A new strain of bacteria isolated from chemically saturated corn rhizosphere under the dominance of the mineral kaolinite

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Abstract. *Sukmawati, Rahim I, Harsani, Syafnur A, Arodhiskara Y, Selao A, Anisa LFN. 2023. A new strain of bacteria isolated from chemically saturated corn rhizosphere under the dominance of the mineral kaolinite. Biodiversitas 24: 1886-1894.* The kaolinite mineral that dominates the cornfield reduces soil fertility and the bacterial community. The discovery of adaptive bacterial strains on kaolinite dominant land is a biological strategy to improve soil fertility because it is part of the root ecosystem. This study aims to obtain adaptive and superior bacteria in producing IAA hormones and fixing nitrogen. The soil analysis method uses X-Ray Diffraction (XRD) and X-Ray Fluorescence (XRF) analysis to characterize clay minerals and soil chemical content. While the bacterial characterization was carried out morphologically and physiologically. After that, followed by the 16S rRNA gene sequence analysis. The type of kaolinite clay mineral dominates the soil samples with a SiO_2 content of >40%. A total of 17 bacterial isolates were purified, 12 had round colony shapes, and 5 had irregular shapes dominated by cream color, 11 of which were identified as Gram-positive and 6 Gram-negative bacteria. RJ1T isolates/isolated produced the highest auxin (0.141 mgL^{-1}), while RJ1P isolates/isolated had the highest nitrogen fixation ability (0.975%) and were not pathogenic. The isolates were closely related to the *Nocardioides marinisabuli* strain RA2816S ribosomal RNA gene (99.57%). Thus, RJ1PI bacterial isolate is an adaptive bacteria that can be formulated as a biological agent to increase maize productivity in the dry land.

Keywords: Biological agents, gram-positive, growth hormone, kaolinite, nitrogen fixation, rhizosphere

INTRODUCTION

The biggest obstacle to maize development is the undulating to hilly land conditions with steep topography and shallow solum (Sufardi et al. 2021). Especially in clay-textured soils dominated by kaolinite with low cation exchange capacity (Kome et al. 2019) and prone to erosion (Aliasghar et al. 2014) caused by a lack of organic matter and mineral composition (Mani et al. 2018; Sufardi et al. 2021). The decreased productivity of corn proves this fact, along with decreasing soil fertility (Nurmiaty et al. 2019). Therefore, to compensate for this, chemical fertilizers act as a vital tool to increase the productivity of corn plants. However, the types and ingredients of fertilizers disrupt the structure of the soil microbial community and nutrient imbalances (Pahalvi et al. 2021). This is evident in the structure of gram-negative bacteria, which are highly affected by high chemical inputs (Islam et al. 2009). On the other hand, soils with high organic matter increase the abundance of gram-positive bacteria (Sun et al. 2004).

Therefore, clay minerals can significantly alter microbial abundance and biosynthetic activity. Clay also could facilitate nutrition and protect against unfavorable physicochemical conditions. Therefore, soil mineral composition is very important for sustainable soil management and productivity (Fomina 2020). It could control the supply and availability of nutrients, absorption and stabilization of soil organic matter, micro-aggregate formation, soil acidity, and soil microbial population and activity (Kome et al. 2019). For example, exopolysaccharides synthesized by bacteria produce biofilm which provides inorganic nutrients and retains soil moisture (Sá et al. 2019; Sukmawati et al. 2020) by retaining water in the pores and interlayer spaces of clay minerals (Fomina 2020). Thus, bacteria can strengthen clay minerals and increase the surface area that can absorb water and bind micronutrients such as Cu, Mn, Mg, Fe, and Ca (Barreca et al. 2014). Therefore, the composition of the soil microbial community is influenced by soil structure, geographical location, soil particle size, mineral composition, and cultivation techniques. In contrast, microbial activity is influenced by plant roots (Ondreičková et al. 2014).

