

Ultisols fertility and morphological characteristics of N-fixing bacteria from oil palm rhizosphere¹

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ABSTRACT

Exploratory studies on microorganisms from the oil palm rhizosphere can be used to increase the nitrogen availability in acidic soils. The present study aimed to determine the soil nutrients and obtain the relationship among the soil chemical characteristics, as well as the morphological and microscopic characteristics of N-fixing bacteria isolates, in Ultisols from the oil palm rhizosphere. The Ultisols fertility was classified as very low to moderate. In addition, the increasing soil pH toward neutral increased the cation exchange capacity, exchangeable cations (Ca^{2+} , K^+ and Mg^{2+}), total N, organic C and available P. Nitrogen-fixing bacteria isolates with varied shapes (circular, concentric, irregular and diffuse) and edges (wavy, slippery and irregular) were found, and the dominant type of isolate presented raised elevation. Four types of isolate had a white color and only one a clear color. Three Gram-positive and two Gram-negative bacteria isolates showed a bacillus shape.

KEYWORDS: *Elaeis guineensis*, soil microorganisms, Gram-positive and Gram-negative bacteria.

Ultisols usually present problems such as acidic soil and low organic matter, macronutrients contents and available P (Fitriatin et al. 2014). Mulyani et al. (2010) stated that, for Ultisols, the cation exchange capacity (CEC), base saturation and organic C are classified as low, while the Al saturation and P fixation are classified as high, and the contents of Fe and Mn approach the toxic limit for plants. Syahputra et al. (2015) also reported that the contents of organic C, total N, total P, available P, exchangeable K, CEC and base saturation in each Ultisol sub-group, such as Typic Hapludults, Typic Paleudults, Psammentic Paleudults, Typic Plinthudults, Typic Ochraquults and Typic Paleaquults, in several areas of North

RESUMO

Fertilidade de Argissolos e características morfológicas de bactérias fixadoras de nitrogênio da rizosfera de dendezeiro

Estudos exploratórios de micro-organismos da rizosfera de dendezeiro podem ser utilizados para aumentar a disponibilidade de nitrogênio em solos ácidos. Objetivou-se determinar os nutrientes e obter a relação entre as características químicas do solo, bem como as características morfológicas e microscópicas de isolados de bactérias fixadoras de N, em Argissolos da rizosfera de dendezeiro. A fertilidade dos Argissolos foi classificada de muito baixa a moderada. Além disso, o aumento do pH do solo rumo ao neutro aumentou a capacidade de troca catiônica, cátions trocáveis (Ca^{2+} , K^+ e Mg^{2+}), N total, C orgânico e P disponível. Foram encontrados isolados de bactérias fixadoras de N com formas (circular, concêntrica, irregular e difusa) e bordas (ondulada, escorregadia e irregular) variadas, e o tipo dominante de isolado apresentou elevação aumentada. Quatro tipos de isolado mostraram cor branca e somente um cor clara. Três isolados de bactéria Gram-positiva e dois de Gram-negativa apresentaram formato de bacilo.

PALAVRAS-CHAVE: *Elaeis guineensis*, micro-organismos do solo, bactérias Gram-positivas e Gram-negativas.

Sumatra, are classified as very low to low, except for the CEC on Typic Paleudults with moderate criteria.

Ultisols problems affect the root growth, which interferes with the absorption of nutrients needed by oil palm plants. Ajeng et al. (2020) reported that the root growth of oil palm seedlings in Ultisols is lower, when compared to Histosols, Spodosols and Oxisols, even with the application of NPK fertilizer (12:12:17). Yahya et al. (2010) reported that oil palm seedlings in denser soils, particularly Oxisols and Ultisols, have lower primary and secondary roots, but produce longer and thicker tertiary and quaternary roots. Naibaho et al. (2019) also reported that Al doses of 1.5-3.0 g could inhibit the size of the root epidermis, cortex and stele, when compared to the control.

