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Bacterial diversity in different growing mediums of oil palm seedlings associated with potassium use efficiency

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Abstract. *Wening S, Setiowati RD, Pratiwi DR, Pangaribuan IF, Ginting EN. Bacterial diversity in different growing mediums of oil palm seedlings associated with potassium use efficiency. Biodiversitas 25: 3386-3393.* Plant genotype, environmental conditions, and agronomical practices influence nutrient use efficiency. Understanding whether specific microbial communities support the nutrient use efficiency of specific genotypes is very important. This study aimed to understand the bacterial diversity in three different growing mediums of oil palm seedlings with potassium-use efficient characters. The three kinds of medium were medium 1 (sandy soil, recommended dose of fertilizer except for potassium (75% of a recommended dose of potassium)); medium 2 (sandy soil, recommended dose of fertilizer except for potassium (0% of potassium)); and medium 3 (topsoil, recommended dose of fertilizer at five months after planting). A metagenomic approach was conducted using a long-read sequencing platform, allowing the full-length 16S rRNA gene to be sequenced. The alpha diversity analysis showed that medium 3 had the most identified species, while medium 2 had the lowest. Based on the relative abundance, *Lactobacillus helveticus* was the most abundant species, and *Lactobacillus* was the most abundant genus in medium 1. *Ralstonia pickettii* was the most abundant species, and *Dyella* was the most abundant genus in medium 2. For medium 3, *Chujaibacter soli* was the most abundant species that dominated the bacteria community, and *Lactobacillus* was the most abundant genus. These findings were related to the bacteria's function in the respective medium for plant nutrient uptake or utilization. The number of shared species between medium 1 and medium 3 was the highest compared to other medium compositions. It suggested the specific relationship of the potassium-use efficient seedling with the characteristic of the growing medium and the most abundant species or genus of bacteria found. Further research is needed to understand the interactions and influences of bacteria and other microorganisms in the mediums, and the specific genus or species found in potassium-use efficient oil palm seedling mediums.

Keywords: Abundance, fertilizer, metagenomic analysis, microbial communities, nutrient use efficiency

Abbreviations: NUE: Nutrient Use Efficiency, OTU: Operating Taxonomic Unit, NCBI: National Center for Biotechnology Information, bp: base pair, LAB: Lactic Acid Bacteria, PCoA: Principal Coordinates Analysis

INTRODUCTION

Plants, including oil palm, need sufficient and balanced nutrients for their metabolism to grow and develop optimally (López-Arredondo et al. 2017; Soumare et al. 2023). For this demand, oil palm planters supply the plant's nutrient needs through fertilizer application. Meanwhile, the costs that must be spent on fertilizer are high, around 40-70% of the total maintenance costs (Ginting et al. 2021). However, there were about 30-70% of nutrient losses in conventional fertilizer applications, depending on the application method and soil condition (Jin et al. 2011). Besides the inefficiency, the excessive application of inorganic fertilizer can adversely affect the ecosystem (Salamat 2021). Therefore, efficient use of nutrients/fertilizers is very essential for sustainable oil palm plantations. Nutrient Use Efficiency or NUE is the interaction between nutrient acquisition and nutrient utilization, which is influenced by several factors such as plant genotype, environmental conditions, as well as technological and agronomic practices (López-Arredondo et al. 2017; Nieves-Cordones et al. 2020). So, nutrient use efficiency can be achieved by comprehensively managing these factors.

Potassium is one of the macronutrients that is often the limiting factor for optimal plant development, including oil palm (López-Arredondo et al. 2017; Irawan and Putra 2020). It is involved in the osmoregulation of water use and the mechanism of plant tolerance to biotic and abiotic stress (López-Arredondo et al. 2017). There have been several studies on the efficiency of nutrient or potassium use. It included the usage of controlled-release fertilizer to minimize the nutrient loss in oil palm cultivation in the field (Bah et al. 2014), transcriptomic and genomic studies of genes associated with nutrient use efficiency (Maryanto et al. 2020; Maryanto et al. 2021), metabolic data for indication of oil palm potassium status and the relation to its potential growth (Cui et al. 2021), and identification of genetic materials with nutrient-use efficient characters (Afrillah et al. 2018; Chankaew et al. 2019).