The condition of the maize rhizosphere is one of the determinants of the diversity of microbial species that coexist (Peiffer et al. 2013). Whether as pathogens, saprophytes, or promoters of beneficial plant growth (García-Salamanca et al. 2013). Furthermore, root exudates

(sugars, organic acids, aromatics, and enzymes) interact with soil properties, such as pH, water potential, texture, and microbial abundance (García-Salamanca et al. 2013). Whereas biogeographical patterns correlate with soil pH affecting the structure and diversity of soil bacterial communities (Baudoin et al. 2003). On the other hand, long-term fertilization increases the availability of nutrients and the abundance of bacteria but reduces biodiversity and changes the bacteria composition in the rhizosphere (Wang et al. 2017). In term of their abundance and activity as an indicator of soil quality (Lori et al. 2017), microbial plays an important role in regulating fertility. Microbials are also important for plant health, the carbon cycle, nitrogen, and other nutrients (Yang et al. 2017). They are important in evaluating ecosystems and maintaining ecological balance (Zhang et al. 2020).

Exploration of corn rhizosphere bacterial strains in a chemically saturated environment is very important to use as a biological agent in overcoming soil fertility problems. For example, the important role of Plant Growth Promoting Rhizobacteria (PGPR) can increase the ability of plants to use water, nutrient availability, and plant resistance to pathogen attack (Setyowati et al. 2017) and to reduce chemical fertilizers on agricultural land (Etesami and Maheshwari 2018; Sukmawati et al. 2020). Furthermore, even gram-positive bacteria can be used as a biological

technique in erosion control (Lajevardia and Shafieib 2023). At the same time, gram-negative bacteria play an important role in nitrogen fixation and drought resistance (Goh et al. 2012; Nosrati et al. 2012; Freeman et al. 2013; Sá et al. 2019). Therefore, studying the adaptive diversity of corn rhizosphere bacteria is a strategy for adapting and evaluating the sustainability of corn cultivation systems on dry land.

MATERIALS AND METHODS

Soil sampling

Three soil samples were taken based on the biogeography of a corn field on sloping dry land in Parenring Village, Lilirilau Sub-district, Soppeng District, South Sulawesi, Indonesia. The selected land is an intensive corn using a monoculture cultivation pattern with the BISI 18 variety. Sampling used the FAO method, where each soil sample was taken at a depth of 0-20 cm (FAO 2008). Soil samples were taken from the back (coordinates Lat 4º20'39" S, Long 120º2'46" E), the middle (coordinates Lat 4º20'209" S, Long 120º2'48" E), and the valley (coordinates Lat 4º20'39" S, Long 120º2'48" E) (Figure 1).
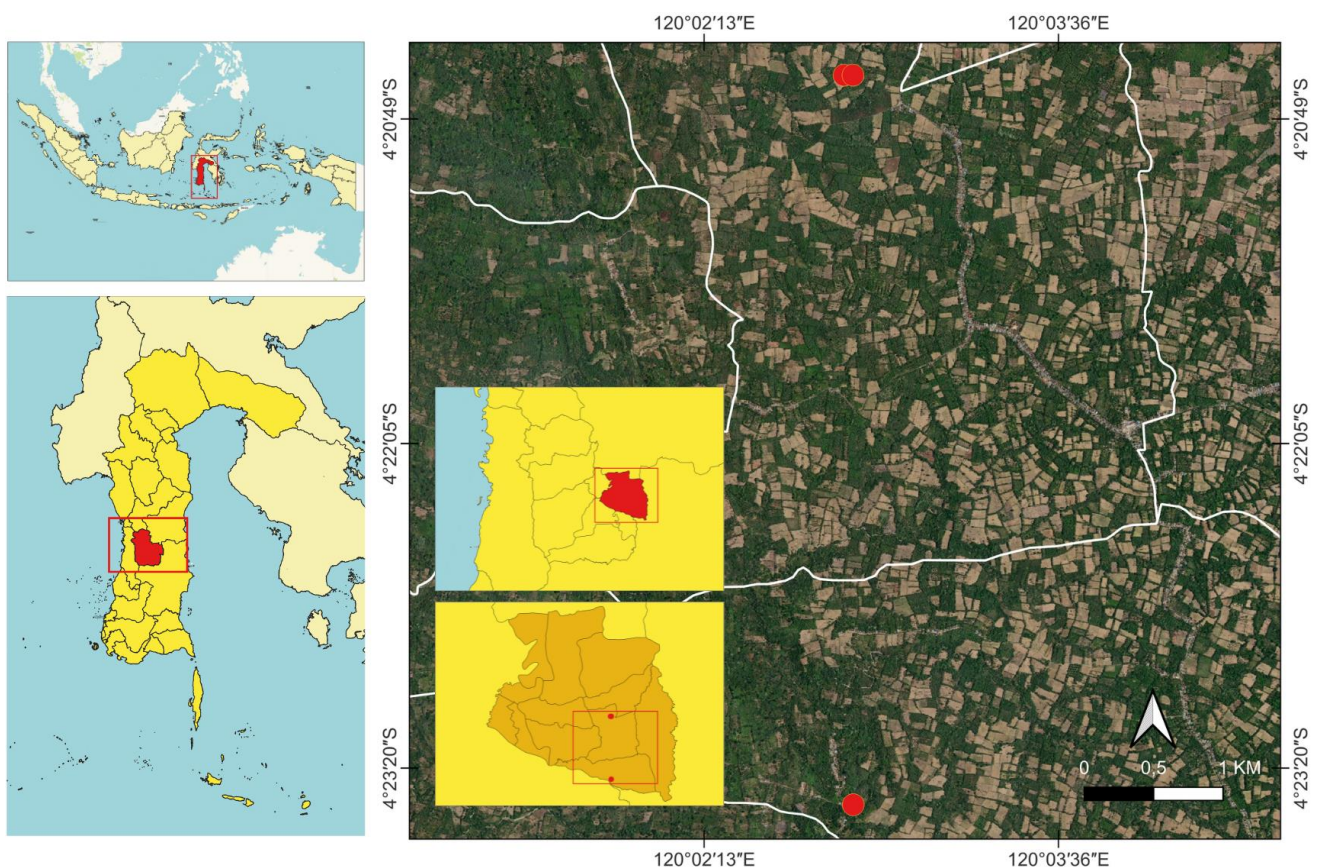

