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Microbiome Structure Analysis of Oil Palm Pollinator *Elaeidobius kamerunicus* (Coleoptera; Curculionidae)

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ABSTRACT

The pollination of oil palm by *Elaeidobius kamerunicus* leads to an increase of over 70% in countries such as India, Malaysia, and Indonesia. The impact of insect-microbiome interactions on the pollination activity and fitness of *E. kamerunicus* is unknown. Our study aimed to gain insight into the bacterial communities of *E. kamerunicus* from two different sites with high and low fruit set percentages, using culturable and Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis. Our result revealed distinctive T-RFs profiles in *E. kamerunicus* from two different sites. Additionally, the culturable approach showed that some of this microbiome were found only in the weevil population from the high fruit set site. Our findings suggest that these bacteria could contribute to the fitness of *E. kamerunicus*, leading to a higher fruit set in oil palm plantations.

1. Introduction

Oil palm is an important crop and a primary source of oil palm industry in Indonesia. This industry has contributed to national and regional income, providing job vacancies, area development, and open investment. Indonesia is one of the world's largest oil palm producers, with up to 15 million ha of oil palm area reported in 2022 with the production of crude palm oil (CPO) reaching up to 48 million tons (Ditjenbun 2021). Oil palm is also an exported commodity to countries such as India, USA, China, and Holland (Kemenperin 2021). As a result, the demand for oil palm from inside and outside the country is increasing yearly.

The demand for oil palm is very high, reaching up to 5 million tons annually. To meet this demand, it is necessary to increase the productivity of oil palm. Increasing pollination effectiveness will help the oil palm to produce more fruit bunch. The degree of pollination effectivity is stated in the fruit set, which is the percentage of pollinated fruit from total fruit in a single fruit bunch (Prasetyo and Susanto 2012). In other words, a high pollination effectivity will increase the fruit bunch and eventually oil production.

The Coleopteran order is the most abundant insect interacting with oil palm as a pollinator. However, oil palm pollination can also occur naturally, through wind, other insects such as thrips and bees, or through the intervention of assisted pollination (Yousefi *et al.* 2020). One of the primary pollinators of oil palm is the weevil, *Elaeidobius kamerunicus* (Coleoptera; Curculionidae) which had shown to improve fruit set by over 70% in countries such as India (Dhileepan 1994), Malaysia (Rizuan *et al.* 2013), and Indonesia (Prasetyo *et al.* 2014). The increase in fruit set is due to the high effectivity of pollination by *E. kamerunicus*. A study in Seram Island, Maluku, showed that the increase in fruit set resulted from the high population growth of *E. kamerunicus* (Prasetyo and Susanto 2016). Additionally, *E. kamerunicus* should be fit and active to transfer the pollen from male to female inflorescence. Therefore, maintaining its fitness is crucial to maintain the weevil population ratio and activity.

The fitness of *E. kamerunicus* can be affected by the insect-microbiome interaction. Microbiome in the insect body maintain metabolism, physiology, adaptation, immunity, and reproduction. Some

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research reported that microbiome can affect the weight and metabolism of social bees, *Apis mellifera* (Zheng *et al.* 2017), the fitness of solitary bees, *Osmia ribifloris* (Dharampal *et al.* 2019), growth and development of red palm weevil *Rhynchophorus ferrugineus* (Habineza *et al.* 2019). In addition, identifying microbiome community structure associated with *E. kamerunicus* has rarely been performed. The last study reported that the pupal stage of *E. kamerunicus* was dominated by *Serratia marcescens* and *Enterobacter cloacae* (Hussein *et al.* 1995). There is no research on microbiome structure in imago phase of *E. kamerunicus*.

This study aimed to analyze the microbiome community structure of adult *E. kamerunicus*, the main pollinator insect in oil palm, using both culturable and unculturable methods. The culturable bacteria from *E. kamerunicus* were identified using 16S rRNA gene profiling. The Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was employed to compare the microbiome community structure between *E. kamerunicus* from low and high-fruit set oil palm plantations. T-RFLP method is used to analyze microbial community structure because it is easy, rapid, and accurate for monitoring changes in the overall structure of the microbial community (Prakash *et al.* 2014). The goal was to investigate the impact of host-microbiome interactions on host activity and fitness, and to determine if the unique bacteria community structure in *E. kamerunicus* from high fruit set oil palms contributes to their improved fitness and increased fruit set percentage.