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Efforts are needed to increase the efficiency and sub-optimal land productivity, in areas with Ultisols, using the potential soil biology to increase the availability and transformation of nutrients that support the plant growth (Herman & Pranowo 2013). The use of microbes as potential biological agents could increase the efficiency of inputs, especially fertilization (Ajmal et al. 2018), to provide nutrients for the plant rhizosphere (Okur 2018). Several microorganisms found in the rhizosphere could increase the growth and nutrient uptake of oil palm, including the use of N-fixing bacteria (Amir et al. 2001, Amir et al. 2005) such as *Acetobacter diazotrophicus* (Om et al. 2009) and *Bacillus sphaericus* (Zakry et al. 2012), and the phyla of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadates*, *Nitrospirae*, *Proteobacteria* and *Thermotogae* (Schneider et al. 2015). Furthermore, Zakry et al. (2019) reported that 7 of 30 PGPR isolates from oil palm roots (RHI1, RHI3, RHI7, EX3, EX9, EN5 and EN9) could function as N fixers. Khairani et al. (2019) also reported that three genera of rhizosphere bacteria (*Bacillus*, *Arthrobacter* and *Pseudomonas*) were found in oil palm aged 14 years.

The exploration research of rhizosphere bacteria in mature oil palm on acidic soils such as Ultisols with potential age is infrequently reported, especially on the experimental field scale. Thus, it is necessary to explore rhizosphere bacteria on acidic soils through conventional approaches such as color, elevation, edges and Gram staining to detect the shape of bacterial cells, as the first stage to identify colony morphological characteristics. Thus, the present study aimed to determine the soil nutrients and obtain the relationship among the soil chemical characteristics, as well as the morphological and microscopic characteristics of N-fixing bacteria isolates, in Ultisols from the oil palm rhizosphere.

The experiment was conducted at the Universitas Labuhanbatu, North Sumatra, Indonesia, from March to June 2020.

Soil samples were collected using the random composite sampling method by taking five locations, from 15-year-old oil palm rhizospheres (N1, N2, N3, N4 and N5), at a soil depth of 0-20 cm and radius of 30-40 cm around plants grown in Ultisols (USDA 2014), totaling 1.0 kg of soil.

Soil samples of 100 g were collected to evaluate the chemical characteristics of Ultisols by

analyzing the soil pH (H_2O and KCl) with the electric method, total N by the Kjeldahl method, organic C by the Walkley and Black method, available P by the Bray-II method, cation exchange capacity (CEC) and base saturation (K, Ca, Mg and Na) with the NH_4OAc extraction method (pH 7).

The growing media was prepared using the Jensen selective method for fixing the non-symbiotic N bacteria by weighing 20 g of sucrose, 1 g of K_2HPO_4 , 0.5 g of $MgSO_4$, 0.5 g of NaCl, 0.1 g of $FeSO_4$, 2 g of $CaCO_3$ and 2 g of nutrient agar inserted into Erlenmeyer flasks, dissolved in 200 mL of distilled water, and cooked using the hotplate until becoming homogeneous. The media was covered with cotton and the research tools were wrapped in aluminum foil to be sterilized by autoclave at the temperature of 121 °C, for 20 min.

Soil samples of 10 g were inserted into a measuring cup, and then 90 mL of distilled water were added and the mixture was homogenized for 10 min. The dilution technique was conducted in the soil suspension by taking 1 mL of the sample suspension, then pipetting it and putting it in a test tube containing 9 mL of distilled water (10^{-1}). Then, 1 mL of the 10^{-1} suspension was pipetted and put into a test tube containing 9 mL of distilled water (10^{-2}), thereby performing the dilution factors from 10^{-3} to 10^{-8} .

One milliliter of each dilution factor was taken using a micropipette, and then inoculated on a Petri dish containing Jensen media, using the pouring method. The soil suspension was incubated at 35 °C, for 48 h, until growing colonies were obtained. The selection of purified microbial colonies was made based on differences in the appearance of the colony morphology, including shape, elevation, edges and color, to obtain pure isolates (Figure 1). The purification of bacterial isolates was conducted by removing and growing bacteria on nutrient agar media, using a sterilized inoculating loop and scratching on the media surface.