Figure 1. Location of soil sampling in Parenring Village, Soppeng District, South Sulawesi, Indonesia

Physicochemical characteristics of soil in the rhizosphere of maize

The soil minerals types and chemical composition

The chemical composition of the soil was determined by X-ray fluorescence spectroscopy (XRF) according to the method Mohsen and El-maghraby (2010), using a Philips PW 2400 apparatus. The bulk clay samples were crushed to a fine powder mixed with lithium tetraborate for chemical analysis. Loss of ignition was measured by calcination at 1000ºC.

X-ray diffraction

X-ray diffraction (XRD) analysis used an automatic diffractometer (Philips type: PW1840) with a Cu Kα radiation source. At a step size angle of 0.02θ, a scan rate of 2^0 in 2-hour units, and the scan range is from 10θ to 70θ (Mohsen and El-maghraby 2010; Tahir et al. 2019). The parameters measured were the clay type and the soil porosity percentage. The great volume of the kaolinite mineral is used in the search for porosity because this mineral dominates the soil sample (Bish 1993).

Characterization of rhizosphere bacteria based on colony morphology, Gram staining, and sensitivity analysis

The bacterial characterization is based on differences in the morphology of colonies that grow on agar media. The morphology of the colonies growing on the media observed was: (i) Size: pinpoint/punctiform (point), small (small), medium (medium), large (large); (ii) Shape: colonies that appear as whorls or filaments; (iii) Elevation: display elevation as flat or raised; (iv) Margins: in the form of waves, smooth and irregular (Setyowati et al. 2017). Gram staining and sensitivity analysis were observed per Sukmawati et al.'s method. Molecular identification of bacterial isolates was carried out through DNA isolation tests using a DNA isolation kit (Promega, USA), and they were sequenced and analyzed using the Blast program (Lee et al. 2007)

The ability of bacteria to fix nitrogen

The ability of bacteria to fix nitrogen was measured using N-free liquid from Burk using a spectrophotometer (wavelength 636nm) according to the method of Sukmawati et al. (2020).

RESULTS AND DISCUSSION

Soil chemical content based on X-ray fluorescence (XRF) spectroscopy

Table 1 shows the chemical composition of clay from corn fields based on the landscape as the ridge, middle, and valley parts. The XRD spectra detect 9 (nine) micronutrients: Si, Fe, Ca, K, Ti, Al, P, Mo, and Mn. The three most dominant oxides are SiO_2, Fe_2O3, and CaO. Even though it is dominant, $SiO2$ oxide is most abundant in the ridge region Fe_2O_3 oxide. At the same time, CaO is most abundant in the valley area. Al_2O_3 oxide was only

detected in the middle area, while P_2O_5 and MnO were only in the back area. Meanwhile, MO_3 was detected in the valley and middle of the land. Micronutrients come in many forms and are usually absorbed by clay minerals.