There have also been studies about beneficial microbes in the availability of nutrients for plants and microbial diversity of plant ecosystems (Situmorang et al. 2016; Herdiyantoro et al. 2018; Sun et al. 2020; Verdiani et al. 2021). López-Arredondo et al. (2017) explained that the nutrients should be in specific form so that plants can utilize them. Potassium is naturally present in the soil but in an inaccessible form. It is available for plant uptake as a positively charged ion (K^+). Potassium solubilizing microbes could enhance potassium uptake for plants by providing soluble potassium from insoluble potassium form (Olaniyan et al. 2022). Specific bacteria fixate N_2 and change to NH_4^+, which can be used in plant metabolism (López-Arredondo et al. 2017). On the other hand, bacteria take advantage of plants for their life. Specific metabolites released by a plant may recruit bacteria and make an association as holobiont (Hassani et al. 2018). The lack or abundance of particular molecules in the rhizosphere can determine the kind of microbes that attract to the plant. Depending on the changing environment, interactions between plant microbes might change dynamically (Jain et al. 2020). There were variabilities of the microbial diversity in different ecosystems, such as in pesticidal and non-pesticidal paddy fields (Susanti et al. 2023), along a gradient of increasing land-use perturbation in 715 sites across 24 European countries (Labouyrie et al. 2023), and in tropical rainforest and oil palm plantation (Wibowo et al. 2022). The microbial diversity analysis could use a metagenomic approach, as it can detect microbes without culturing them (Garza and Dutilh 2015). The approach could use functional gene screening or sequencing analysis such as next-generation sequencing (Karthikeyan et al. 2021; Singh et al. 2022) and denaturing gradient gel electrophoresis (Wibowo et al. 2022). Despite the numerous studies on microbial diversity analysis in various ecosystems, there has been a lack of knowledge regarding how microbial diversity supports the nutrient use efficiency achieved by specific genotypes in particular environmental conditions.

This study aimed to understand the bacterial diversity in different growing mediums of an oil palm progeny with potassium-use efficient characters in the nursery stage. A metagenomic analysis using a full-length 16S rRNA gene (V1-V9) region sequencing approach, would provide an accurate taxonomical identification (Matsuo et al. 2021) and the abundance of bacteria in each medium.

MATERIALS AND METHODS

Soil sampling

Soil samples were taken from the growing medium of an oil palm nursery trial. Different kinds of growing medium of a DxP progeny seedlings: 1) sandy soil, recommended dose of fertilizer except for potassium (75% of a recommended dose of potassium); 2) sandy soil, recommended dose of fertilizer except for potassium (0% potassium); and 3) topsoil, recommended dose of fertilizer at 5 months after planting, were used in this study. The DxP progeny had potassium (kalium)-use efficient characters at the nursery stage (at eight months after planting). It showed the best vegetative characteristics in sandy soil, recommended dose of fertilizer except for potassium (75% of a recommended dose of potassium) (Medium 1, Table 1), compared with other dose levels of potassium in sandy soil, in a nursery trial (Figure 1). Each seedling was planted in each respective polybag. For metagenomic analysis, there were three replicates of soil samples for each kind of medium (Table 1).

The soil was taken from three different sites of the medium of each polybag, from the region around 1 cm from the root, as much as 1 g. The soil samples of three different medium sites in a polybag were subsequently mixed to be a sample.

Metagenomic analysis

The metagenomic analysis, including bioinformatics analysis, was conducted using a commercial service provided by PT Genetika Science (Indonesia). According to the manufacturer's protocol, the samples proceeded to the DNA extraction procedure using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082). At the same time, the DNA quantification was conducted using a NanoDrop spectrophotometer and Qubit Fluorometer. The quality of the extracted DNA was checked by the value of 260/280 and 260/230 ratios and the amplicon of the full-length 16S rRNA gene. The region of the 16S rRNA gene of the DNA samples was amplified using the 27F/1492R primer pair to obtain the full-length 16S rRNA gene (V1-V9) region.