2. Materials and Methods

2.1. Sample Collection

The weevil *E. kamerunicus* were collected from two areas with different fruit formation in Wilmar Oil Palm plantation, West Sumatra, Indonesia. The high fruit set (80.69%) weevil were collected from PT Agro Masang Plantation (AMP), meanwhile the low fruit set (<50%) weevils were collected from PT Gersindo Minang Plantation (GMP). The weevils were put into a sterile Eppendorf tube before proceeding to isolation.

2.2. Isolation of Culturable Bacteria

The study used two types of media for bacteria isolation: Plate Count Agar (PCA) for common bacteria and De Man Rogosa Agar (MRSA) for lactic acid bacteria (Yanti *et al.* 2020; Gorrens *et al.* 2021). A total of 25 *E. kamerunicus* weevil was dissolved in 1 ml of sterile saline (0.9%) solution. Then, the mixture was swirled at room temperature for 1 minute. The sterilized weevil was then transferred into a new tube containing 1.5 ml sterile phosphate buffer saline, crushed using a sterile micro pestle and vortexed for 5 minutes. The crushed weevil was then divided into two parts for T-RFLP analysis (0.5 ml) and 1 ml for bacteria isolation.

About 1 ml sample of crushed weevil material sample then added to 9 ml sterile PBS. The mixture was then vortexed for 10 seconds. Subsequently, 100 µl of the sample was spread onto a sterile Plate Count Agar (PCA) media using the spread plate technique. This media is used to count total aerobic bacteria. PCA plate was incubated aerobically for 24 hours (Gorrens *et al.* 2021). Additionally, lactic acid bacteria were isolated using De Man Rogosa Agar (MRSA) media (Yanti *et al.* 2020). A 100 µl sample was spread onto a sterile MRSA media and incubated anaerobically for 48 hours using a candle anaerobic jar system (Ermenlieva *et al.* 2021).

Morphologically different colonies were streaked on the same media to obtain individual colonies. The identification of lactic acid bacteria was performed by streaking bacteria colonies on MRSA media with 1% $CaCO_3$ for a clear zone test. The clear zone around the colony would be considered an indicator of the lactic acid bacteria's presence (Hwanhlem *et al.* 2011).

2.3. Identification of Culturable Bacteria Using 16S rRNA Gene Profiling

The 16S rRNA genes were isolated using PCR colony-modified protocol from Bethe *et al.* (2010) and amplification was done using universal forward primer 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and universal reverse primer 1387r (5'-GGGCGGWGTGTACAAGGC-3'). The DNA of bacteria for 16S rRNA PCR amplification was extracted from a single colony on agar plates. A single colony of bacteria from an overnight culture was picked using a skewer and placed into 50 µl sterile of NFW, then heated in a hotplate to 65°C for 10 minutes and vortexed.

The PCR reaction mixtures contained 3 µl of a sample, 5 µl GoTaq Green Mastermix 2x (Promega), and 1 µl (10 pmol) of each primer, for a final volume of 10 µl. The PCR was performed on a Thermal Cycler (Biorad) with an initial denaturation at 95°C for 5

minutes followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 90 seconds. A final extension at 72°C was performed for 7 minutes to yield a 1,500 bp PCR product. The product was then visualized by agarose gel electrophoresis stained with ethidium bromide under UV light.

The amplified PCR product was purified using a Qiaquick DNA purification kit and treated with ExoSAP-ITTM (Applied Biosystem). The purified amplicon was sequenced using the dideoxy method with the BigDye X-terminator™ Purification Kit (Applied Biosystem) and analyzed on ABI PRISM 3100 genetic analyzers (Applied Biosystem). The obtained 16S rRNA sequence was then compared with sequences in the NCBI database using the BLASTN tool. The DNA sequence of each identified bacteria was aligned using Clustal W program and the phylogenetic tree was constructed based on Maximum Likelihood (ML) model using Molecular Evolutionary Genetics Analysis (MEGA) X software with 1000x bootstrap values. Bootstrap value was categorized as strong (>85%), moderate (70-85%), weak (50-70%), or poor (<50%) (Kress *et al.* 2002).