Mineral characterization based on the X-ray diffraction (XRD) method

The soil corn rhizosphere types are clay consisting of hematite, kaolinite, montmorillonite, and illite. The results of the XRD spectra showed that the mineral kaolinite dominates the soil around the corn roots, both in the back, middle, and valley areas. The kaolinite mineral begins to be detected at several diffraction angles starting from $15-60\theta$. However, kaolinite minerals with intensities above 600a.u were observed in the valley area (red graph in Figure 2). The montmorillonite mineral was detected at diffraction angles between $15-25\theta$. Meanwhile, the mineral hematite was detected at a diffraction angle between $35-50\theta$. Illite minerals were read through spectra between diffraction angles of $15-20\theta$ and $25-30\theta$. The presence of clay mineral types indicated the nutrient status in the soil, which can be used to measure the surface area of the soil.

Table 1. Chemical composition of clay on chemically saturated corn fields based on landscape

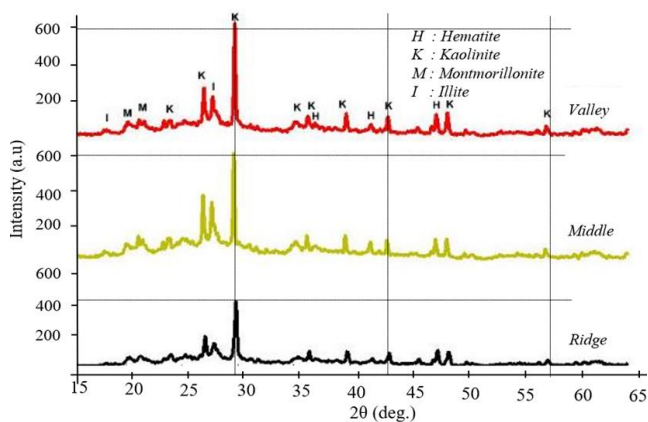| Material	Sampling area		
	Valley	Middle	Ridge
SiO_2 (%)	44.42	47.66	48.69
Fe_2O_3 (%)	26.4	20.46	27.6
CaO (%)	24.04	20.86	16.79
$K2O$ (%)	2.66	2.51	2.15
TiO_2 (%)	1.76	1.46	1.97
Al_2O_3 (%)	-	6.81	-
P_2O_5 (%)	-	-	1.29
MoO_3 (%)	0.105	0.029	-
MnO	-	-	0.26

Figure 2. X-ray diffraction pattern of clay based on the landscape of a corn plantation

Characterization of rhizosphere bacteria based on colony morphology, Gram staining, and sensitivity analysis

Isolation of soil bacteria from corn rhizosphere based on the landscape (valley, middle, and ridge) was successfully purified as many as 17 bacterial isolates, 5 (five) isolates (valley), 5 (five isolates (middle), and 7 isolates (back)). The bacterial isolates had diversity in colony morphology, gram staining, and sensitivity (Table 2.) For example, bacterial isolates from the valley area had the same shape and colony margins (circular; lobate) dominated by convex elevation with medium colony size (RJ1L; RJ3L; RJ5L). On the other hand, the sensitivity analysis showed potential pathogens from one bacterial isolate, namely RJ5L, which is classified as a gram-negative bacterium.

Moreover, the circular shape, with lobate margins and convex elevation, dominated the shape of the five rhizosphere bacterial isolates from the middle of the field. The difference between the five isolates was seen in the colony's size, where there was one isolate of medium size (RJ1T) and only one isolate that secreted yellow pigment (RJ4T). The Gram staining results detected four isolates identified as gram-positive bacteria with coccus cell form. Furthermore, only one gram-negative bacteria has bacilli cell form (RJ3T). All isolates did not show potential for plant pathogens.

The bacterial isolates isolated in the back area tended to vary in irregular (5 isolates) and round (2 isolates) colony shapes. Of the seven isolates, there were five isolates with undulate and two lobate margins. Four isolates with medium colony size and three had convex elevation (RJ1P; RJ3P, RJ6P). Only one isolate released a yellow pigment, namely RJ6P. Although the seven isolates did not have pathogenic potential, only one was classified as a gram-negative bacterium (RJ3P) with a bacillus cell shape.