Table 1. List of samples and medium treatment

Sample ID	Medium	Remarks
Sample 1 Sample 2 Sample 3	1	Sandy soil, recommended dose of fertilizer except for potassium (75% of a recommended dose of potassium)
Sample 4 Sample 5 Sample 6	2	Sandy soil, recommended dose of fertilizer except for potassium (0% potassium)
Sample 7 Sample 8 Sample 9	3	Topsoil, recommended dose of fertilizer at 5 months after planting

Figure 1. Vegetative characters of potassium-use efficient seedlings (8 months after planting) at mediums with: 1: sandy soil, without any fertilizer applications; 2: sandy soil, recommended fertilizer but with no potassium; 3: sandy soil, recommended fertilizer; 4: sandy soil, recommended fertilizer but with 75% of a recommended dose of potassium; 5: sandy soil, recommended fertilizer but with 50% of a recommended dose of potassium; 6: topsoil, recommended fertilizer at five months after planting

Library preparations were conducted using Kits from Oxford Nanopore Technology (ONT), following the manufacturer's instruction, and Nanopore sequencing was operated by MinKNOW software version 23.04.5. ONT sequencer is a long read sequencer platform that allows reading long DNA sequences so that the base sequence read varies depending on the length of the library fragment. The length of the 16S rRNA full-length target library fragment ranged from 1300 to 1700 base pairs (bp). DNA reading was carried out on a single-strand DNA (single-end). The sequencing was conducted with 100,000 depth of raw reads. The reads were filtered based on the length (1300 to 1700 bp) and the minimum quality standard (Q10). Basecalling was performed using Guppy version 6.5.7 with the high-accuracy model (Wick et al. 2019). FASTQ file quality was visualized using NanoPlot, and quality filtering was performed using NanoFilt (de Coster et al. 2018; Nygaard et al. 2020). Filtered reads were classified using a centrifuge classifier (Kim et al. 2016). Determination of the origin of the sequences was conducted using Operating Taxonomic Unit (OTU) clustering, while the bacteria and Archaea indexes were built using the NCBI 16S RefSeq database (https://ftp.ncbi.nlm.nih.gov/refseq/TargetedLoci/). Alpha and beta diversity analysis and visualizations were performed using Pavian (https://github.com/fbreitwieser/pavian) and RStudio using R version 4.2.3 (https://www.R-project.org/).

RESULTS AND DISCUSSION

Figure 2 shows the alpha diversity analysis of the bacteria in the three mediums. The Chao1 index measures richness or the number of species identified (Chao 1984), while the Shannon index considers both richness and evenness, with a heavier emphasis on richness (Lemos et al. 2011; Magurran 2004). On the other hand, the Simpson index also considers richness and evenness but puts more weight on evenness (Simpson 1949; Lemos et al. 2011). The median Chao1 index value was the lowest for medium 2 (1,635.72) and highest for medium 3 (3,329.86), indicating that the number of species identified in medium 2 was the lowest and the highest in medium 3. As for the Shannon index, the median value was the lowest for medium 1 (3.94) and the highest for medium 3 (5.59). Regarding the Simpson indexes, the lowest median value was for medium 2 (0.962) and the highest for medium 3 (0.992). The alpha diversity analysis suggested that medium 3 had the most identified, but a species was dominant.

Figure 3 shows the top 10 most abundant species and genus among the three mediums. The medium 1 had *Lactobacillus helveticus* as the most abundant species (overall relative abundance was 22.84%). At the same time, *Lactobacillus* was the most abundant genus. *Ralstonia pickettii* was the most abundant species (overall relative abundance was 26.42%), and *Dyella* was the most abundant genus in medium 2. In medium 3, *Chujaibacter soli* was the most abundant species (overall relative abundance was 54.95%), and *Lactobacillus* was the most abundant genus.