Microscopic observations were made on nutrient agar through Gram staining. The slides were prepared with 70 % alcohol, and then sterilized over an alcohol flame. The bacterial suspension was placed on the slides using an inoculating loop and then flattened. It was stained with 2 to 3 drops of a crystal violet solution for 1 min and then washed with water and dried, as well as with an iodine solution and left to rest for 1 min, then washed with

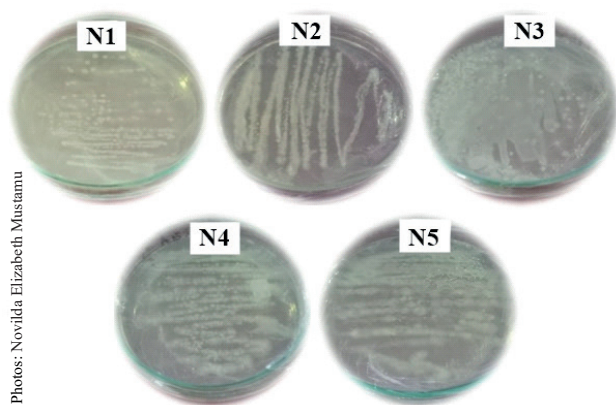


Figure 1. Pure isolates of N-fixing bacteria (N1-N5 isolates) from the oil palm rhizosphere.

water and dried. The sample was washed with 70 % alcohol for 30 s, then washed with water and dried. It was then stained with a safranin solution for 2 min, then washed with water and dried. The slide was observed under a microscope with magnification of 50-100X. Gram-positive bacteria absorbed the violet color, while Gram-negative bacteria absorbed the red color.

The Ultisols chemical characteristics were analyzed using standard errors, processed by natural logarithms, and then processed using the Kendall's tau for correlation analysis with the SPSS statistical software v.20 (IBM 2011), while the isolate data and morphological characteristics of each isolate were analyzed descriptively.

The chemical characteristics of the Ultisols from the oil palm rhizosphere are presented in Table 1.

The results showed that the chemical characteristics of the Ultisols from the oil palm rhizosphere were classified as very low to moderate. However, this was in contrast to the available P content, which was classified as very high. This resulted in a difference of -0.48 for soil pH (between KCl and H₂O), which means that the Ultisols in the study location were dominated by variably charged minerals. This is supported by the correlation coefficient value of soil pH with available P of 0.738 (Table 2). According to Ueharaan & Gillman (1981), if the difference for soil pH is positive, zero or smaller than -0.5, the soil is dominated by variable charge minerals. Gillman (2007) stated that the negative charge is dominated at high pH; while the positive charge is found at low pH values. Tampubolon et al. (2019) reported that the soil pH of oil palm, in the Teluk Panji state (south Labuhanbatu district, North Sumatra province, Indonesia), was classified as acidic, with a pH difference of -0.46, and the available P was classified as very high. Furthermore, Allen et al. (2015) stated that the soil pH from oil palm in the Jambi province, Indonesia, ranged from 4.4 to 4.6. Kurniawan et al. (2018) reported that the soil pH at three locations in oil palm plantations (weeded circles, frond pile and harvest path) ranged from 4.41 to 4.53.

The correlation analysis for the chemical characteristics of Ultisols from the oil palm rhizosphere are presented in Table 2.

The results showed that the soil pH was significantly and positively correlated with the CEC and exchangeable Ca of 0.949 and 0.882, respectively, while there was an insignificant effect

Table 1. Chemical characteristics of the Ultisols from the oil palm rhizosphere (0-20 cm depth).

Chemical characteristics	Value \pm standard error	Category
pH-H ₂ O	4.18 \pm 0.09	Very acidic
pH-KCl	3.70 \pm 0.11	-
Δ pH	-0.48 \pm 0.17	-
Total N (%)	0.26 \pm 0.03	Moderate
Organic C (%)	1.09 \pm 0.34	Low
Available P (mg kg ⁻¹)	26.66 \pm 8.67	Very high
CEC (cmol kg ⁻¹)	19.23 \pm 0.79	Moderate
Exchangeable K (me 100 g ⁻¹)	0.35 \pm 0.14	Low
Exchangeable Ca (me 100 g ⁻¹)	0.25 \pm 0.16	Very low
Exchangeable Mg (me 100 g ⁻¹)	0.16 \pm 0.06	Very low
Exchangeable Na (me 100 g ⁻¹)	0.21 \pm 0.04	Low

pH-H₂O: very acidic < 4.5; total N: moderate = 0.21-0.5 %; organic C: low = 1-2 %; available P: very high > 15 mg kg⁻¹; CEC: moderate = 17-24.99 me 100 g⁻¹; exchangeable K: low = 0.1-0.39 me 100 g⁻¹; exchangeable Ca: very low < 2 me 100 g⁻¹; exchangeable Mg: very low < 0.4 me 100 g⁻¹; exchangeable Na: low = 0.1-0.39 me 100 g⁻¹. Source: ISRI (2009).