The ability of bacterial isolates to produce auxin

Figure 3 shows the ability to produce auxin from 17 isolates differed between isolates and the origin of the sampling area. The auxin production ability of the five bacterial isolates from the valley section only ranged from 0.033 -0.065 mg.L^{-1}. While the ability to produce auxin from five bacterial isolates from the middle section ranged from 0.032-0.141 mg.L^{-1}. The seven bacterial isolates from the dorsal section could produce auxin between 0.037-0.081 mg.L^{-1}. Isolates with the code RJ1T had the highest auxin production capacity, 0.141 mg.L^{-1}, followed by isolates RJ3T and RJ3P. Auxins comprise a class of plant hormones that play an important role in the physical development of plants.

The ability of bacteria to fix nitrogen

Figure 4 shows the ability of 17 bacterial isolates from the corn rhizosphere to fix nitrogen. The isolate with the RJ5L code had the highest nitrogen fixation ability 0.979%), almost the same as the isolate coded RJ1P (0.975%). However, the RJ5L bacterial isolate showed positive results on sensitivity to tobacco leaves, so it has the potential to be a plant pathogen (Table 3). Thus, the RJ1P bacterial isolate can be a biological plant growth and production agent.

Molecular identification of bacteria based on 16S rRNA gene sequencing

BLAST analysis of isolates with code RJ1P was performed to find similarities in bacterial names and nucleotide sequences from isolate DNA juxtaposed with nucleotide sequences from bacterial species in the Gen Bank database. The results of the 16S rRNA gene sequence of the PGPR bacterial isolate from the rhizosphere of corn are presented in Table 3. Based on the alignment results in the NCBI database through the BLAST-N program, RJ1P has a similarity (max identity value) of 99.57% to the *Nocardioides marinisabuli* strain *RA2816S ribosomal RNA gene*. In addition, a phylogenetic tree was constructed based on the 16S rDNA sequence obtained where the RJ1P isolate represents the *Nocardioidaceae* family (Figure 5). With the similarity of the 16S rRNA gene sequence, its phylogenetically closest neighbor is *Nocardioides sp.* S2-43 (99.21%), *Nocardioides lianchengensis* (99.14%).
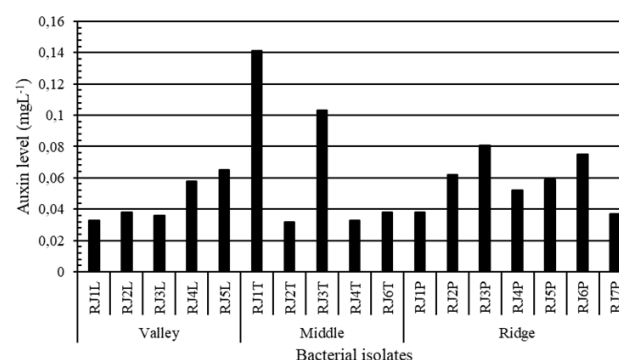

Figure 3. Ability to produce auxin isolates of corn rhizosphere bacteria isolated based on landscape
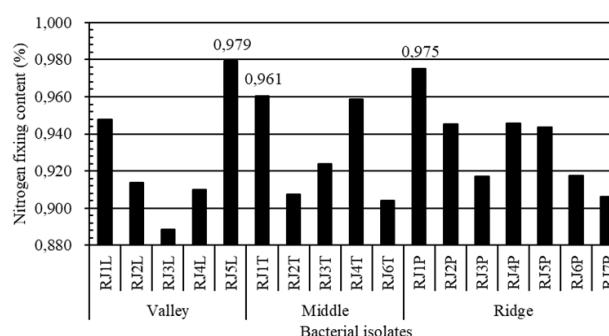