2.4. Unculturable Approach Using T-RFLP Analysis of 16S rRNA Gene

T-RFLP analysis was carried out using a modified protocol by Egert *et al.* (2003). DNA was extracted from a crushed weevil sample of *E. kamerunicus* by homogenizing it in a buffer containing 200 µl of Tris and 2 microbeads in a 2 ml tube, then extracted using TissueLyser II (Qiagen) for 10 minutes with 30 Hz frequency. Cell lysis was performed by adding 50 µl Proteinase K (20 mg/ml) into a homogenized sample and incubating for 1 hour at 37°C. DNA then was extracted according to the Promega DNA Extraction kit (Promega) and the DNA concentration was measured using NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific).

16S rRNA genes were amplified using universal bacterial forward primer: 27f (5'-GAGTTTGATCCTGGCTCAG-3'), labeled with fluorescein carboxyfluorescein (FAM) and universal bacterial reverse primer 1387r (5'-GGGCGGWGTGTACAAGGC-3'), labeled with fluorescein hexa-chloro derivative (HEX). PCR mixtures contained: 25 µl GoTaq Green Mastermix 2x, 5 µl (10 pmol) of each primer, and nuclease-free water (NFW) up to a final volume of 50 µl. The PCR assay used the same PCR condition with culturable bacteria identification (2.3). The result was analyzed by performing 1% agarose gel electrophoresis to visualize PCR product stained with ethidium bromide under UV light. PCR fragment representing the 16S rRNA gene was cut at 1,500 bp and then purified using QIAquick Gel Purification Kit (Qiagen). DNA concentration was read using NanoDrop™ 2000/2000c Spectrophotometers (Thermo Scientific).

The digestion step was carried out separately using 500 ng of isolated DNA with 20 U of restriction enzyme (performed using *Msp*I and *Hha*I) in the 1x buffer rCut Smart (NEB) in a total reaction mix of 10 µl and incubated overnight at 37°C. DNA fragments were analyzed using 1 µl DNA incubated samples on ABI PRISM 3100 genetic analyzers (Applied Biosystem). The data was presented as peaks in an electropherogram in each direction primer. Peak size (bp), height, and area were estimated by comparing with the internal size standard of GeneScan - 500 ROX (Applied Biosystems). Every peak was represented as Terminal Restriction Fragment (T-RF).

The T-RF were sorted using Peak Scanner™ Software v1.0, and only fragments with lengths of about 50-500 bp were selected. Each T-RF was assumed as one phylotype and identified in silico using the MiCA III tool (http://mica.ibest.uidaho.edu/) at the phylum level. T-RFLP phylogenetic assignment tool (PAT+) option was used in MiCA. Ribosomal Database Project (RDP) (R10, U27) database consisting of 1,519,357 bacterial 16S rRNA was used with 0 mismatches in digest and ±1bp bin tolerance for all fragments (Shyu *et al.* 2007).

The similarity index of T-RFs was also calculated using Sorensen and Jaccard index with the following equation:

$$\text{Jaccard Index} = \frac{a}{a + b + c}$$

$$\text{Sorensen Index} = \frac{a}{2a + b + c}$$

Where a represents the total shared T-RF from both oil palm plantations, b represents the total T-RF in AMP oil palm plantation, and c represents the total T-RF in GMP oil palm plantation (Magurran 2004).

3. Results

3.1. T-RFLP Analysis

T-RFLP analysis was conducted on weevil *E. kamerunicus* samples from oil palm plantations with high (AMP) and low (GMP) fruit sets. The 16S rRNA genes were restricted with *Msp*I and *Hha*I enzymes, yielding 23 forward and 11 reverse T-RFs from *Msp*I, 8 forward and 9 reverse T-RFs from *Hha*I.

The T-RF analysis using *Msp*I (Figure 1A and B) showed that some T-RFs were only found in the high fruit set AMP estate (81.17, 135.41, 137.1, and 489.17 bp in forward fragments, 241 and 485 bp in reverse fragments). Meanwhile, 16 T-RFs were only found in the low fruit set GMP estate (141.58, 150.89, 159.68, 161.14, 163.02, 273.46, 274.7, 284.55, 392.54, 395.19, 428.81, 433.98, 450.69, 461.82, 480.33, and 484.69 bp in forward fragments, 230, 237, and 397 bp in reverse fragments). The rest of the T-RFs were presented in both AMP and GMP weevil populations (12 T-RFs in forward fragments at 120.52, 123.81, 150.89, 159.68, 161.14, 273.46, 391.54, 395.19, 450.69, 461.82, 480.33, 484.69 bp, and 6 T-RFs in reverse fragments at 96, 109, 160, 228, 240, and 262 bp).

