters that indicate the genus Aspergillus. Isolated colonies are white in color with black granules above the mycelium. Aspergillus has insulated vegetative hyphae, single conidiophores, round hyaline vesicles, phialids located at the apical end of the metula, conidia are round (globose) [35]. The Aspergillus genus can live in various conditions, including extreme conditions [36]. The isolates JMPET2, JMSUM1.2, JPSAL1 and JPSAL4 had similar characteristics to Trichoderma. The mushroom colonies were green and growing fast filling the petri dish. conidiophores have an erect, branched structure arranged vertically, short and thick phialids, and oval-shaped conidia [37].

The phosphate solubilization ability of the fungal isolates obtained was seen from the clear zone (halozone) formed around the colony. The clear zone was formed because the fungus was able to dissolve the P contained in the media [38]. The wider and brighter the clear zone produced, the greater the ability of the fungus to dissolve P in the medium, although it was not enough to be the basis for the ability of the fungus to dissolve the actual P [39}. The phosphate solubilization ability of fungal isolates after incubation for 3 days can be seen in the Fig. 1.

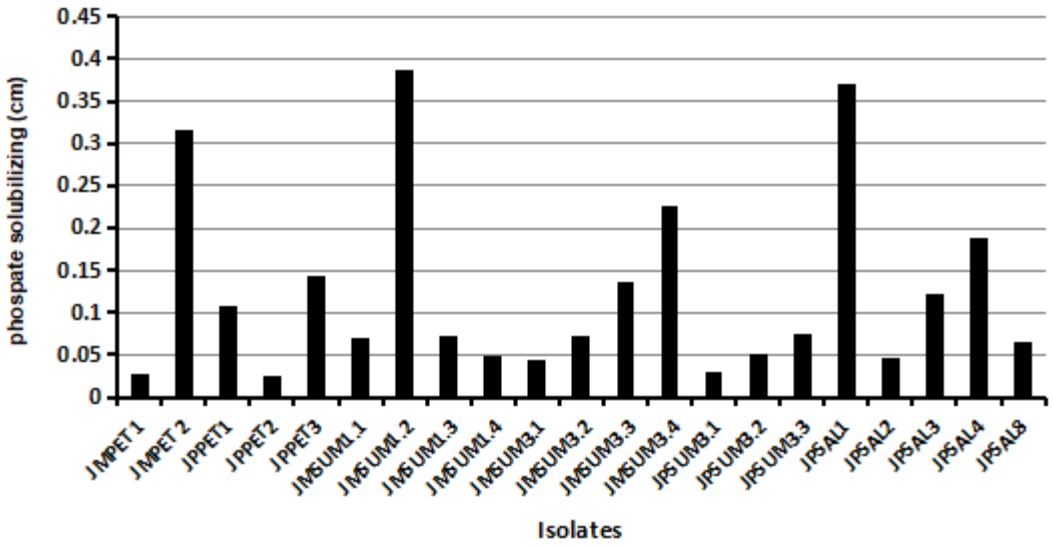

FIGURE 1. The ability for phosphate solubilizing each isolate.

Fungal isolates have varying abilities in dissolving phosphate. Some isolates produced clear and slightly opaque dissolution zones. In addition, the resulting clear zone area also varies, namely 0.021739 - 0.385993 cm. Islamiati (2015) explained that genes are the main factor that causes each isolate to have the ability to produce different organic acids. The amount and type of organic acid secreted affected the phosphate solubilization ability of the isolate.

Phosphate solubilizing microorganisms will produce various organic acids such as malic, citric, oxalic, acetic, and propionic [39]. The high organic acid content in the soil causes the soil pH to be low. The organic acids will also react with phosphate binding ions, such as Fe3+, Mg2+, Al3+ and Ca2+, so that phosphate ions will no longer be made [7]. In addition to phosphoric acid, phosphatase and phytase enzymes also play a role in breaking phosphate with organic compounds into available forms. The free phosphate ion can be absorbed and utilized by plants. JMSUM1.3 isolate had the largest dissolution zone compared to other isolates. These isolates have the potential to be applied to soils that are poor in available P.

CONCLUSION

Potential soil microorganisms isolated from pepper rhizosphere consisted of nitrogen fixing bacteria, phosphate solubilizing bacteria, and phosphate solubilizing fungi. The highest density of soil microorganisms was found in the Mono Sumasang 3 area, which was 2.7 x 107 cfu/unit, while the lowest was in the Poly Sumasang 3 area, which was 8.8 x 105. cfu/unit. The nitrogen fixing bacteria obtained consisted of the genera Rhizobium, Bradyrhizobium, Clostridium, Acetobacter, and Azospirillum. Phosphate solubilizing bacteria that have been isolated consist of the genera Azotobacter, Bacillus, Pseudomonas, and Thiobacillus. In contrast, the phosphate solubilizing fungi obtained consisted of the genus Aspergillus, Penicillum and Trichoderma. The potential test of potential microorganisms needs to be carried out to determine the nitrogen fixing ability of nitrogen fixing bacteria and the phosphate solubilization ability of phosphate solubilizing bacteria and fungi.

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