Figure 4. The nitrogen-fixing ability of corn rhizosphere bacteria isolates based on landscape
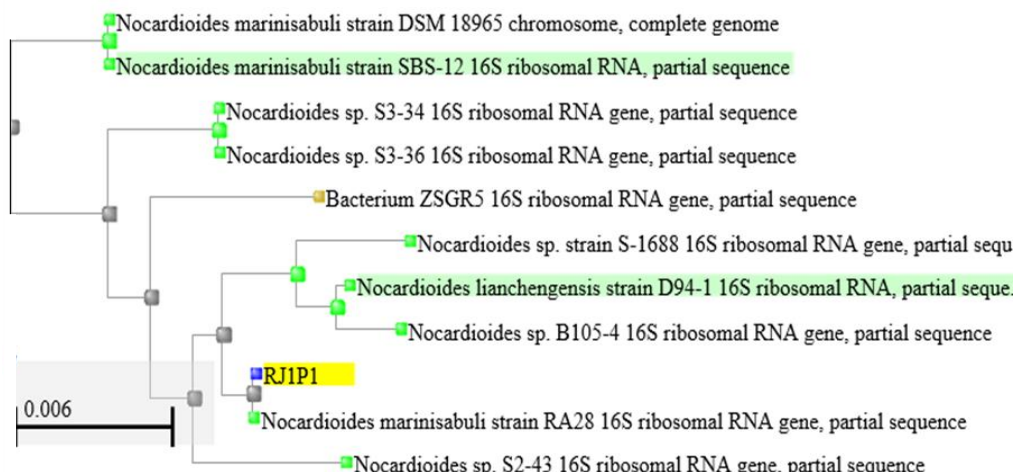
Figure 5. Phylogenic tree of bacterial isolate RJ1P based on 16S rDNA sequence

Table 2. Characterization of bacteria from the maize rhizosphere based on morphology, Gram staining, and sensitivity (Isolate code followed by letters (L=Valley; P=Ridge; T=Middle) indicates the position of the landscape

Isolates code	Morphological characteristic					Gram stain		Sensitivity analysis
	Colony shape	Margin	Elevation	Small	Color	Cells shape	Gram	
RJ1L	Circular	Lobate	Convex	Medium	Orange	Baciliicoccus	+	-
RJ2L	Circular	Lobate	Convex	Small	Beige	Bacilli	-	-
RJ3L	Circular	Lobate	Convex	Medium	Beige	Bacilli	-	-
RJ4L	Circular	Lobate	Flat	Small	Beige	Bacilli	-	-
RJ5L	Circular	Lobate	Convex	Medium	Beige	Baciliicoccus	-	+
RJ1T	Circular	Lobate	Convex	Medium	Beige	Coccus	+	-
RJ2T	Circular	Lobate	Convex	Small	Beige	Coccus	+	-
RJ3T	Circular	Lobate	Convex	Small	Beige	Bacilli	-	-
RJ4T	Circular	Lobate	Convex	Small	Yellow	Coccus	+	-
RJ6T	Circular	Lobate	Convex	Small	Beige	Coccus	+	-
RJ1P	Irregular	Undulate	Convex	Medium	Beige	Coccus	+	-
RJ2P	Irregular	Undulate	Flat	Small	Beige	Coccus	+	-
RJ3P	Irregular	Lobate	Convex	Medium	Beige	Bacilli	-	-
RJ4P	Circular	Lobate	Flat	Small	Beige	Bacilli	+	-
RJ5P	Irregular	Undulate	Flat	Small	Beige	Bacilli	+	-
RJ6P	Circular	Undulate	Convex	Medium	Yellow	Coccus	+	-
RJ7P	Irregular	Undulate	Convex	Small	Beige	Bacilli	+	-

Table 3. BLAST analysis RJ1P bacterial isolate isolated from corn rhizosphere

Scientific name	Query cover	E value	Percent identification	Accession
Nocardioides marinisabuli	99%	0.0	99.57%	JN585687.1
Nocardioides sp. S2-43	99%	0.0	99.21%	JQ660057.1
Nocardioides lianchengensis	99%	0.0	99.14%	NR_118013.1
Nocardioides sp. S3-34	99%	0.0	98.92%	JQ660094.1
Nocardioides sp. S3-36	99%	0.0	98.92%	JQ660097.1
Bacteriun ZSGR5	100%	0.0	98.71%	KC577563.1
Nocardioides sp.B105-4	99%	0.0	98.85%	KJ191042.1
Nocardioides sp.	99%	0.0	98.07%	MK621198.1
Nocardioides marinisabuli	99%	0.0	97.93%	CP059163.1
Nocardioides marinisabuli	99%	0.0	97.93%	NR_042590.1