The T-RF analysis using the *Hha*I enzyme (Figure 1C and D) showed that T-RF from the AMP oil palm plantation was more abundant than the GMP oil palm plantation. All T-RFs from GMP oil palm were found in AMP oil palm, while some reverse T-RFs were only found in AMP oil palm (62.7, 290.4, 293.9, and 301.3 bp).

Furthermore, to obtain more information regarding the microbiome structure of *E. kamerunicus* between AMP and GMP oil palm plantations, each of T-RF was identified *in silico* using MiCA III tool. Each T-RF assumed as one phylotype represented a bacteria phylum. There were four bacteria phylum presented in *E. kamerunicus* from both sites, but the abundance was different. *E. kamerunicus* from AMP oil palm plantation was dominated by *Bacillota* and *Pseudomonadota*. Meanwhile, *E. kamerunicus* from GMP plantation was dominated by *Actinomycetota* and *Bacteroidota* (Figure 2). There were also some T-RFs identified as uncultured bacteria from both sites, but it was more abundant in AMP compared with GMP oil palm plantation.

The similarity of *E. kamerunicus* T-RF profiles from AMP and GMP oil palm plantations was calculated using Jaccard and Sorensen index. The range for the similarity index is 0 to 1, with values below 1 (Table 1), indicating that the bacterial community structure between the two plantations differed.

3.2. Bacteria Identification Using 16S rRNA Amplification and Sequencing

T-RFLP analysis had proven that the bacteria community structure of *E. kamerunicus* that lived in different fruit set values of oil palm plantation (AMP and GMP oil palm plantation) was different. To explain the difference, the culturable approach was conducted to evaluate bacteria community structure in imago phase of *E. kamerunicus* in AMP and GMP oil palm plantations. Culturable bacteria of *E. kamerunicus* from AMP and GMP oil palm plantations were identified using 16S rRNA genes.

The result showed that some bacteria are unique to either AMP or GMP weevil populations, while some are found in both areas (Figure 3). Bacteria found in both plantations with higher average abundance are considered the core microbiome of *E. kamerunicus*. Those are *Bacillus cereus, Bacillus megaterium*, and *Lysinibacillus fusiformis*. Based on the phylogenetic tree (Figure 4), some core bacteria of *E. kamerunicus* from AMP and GMP weevil populations have similar DNA sequences. *B. megaterium* 1 from AMP weevil population shared a similar DNA sequence with *B. megaterium* 3 from GMP weevil population with 74% bootstrap value. *L. fusiformis* 1 from AMP weevil population also shared a similar DNA sequence with *L. fusiformis* 5 from GMP weevil population with 92% bootstrap value. Likewise, *B. cereus* from AMP weevil population has a similar DNA sequence with GMP weevil population with bootstrap value above 50%. Those are *B. cereus* 12 and 19 (58%); 4 and 17 (93%); 14 and 16 (93%); 2 and 22 (92%); 5 and 22 (92%); 9 and 23 (92%); 4 and 16 (93%). Thus, *B. cereus*, *L. fusiformis*, and *B. megaterium* can be referred to as