Table 2. Relationship among the chemical characteristics (0-20 cm depth) of the Ultisols from the oil palm rhizosphere.

Chemical characteristics	pH	TN	OC	AP	CEC	K-Ex	Ca-Ex	Mg-Ex	Na-Ex
pH	1								
TN	0.667	1							
OC	0.527	0.738	1						
AP	0.738	0.949*	0.800	1					
CEC	0.949*	0.738	0.600	0.800	1				
K-Ex	0.354	0.354	0.447	0.447	0.447	1			
Ca-Ex	0.882*	0.882*	0.598	0.837	0.837	0.267	1		
Mg-Ex	0.667	1.000**	0.738	0.949*	0.738	0.354	0.882*	1	
Na-Ex	-0.316	-0.527	-0.800	-0.600	-0.400	-0.224	-0.359	-0.527	1

* and **: significant at the 0.05 and 0.01 levels, respectively; pH (pH-H₂O); TN: total N; OC: organic C; AP: available P; CEC: cation exchange capacity; K-Ex: exchangeable K; Ca-Ex: exchangeable Ca; Mg-Ex: exchangeable Mg; Na-Ex: exchangeable Na.

and a positive correlation between the soil pH and the total N, organic C, available P, exchangeable K and exchangeable Mg, as well as an insignificant effect and a negative correlation with the exchangeable Na. The soil pH increased in value toward neutral with the increase in the CEC, as well as the exchangeable Ca, total N, organic C, available P, exchangeable K and exchangeable Mg of the Ultisols. According to Kinraide (1993) and Silva (2012), very acidic soils can be caused by Al³⁺, Cu²⁺, Fe³⁺ and Mn²⁺ ions, which could be toxic in plants. Andersson et al. (2000) pointed out that the soil pH could increase the solubility of the soil organic matter by increasing the dissociation of acidic functional groups. Tsado et al. (2014) and Desta (2015) stated that organic acids in acidic soils would chelate Al and Fe, thus increasing the soil pH. Prasetyo et al. (2005) emphasized that the relationship between the CEC and the organic C content tends to reach a positive effect with $r^2 = 0.47$, and that the addition of organic matter increases the CEC of Ultisols.

The morphological characteristics of the N-fixing bacteria isolates from the oil palm rhizosphere, based on shape, elevation, edges and color, are presented in Table 3.

The results showed that the shape of the N-fixing bacteria isolates varied widely, namely

circular, concentric, irregular and diffuse. The dominant types of isolates (N1, N3, N4 and N5) showed raised elevation, and only one isolate (N2) had flat elevation. The edges of the isolates also varied, namely wavy, slippery and irregular. It was found that four isolates (N1-N4) had a white color, and only one isolate (N5) had a clear color. This suggests that the N-fixing bacteria isolates from the oil palm rhizosphere are still able to survive in very acidic soil conditions (pH 4.18), or the bacteria were classified as acidophilic microbes. According to Waluyo (2004), microbes that could be growing within a soil pH ranging from 2 to 5 are classified as acidophilic.

Agustian et al. (2012) reported that the *Azotobacter* isolate A₃b from the *Tithonia diversifolia* rhizosphere on Ultisols is more tolerant, when compared to the other isolates in acidic soils (pH 4). Wolff et al. (1993) stated that several strains of rhizobia were still viable at pH 5, while most rhizobial strains did not develop in the soil at a pH of 4.4, and the infection process was also inhibited. The optimal pH for rhizobium ranges from slightly neutral to slightly alkaline. Several rhizobia are susceptible to low pH and do not infect the root hairs in acidic soils. Weisany et al. (2013) stated that acidic soils cause Ca deficiency, as well as Al and

Table 3. Morphological characteristics of N-fixing bacteria isolates from the oil palm rhizosphere.