Discussion

The interaction of clay minerals with soil organic carbon is an important mechanism for maintaining soil fertility and ecosystems. The analyses on the maize rhizosphere area show that kaolinite dominated the intervention area. The XRD spectrum is used to identify the type of mineral in the soil sample. The kaolinite mineral diffracted evenly between 25-60θ. X-ray diffraction techniques are useful in determining clay minerals' shape and size characteristics (Ali et al. 2022). Therefore, it can be assumed that kaolinite minerals dominate clay in the rhizosphere region. In addition, the absorption distribution increases in the valley area. It was then followed by decreasing from the middle to the ridge area. This follows the report of Horabik and Jozefaciuk (2021) that kaolinite interacts with soil structure and spreads widely in influencing soil hydrology. Kaolinite is a representative 1:1 layer-type clay mineral, the main clay mineral in tropical and subtropical soils (Wu et al. 2014). Kaolinite does not swell in water and has a low surface area and cation exchange capacity (<1 centimole/kg). Kaolinite has a low capacity to adsorb ions, only around 10-70 $m^2.g^{-1}$ (Kumari and Mohan 2021)) and a low ability to bind water. Because kaolinite is a 1:1 clay mineral with tetrahedral silicate sheets and octahedral hydroxide sheets. That greatly affects soil moisture because water stored between silicate sheets has a low capacity to absorb cations and has low fertility. The role of clay minerals in stabilizing soil organic matter differs according to surface area, namely allophane>smectite>illite>kaolinite.

The kaolinite mineral dominance, also shown by the XRF spectrum, detects the presence of silicate minerals with a content of >40% in all parts of the landscape (Table 1). The ratio of SiO2 in a formula is a key factor determining the clay minerals kaolinite type. That included halloysite, illite, and montmorillonite. In addition, clay, which is dominated by kaolinite minerals, can bind more than 75% of P. Fe_2O_3 content >20% (Table 1), which according to Mohsen and El-maghraby (2010), is relatively high. Fe oxide is a charged mineral that can retain element P but is unavailable to plants (Singh and Schulze 2015). It can be seen that the P_2O_5 compound was only detected in soil originating from the back of the land. Important factors facilitating soil particle aggregation are clay particles and clay minerals type; cations such as Ca; om; colloidal matter such as Fe and Al oxides; plant roots; and soil microbes (FAO 2008). Clay mineral interactions are involved in soil degradation, distribution and type of microbial biomass in the soil and water retention (Bradford et al. 2013; Patel et al. 2017)

The diversity of bacterial isolates from the corn rhizosphere varied in the morphology of the bacterial colonies from the three parts of the field. Corn rhizosphere bacteria isolates from the valleys, and the middle of the field tended to have almost the same morphology, namely circular, lobate margin margins, and convex elevation shapes. At the same time, the average grain was unpigmented (beige). On the other hand, the dorsal area is dominated by irregularly shaped bacterial colonies with undulate margins and convex elevation. The rhizosphere

microbial diversity is influenced by their physical and chemical properties, which are partly determined by the genetics of the host plant. Community composition differs significantly by mineral type mineral (Whitman et al. 2017). The relative abundance of mineral-associated microbial communities differs from that of soil communities.

Actinobacteria show both positive and negative responses. Spatially minerals are a key factor in the success of bacterial colonization. The relatively rare distribution of resources in the mineral kaolinite may result in stronger selective pressures for microbial attachment, also contributing to the increasing importance of variable selection and competition in these communities. Soil microbial community composition is influenced by soil structure and geographic location (Gelsomino et al. 1999), soil particle size, mineral composition (Carson et al. 2010), and agricultural practices (Benizri and Kidd 2018), while microbial activity is strongly influenced by plant roots (Bais et al. 2010). Clay dominated by kaolin tends to be infertile, so it does not support the diversity of bacteria. That indicated the distribution of bacteria in this study tended to be uniform in terms of colony morphology and cell shape. The uniform colony shape indicates that the environment does not provide a variety of nutrient sources. Cell size and shape control is essential for bacterial growth, proliferation, nutrient access, motility, and adhesion (Wu et al. 2014). It is known that bacterial morphology is highly adaptive and varies with growth conditions. Corn cultivation in monoculture causes the soil organic matter content to decrease. In addition, the dominant kaolin content with a low specific surface area causes a lack of organic elements adsorbed on the soil surface, where clay minerals provide nutrients and habitat for microorganisms (Mcmahon et al. 2016). This results in a less diverse energy source for bacteria. This is reflected in the uniform colony shape based on the landscape. The specific shape is a consequence of adaptive pressures that optimize bacterial growth, including critical biological functions, nutrient acquisition, dispersion, stress resistance, and interactions with other organisms (van Teeseling et al. 2017)