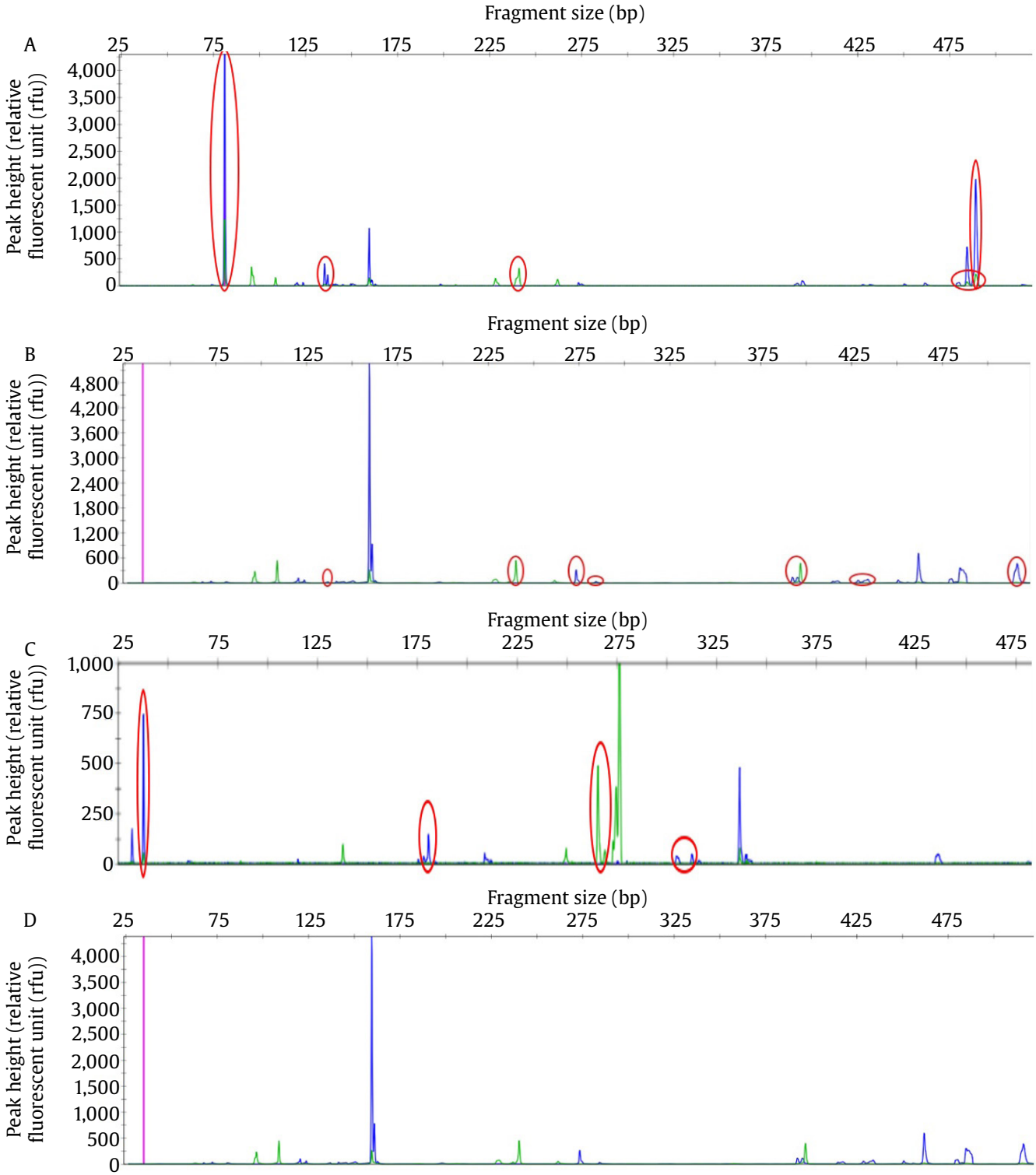

Figure 1. The result of T-RFLP analysis. (A) T-RF forward and reverse fragments of AMP oil palm plantation cut with *Msp*I, (B) T-RF forward and reverse fragments of GMP oil palm plantation cut with *Msp*I, (C) T-RF forward and reverse fragments of AMP oil palm plantation cut with *Hha*I, (D) T-RF forward and reverse fragments of GMP oil palm plantation cut with *Hha*I. The red circle showed different fragments between AMP and GMP

core microbiome of *E. kamerunicus* because it had a high similarity of DNA sequence and were found in both sites.

Bacteria specific to AMP weevil population are *Staphylococcus sciuri, Bacillus wiedmannii, Lysinibacillus macroides*, and *Bacillus toyonensis*. Those specific to GMP weevil population are *Bacillus nealsonii* and *Bacillus subtilis* (Figure 3). Nevertheless, bacteria with low abundance and only found specifically in AMP or GMP weevil populations might affect the fitness of *E. kamerunicus* in pollinating oil palm.