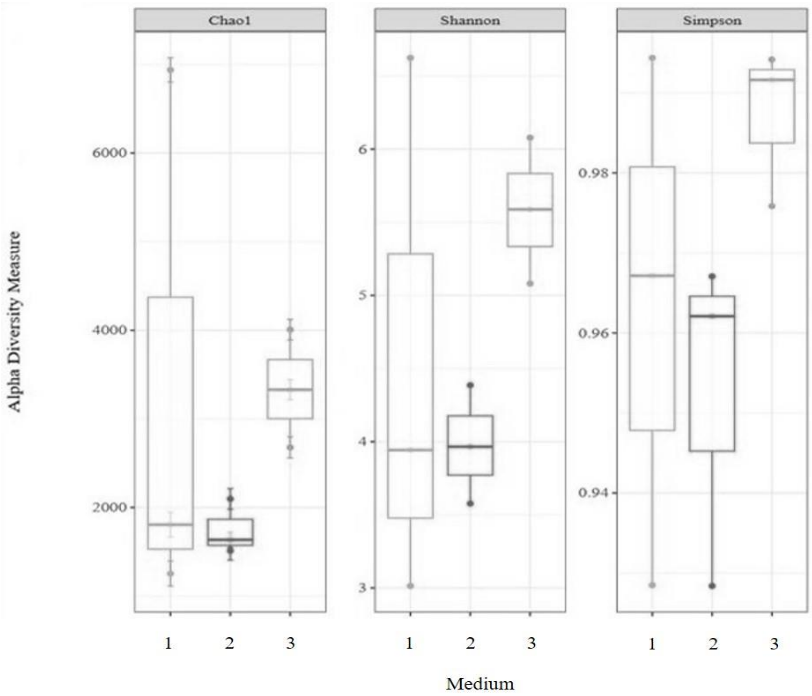
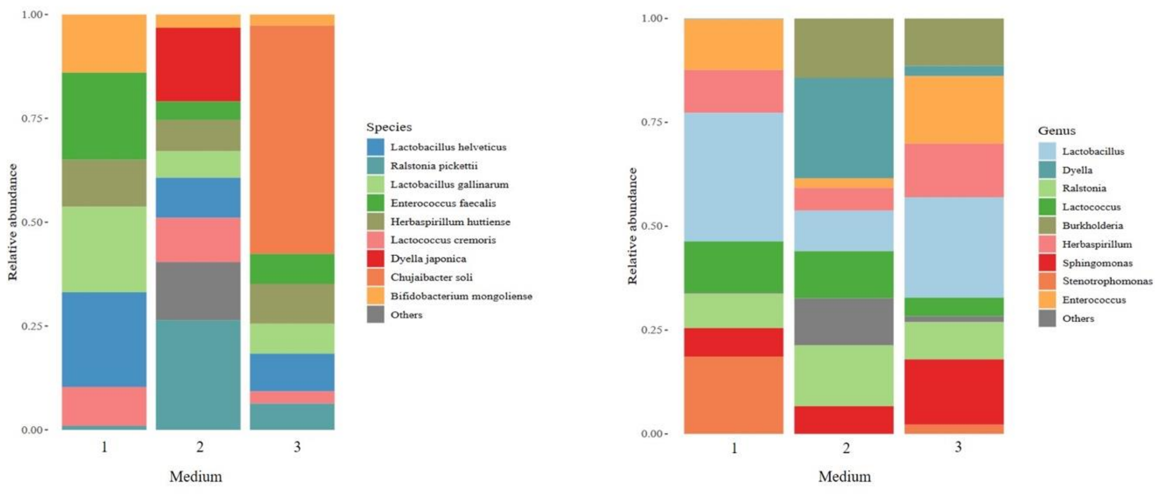


Figure 2. Alpha diversity measures of the three mediums (1, 2, and 3; See Table 1) based on Chao1, Shannon, and Simpson indexes



A **B**

Figure 3. Top ten relative abundance from species level (A) and genus level (B) among mediums