Isolates	Shape	Elevation	Edges	Color
N1	Circular	Raised	Wavy	White
N2	Circular	Flat	Slippery	White
N3	Circular	Raised	Wavy	White
N4	Concentric	Raised	Slippery	White
N5	Irregular and diffuse	Raised	Irregular	Clear

Mn toxicity, thus inhibiting nodulation and nitrogen fixation. Khairani et al. (2019) found 18 bacteria isolates from the oil palm rhizosphere at the ages of 8, 11 and 14 years within the pH range of 6.2 to 6.8. Furthermore, Schneider et al. (2015) found that Firmicutes, Proteobacteria and Actinobacteria phyla were found in the rhizosphere of oil palm with soil pH ranging from 6.2 to 7.5. Gao et al. (2016) also reported that Proteobacteria and Actinobacteria were found in environment with pH ranging from 5.5 to 8.2 and 7 to 8, respectively.

The microscopic characteristics of the N-fixing bacteria isolates from the oil palm rhizosphere are shown in Table 4, and the Gram staining of the isolates in Figure 2.

The results showed three Gram-positive (N1, N4 and N5) and two Gram-negative (N2 and N3) N-fixing bacteria isolates from the oil palm rhizosphere and all isolates had a bacillus shape. The difference between the Gram-positive and Gram-negative bacteria indicates differences in the structure of the cell wall, with thicker peptidoglycan contents for the Gram-positive and higher lipid contents for the Gram-negative bacteria. This suggests that the soil conditions for the oil palm plants in the present

experiment are classified as fertile, based on the microbial status supporting the root growth and indirectly increasing the nutrient uptake.

According to Trivedi et al. (2010), the Gram-positive bacteria have more peptidoglycan inside the cell wall than the Gram-negative ones. The cell walls of the Gram-positive bacteria had peptidoglycan ranging from 40 to 80 % by dry weight and were thicker when compared to the cell walls of Gram-negative bacteria, but the Gram-negative bacteria had higher lipid percentages when compared to the Gram-positive ones. Seung et al. (2015) reported rhizosphere bacteria from oil palm consisting of the genera *Pseudomonas*, *Bacillus* and *Burkholderia*. Susanti (2014) pointed out that the bacterial isolates B6, B9, B3, B7, A7, A10, C1 and C4 that were explored from the oil palm rhizosphere were non-pathogenic. Himawan & Mawandha (2018) also reported one species of bacteria from the oil palm rhizosphere in mineral soil using the PCR-RISA method, but the species was unknown.

The chemical characteristics of the Ultisols from the oil palm rhizosphere were classified as very low to moderate, but the available P content was very high, and the correlation analysis showed that the increase in the soil pH value toward neutral resulted in an increase in the CEC, exchangeable cations (Ca^{2+} , K^+ and Mg^{2+}), total N, organic C and available P.

In the present research, five N-fixing bacteria were isolated from the oil palm rhizosphere, with varied shapes (circular, concentric, irregular and diffuse) and edges (wavy, slippery and irregular), and the dominant type of isolates showed a raised elevation. Four types of isolates presented a white color and only one a clear color. Three Gram-positive and two Gram-negative bacteria isolates showed a bacillus shape.

Table 4. Microscopic characteristics of N-fixing bacteria isolates from the oil palm rhizosphere.

Isolates	Gram staining	Cell form
N1	+	Bacillus
N2	-	Bacillus
N3	-	Bacillus
N4	+	Bacillus
N5	+	Bacillus

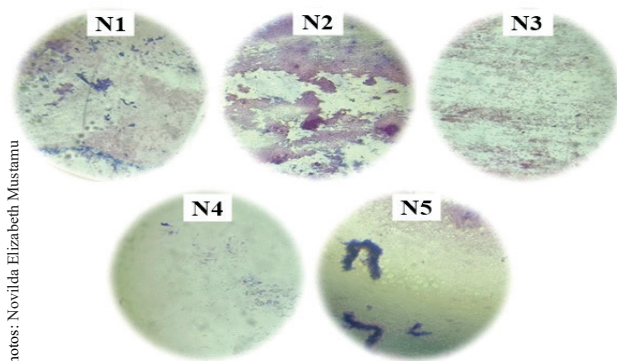


Figure 2. Gram staining of the N-fixing bacteria isolates from the oil palm rhizosphere: Gram-positive (N1, N4 and N5 isolates) and Gram-negative (N2 and N3 isolates).

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