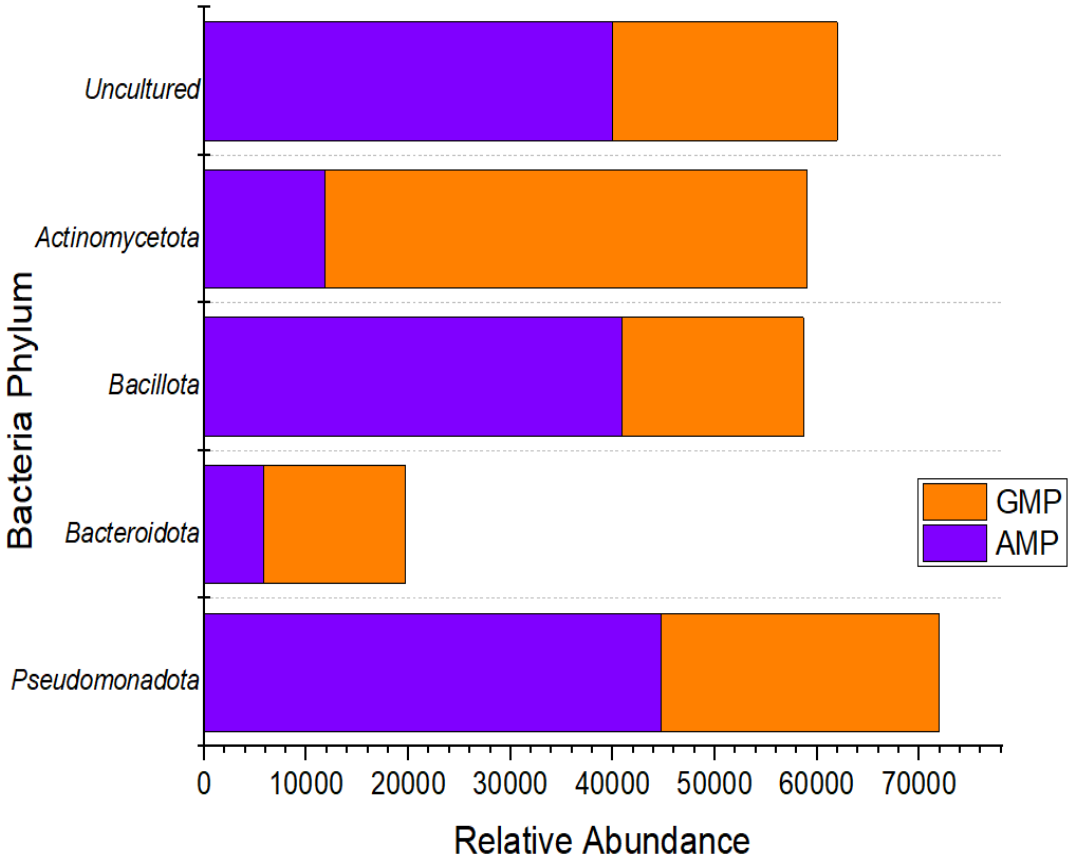

Figure 2. Bacteria phylum found in *E. kamerunicus* from AMP and GMP oil palm plantation based on T-RF *in silico* analysis using MiCA III tool that matched the database

Table 1. Similarity index of T-RF fragments using Sorensen and Jaccard indexes

Fragment	Sorensen		Jaccard	
	*Msp*I	*Hha*I	*Msp*I	*Hha*I
forward fragment	0.69	0.69	0.52	0.38
reverse fragment	0.71	0.71	0.55	0.56