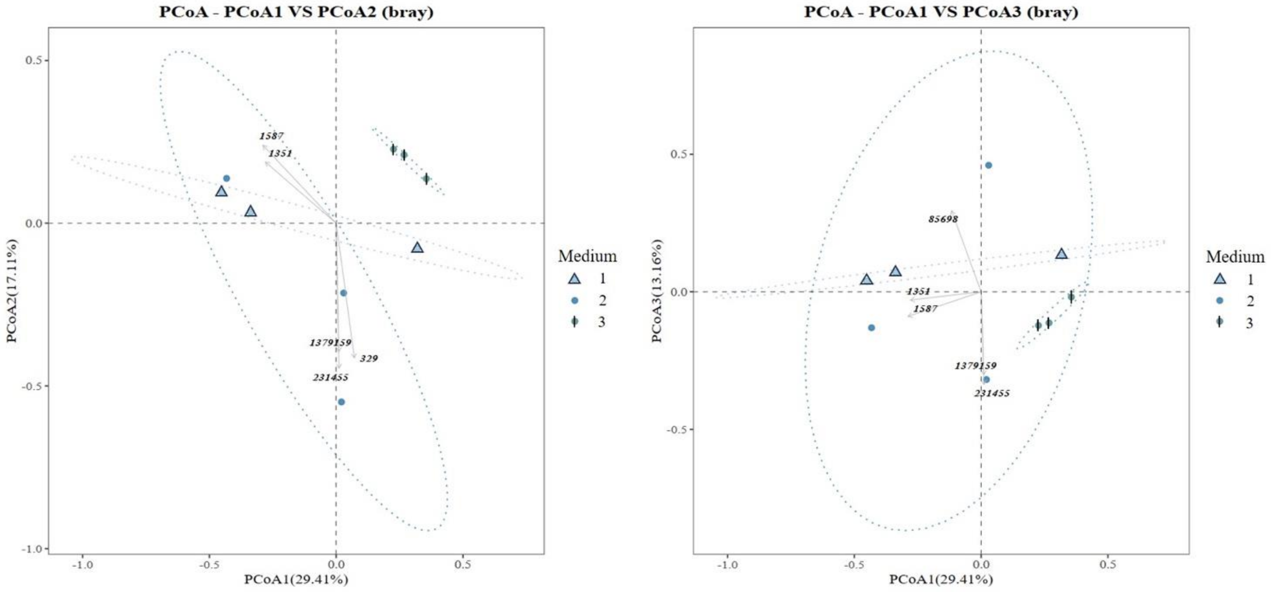


Figure 4. PCoA of the samples analyzed, based on Bray-Curtis dissimilarity

In more detail, PCoA analysis (Figure 4) shows that the samples of medium 3 were in the same quadrant of the PCoA1 vs PCoA2 and the PCoA1 vs PCoA3. On the other hand, there was higher dissimilarity among samples within medium 1 and medium 2 since the samples within each medium were in different quadrants of the PCoAs. The Venn diagram (Figure 5) shows that the abundance of the bacteria in sample 1 was more similar to medium 3 since the number of the shared operational taxonomic unit between medium 1 and medium 3 (1,602) was the highest, compared to the number of the shared operational taxonomic unit between medium 1 and medium 2 (682) or between medium 2 and medium 3 (239). The number of shared operational taxonomic units between medium 1 and medium 3 was very close to the total number of shared operational taxonomic units among the three mediums (1,051).

Discussions

Microbial diversity of the growing medium, in this case, soil, is essential for nutrient use efficiency study, as the knowledge of soil microorganism diversity is a bioindicator of carbon and nutrient cycling, as well as soil fertility (Situmorang et al. 2016; Dong et al. 2023). This study analyzed more on bacterial diversity since bacteria are involved in plant nutrient use pathways and function as plant growth promoters (López-Arredondo et al. 2017; Hidayat et al. 2023). The same approach was used by Berkelmann et al. (2020) in their analysis of soil bacterial community structures concerning different oil palm management practices.