The effect of PGPR increases plant growth through various mechanisms, including producing plant hormones, such as auxin and free nitrogen fixation which are transferred into plants. In addition, growth hormone is produced as a bacterial response to extreme environments. This study identified 17 bacterial isolates isolated from chemically saturated corn rhizosphere as capable of producing auxin. Isolates coded RJ1T, and RJT3 produced the highest auxin, 0.141 mg.L^{-1} and 0.103 mg.L^{-1}. However, Auxin production from these bacterial isolates is still relatively low, <2 mgL^{-1} (Fatmawati et al. 2019). RJ1T bacterial isolate is a gram-positive bacterium with a coccus cell shape (Figure 6). Research by Kesaulya et al. (2017) confirmed auxin production from several gram-positive bacteria ranging from 0.39-5.8 mg.L^{-1}. Actinomecetas bacteria can produce IAA ranging from 0.33-36 mgL^{-1} (Fatmawati et al. 2019).

Figure 6. Characteristics of three superior bacterial isolates in producing the hormone auxin and nitrogen fixation based on morphology, Gram staining, and sensitivity analysis

The study's low auxin production of the bacterial isolates was probably caused by the extreme environment, where corn fields received very high chemical fertilizer inputs with eroded land conditions. Rhizobacteria must modify their phytohormonal activity to induce plant drought tolerance (Vurukonda et al. 2016) evertheless, the production of Indole-3-Acetic Acid (IAA), thus Actinomycetes bacteria which colonize the rhizosphere of plants has a vital role in increasing plant growth. Where auxin-producing PGPR is a better alternative to fight abiotic stress (Raheem et al. 2017).

One of the advantages of rhizosphere bacteria is their ability to fix free nitrogen. From the results of this study, the highest range of free nitrogen fixation was 0.961-0.979%. Corn rhizosphere bacteria isolates coded RJ5L fix nitrogen as much as 0.979%. Higher than the results of a study by Sukmawati et al. (2020), where it was reported that gram-negative bacterial isolates from the corn rhizosphere had nitrogen content (0.36%). Changes in these bacterial species may have a close relationship with root exudates from maize. For example, nitrogen-fixing bacteria are sensitive to sugars and amino acids in root exudates. That results in many bacteria with a nitrogen-fixing function in the rhizosphere (Gupta Sood 2003). The RJ1P isolate isolated from the corn rhizosphere in the back area of the field was able to fix nitrogen almost the same as the RJ5L isolate. The advantage of this isolate is that it does not cause a positive reaction from sensitivity to tobacco leaves. RJ5L isolate is a Gram-positive bacterium with a coccus cell shape. The results of nitrogen fixation vary depending on the bacterial strain and environmental conditions (Navarro-Noya et al. 2012). Microbial biomass carbon and nitrogen are indicators for assessing soil microbial community size (Fierer et al. 2012).

Moreover, with various considerations, the intervention area contains high clay with the dominance of the infertile mineral kaolinite and high input of urea; RJ1P isolate can be developed as a biological agent on dry land. Therefore, genetic tracing was carried out to find out the species of this isolate. Based on the 165rRna gene sequence, it was identified that RJ1P isolate had a very close relationship with *Nocardioides marinisabuli* strain RA2816S ribosomal RNA gene by 99%. The results of the morphological characterization of RJ1P isolate were irregular colony shapes, undulate edges, convex elevation, medium size with a cream color belonging to a gram-positive bacterium with a coccus cell shape. This isolate is almost the same morphological character as *Nocardioides marinisabuli sp.* isolated by Lee et al. (2007), with similarities as a Gram-positive rod, does not form spores, and is immobile (0.6-0.8×1.4-2.1 μm) colonies of opaque, convex, circular and pale yellow cells with an entire margin, and reaching a diameter of about 1.0-1.5 mm. On the other hand, research by Jun et al. (2021) claims a new species from the genus Nocardioides isolated from kaolinite, is gram-positive, non-motile, rod-shaped, and short bacterium.This bacterium is also found in the rhizosphere of corn (Yang et al. 2017). It belongs to the *Actinobacteria* genera, which are abundant in soil and water ecosystems and play an important role in decomposition. humus position and formation (Rousk et al. 2010). Gram staining showed gram-positive rod-shaped bacteria could not form spores (Dubourg et al. 2016). Nevertheless, most *Nocardioides* species usually exhibit a coccoid-stem morphogenetic cycle and can move with gram-stain-positive cell walls with non-pigmented colonies and cream markings (Evtushenko and Arskina 2015).

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