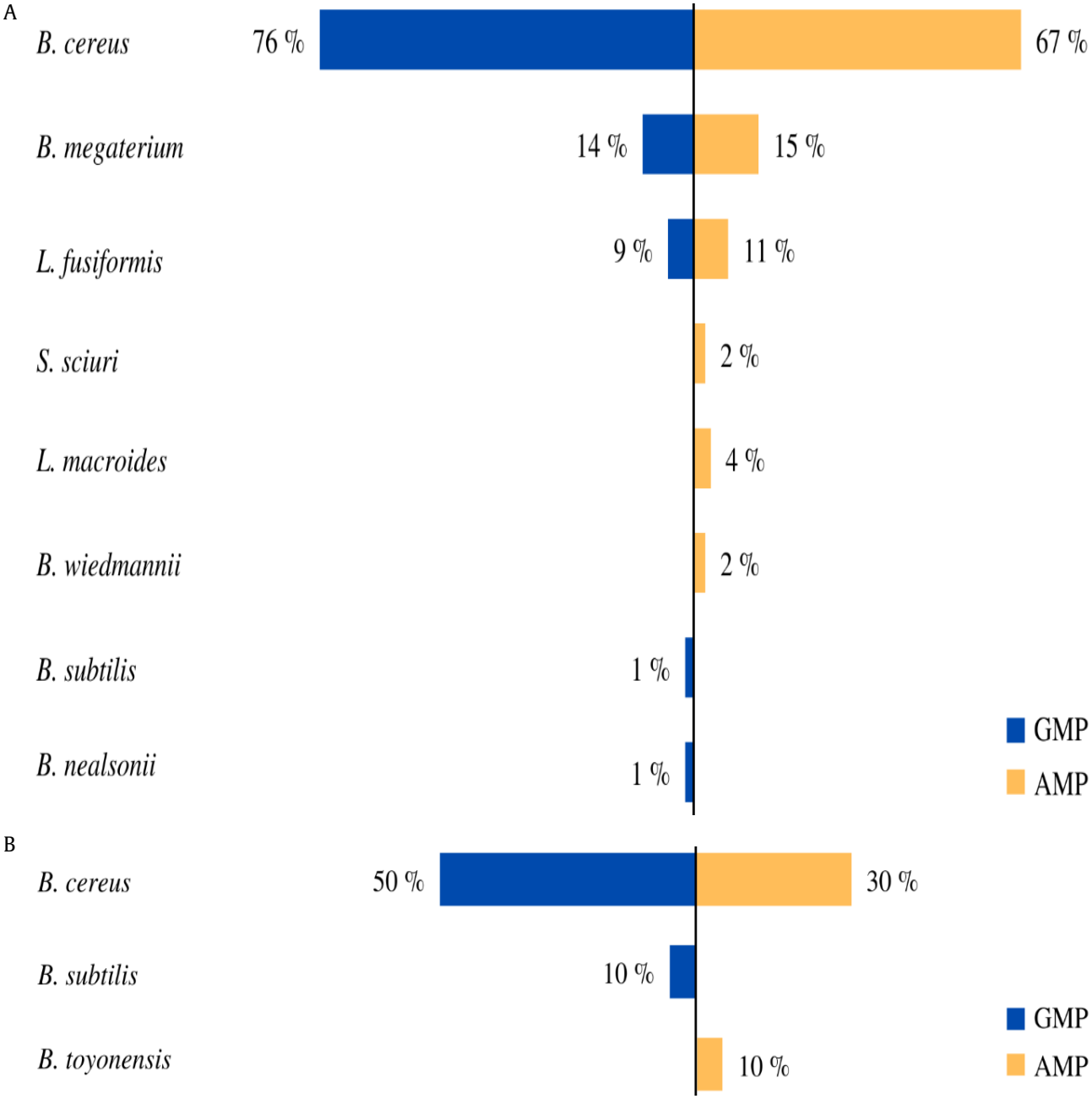

Figure 3. Bacteria isolated from *E. kamerunicus* in AMP (yellow; right) and GMP (blue; left) oil palm plantation. (A) Bacteria found in PCA media, (B) bacteria found in MRSA media. The percentage showed relative abundance of the bacteria