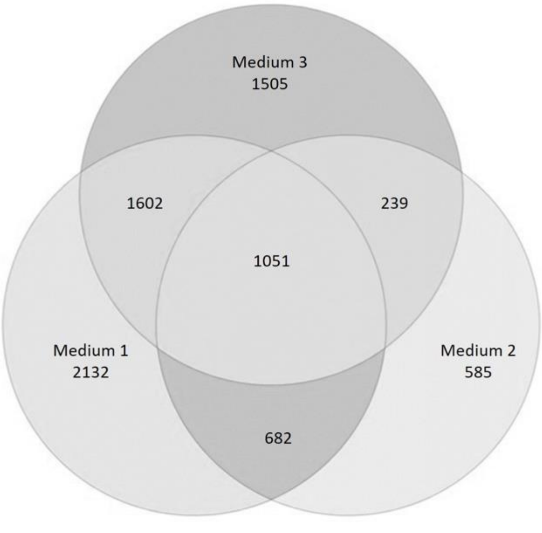


Figure 5. Venn diagram of the bacteria's operational taxonomic unit of the three mediums analyzed

Bacteria's growth is influenced by the environment where they live (Gonzalez and Aranda 2023). The experiment was conducted in a designed trial and controlled environment to minimize the environmental factors affecting bacterial diversity. The sampling method was conducted to provide representative samples using replications (3 polybags) for each medium treatment. For each polybag, the soil was taken from different areas.

The identification of organisms during the study used information from the 16S rRNA gene, as the gene has been widely used as a DNA barcode in bacteria identification (Lebonah et al. 2014; Tran et al. 2017). The 16S rRNA consists of nine hypervariable regions, and the choice of the region will affect the accuracy of the taxonomy identification (López-Aladid et al. 2023). So, the full-length sequencing strategy conducted using the Oxford Nanopore Technology Platform will higher the accuracy of the identification (Chen et al. 2015; Kai et al. 2019; Lin et al. 2021; Ciuffreda et al. 2021). The same approach was used by Chavan et al. (2022) in the analysis of full-length 16S rRNA gene sequences generated by Oxford nanopore system to study the effect of nanoparticles on soil microbial communities.

The alpha diversity was analyzed based on Chao1, Shannon, and Simpson indexes to understand each medium's bacterial community's richness and evenness (Thukral 2017). The results (Figure 2) show that the topsoil medium had more bacteria species than sandy soil medium treatments. The lower porosity level of topsoil leads to higher water retention than sandy soil. Supported by higher organic biomass in the topsoil, bacteria can grow and develop better in the topsoil than in sandy soil. The physical and chemical characteristics of the topsoil, supported by its higher number of bacteria, could make fertilizer uptake in topsoil easier compared with sandy soil (Fageria and Stone 2006). The vegetative characteristics of the progeny proved it in different mediums, in which the progeny was the highest in the topsoil medium, compared to the other treatment in sandy soil with various doses levels of potassium (Figure 1). Higher bacterial abundance at the mediums was better characteristic of the oil palm seedlings. It suggested that the potassium-use efficient seedlings should be planted in a medium that supported the growth and development of bacteria.

The results highlight that the most abundant genus or species in each medium (Figure 3) were related to their function in the medium for plant nutrient utilization. These findings were consistent with information reported in previous studies. There may be interactions and influences of other microorganisms in the soil that may impact nutrient use efficiency and bacterial diversity in oil palm seedlings. This experiment considered the top ten relative abundances of bacteria at the species and genus level. Further study is needed to fully understand the interactions and influences of the bacteria and other microorganisms in the mediums.

The medium 1, sandy soil with standard fertilizer but 75% of a recommended dose of potassium, had *Lactobacillus helveticus* as the most abundant species and *Lactobacillus* as the most abundant genus. Genus *Lactobacillus*, including *Lactobacillus helveticus*, is grouped as lactic acid bacteria (LAB) since its metabolism majority product is lactic acid. It has diverse habitat ranges, such as fermented dairy products, wine, sourdough, soil, plants, and the human gastrointestinal tract (Slattery et al. 2010; Raman et al. 2022). LAB metabolites promote plant growth and stimulate shoot and root growth. It promotes biodegradation, accelerates the soil's organic content, and produces organic acid and bacteriocin metabolites (Raman et al. 2022). So, it supports why the progeny showed the best vegetative characteristic in medium 1 compared to the other kind of sandy soil medium.

Ralstonia pickettii, the most abundant species in medium 2, was determined to be a novel phosphate solubilizing bacterium from a unique source rhizosphere soil of pomegranate (Kailasan et al. 2015). It is categorized as an oligotroph bacterium that can survive deficient nutrient conditions (McAlister et al. 2002). The result was the following condition of medium 2, sandy soil with a 0% dose of potassium. Indeed, the oligotroph species was highly abundant in the medium with minimum potassium supply. Genus *Dyella,* the most abundant genus in medium 2, was also isolated from soil (Weon et al. 2009), suggesting the reliability of the sequencing analysis during this study. Sembiring and Sabrina (2022) used *Dyella japonica* in a biofertilizer trial for maize, as their previous research showed that the species could convert atmospheric nitrogen to ammonia, so there was an increment in the level of nitrogen in the soil (Sembiring and Sabrina 2021). As medium 2 was supplied with the standard dose of nitrogen fertilizer, the bacteria could provide available nitrogen to the seedling.

In medium 3, the top soil medium with complete/a recommended dose of fertilizer at five months after planting, *Chujaibacter soli* was the most abundant species, and *Lactobacillus* was the most abundant genus. *Chujaibacter soli* is a species of bacterium found in soil (Wu et al. 2023), while the genus *Chujaibacter* was highly abundant in paddy's chemical fertilizer treated-soil medium (Prasedya et al. 2022); the same case with medium 3, which treated with complete/standard fertilizer at five months after planting. The abundance of the genus *Lactobacillus* makes sense, as their existence is essential for plant growth.

The similarity of bacteria community in mediums 1 and 3 could be explained by more sufficient nutrients than in medium 2. Seedlings with sufficient nutrients could produce beneficial metabolites for certain bacteria, while the seedlings need the bacteria to make the nutrients available for the plant (Pang et al. 2021; Weigh et al. 2023). Since the dose level of fertilizer applied for medium 1 and medium 3 was more similar to medium 2, the bacteria community was also more similar. Identification of unique bacteria in medium 1 is also valuable knowledge since there could be specific metabolites produced by the potassium-use efficient progeny, which had the best growth at the 75% of a recommended dose of potassium, that attracted or were liked by a specific bacteria community (Pang et al. 2021).

Studies for understanding oil palm soil microbial diversity were also done by Verdiani et al. (2021) in a comparative metagenomic analysis of rhizosphere bacterial communities between suppressive and conducive soil towards *Ganoderma boninense* infection, by Salamat et al. (2021) in the analysis of the effect of inorganic fertilizer application in an oil palm plantation, and Wibowo et al. (2022) in their analysis of soil bacterial diversity from tropical rainforest and oil palm plantation In Jambi.

Finding *Lactobacillus helveticus* as the most abundant species in the medium where the progeny had the best growth despite the low dose of potassium (75% of a recommended dose) suggested its role in potassium optimization. The potassium-use efficient progeny is also suspected to produce metabolites that attract the most *Lactobacillus helveticus* or *Lactobacillus* (Hu et al. 2018; Pang et al. 2021).

This study observed the bacterial diversity at different mediums planted with an oil palm progeny with potassium-use efficient characters at the nursery stage. The topsoil medium with a recommended fertilizer had more bacteria than sandy soil with a recommended fertilizer but less potassium dose. The abundance of species and genus of bacteria were different in each kind of medium, and they were related to their function in the medium for plant nutrient uptake or utilization. It suggested the specific relationship of the seedlings with the characteristic of the growing medium, as well as the most abundant species or genus of bacteria found. Investigating bacteria with an essential role in potassium utilization could also be done by observing the unique bacteria or microbiome found only in the medium 1.

To the best of the authors' knowledge, this is the first report on the microbial diversity of different mediums of oil palm seedlings with potassium-use efficient characters. Further research is needed to understand the interactions and influences of bacteria and other microorganisms in the mediums, microbial community structure across different mediums, and the specific genus or species found in potassium-use efficient oil palm seedling mediums. In this experiment, the seedlings had better vegetative growth and nutrient uptake (Picture 1; data is not shown) in the sandy soil medium with a 75% recommended dose of potassium compared with sandy soil medium with a full/recommended dose of potassium. It suggested that the planting material's dose of anorganic potassium fertilizer can be decreased. Further researches are needed to study the practical applications to improve the efficiency and productivity of oil palm plantations.

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