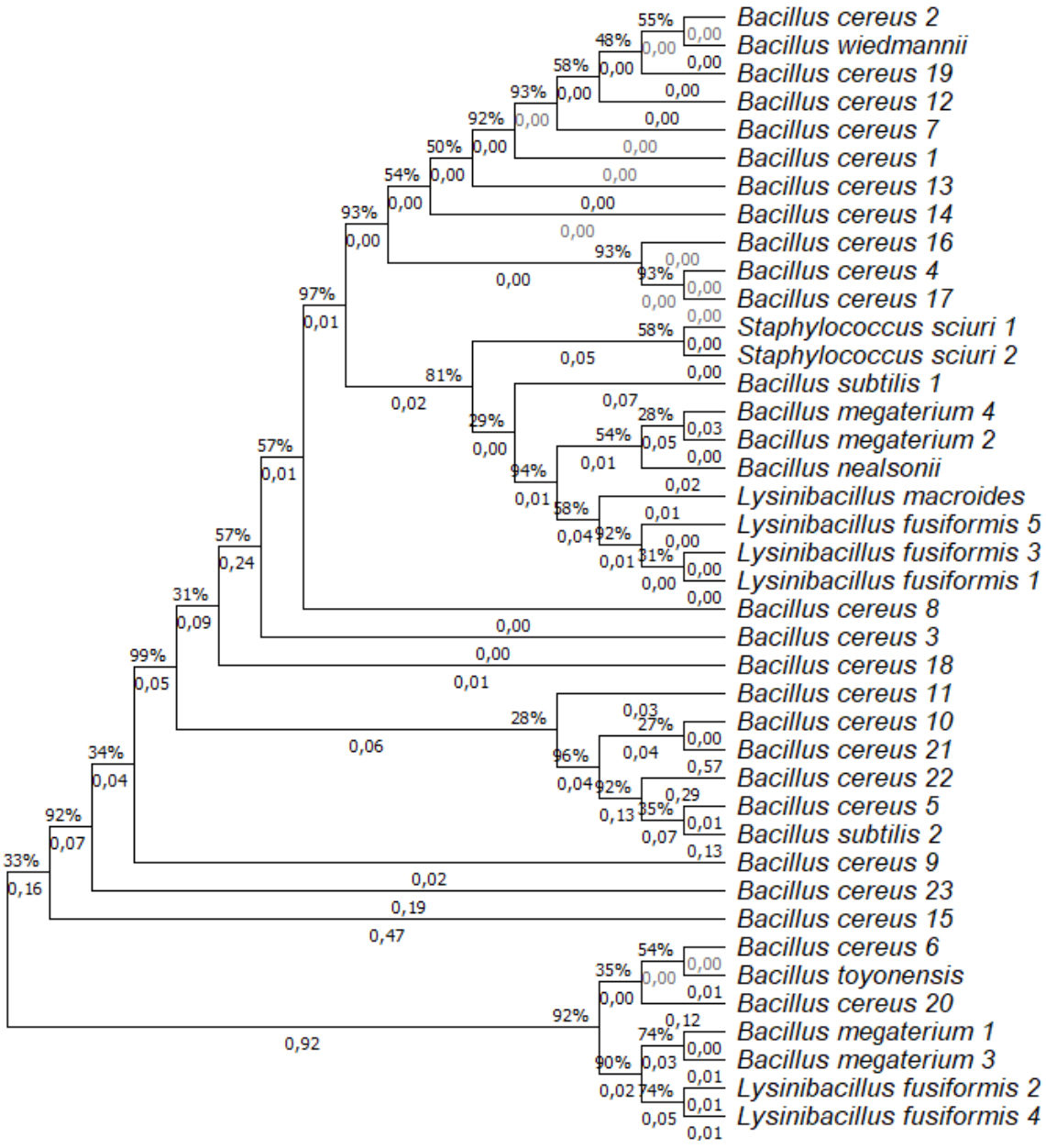

Figure 4. Phylogenetic tree of culturable bacteria from *E. kamerunicus* using Maximum Likelihood (ML) model. *B. cereus* 1-14: AMP, 15-23: GMP. *L. fusiformis* 1: AMP, 2-5: GMP. *B. megaterium* 1-2: AMP, 3-4: GMP

4. Discussion

E. kamerunicus is an African oil palm weevil, introduced to Indonesia in 1983. It lives in oil palm inflorescences. It lays eggs in the feeding pits of oil palm male inflorescences or outside the anther tube. The three instar larval stages occurred in the anther of male inflorescences (Zulkefli *et al.* 2020). Adult weevils (imago phase) stayed on spikelet of male inflorescences for matting and oviposition until 3rd day of anthesis, then they moved to female inflorescences because of anise-like odor (Yue *et al.* 2015). The anise-like odor was identified as estragole, a volatile organic compound from plants that attract *E. kamerunicus* (Fahmi-halil *et al.* 2021). Female inflorescences emitted the strongest estragole on the 2nd day of anthesis. While it perched on female flowers, the pollen from its body fell, and pollination occurred (Yue *et al.* 2015; Swaray *et al.* 2021).

E. kamerunicus is one of the most effective pollinator of oil palm. It had increased fruit set value by more than 70% in some countries including

Indonesia. After introducing *E. kamerunicus*, the fruit set value of oil palm reached up to 75% in Riau, West Sumatra (Solin *et al.* 2019) and Seram Island, Maluku (Prasetyo dan Susanto 2016). It was also reported that introducing *E. kamerunicus* in North Sumatra increased the fruit set from 69% to 80% (Wahyuni 2018).

E. kamerunicus can effectively pollinate oil palm because its size, shape, and structure match the size and structure of oil palm flowers, supported by high populations due to its proliferation in male oil palm flowers (Siswanto and Soetopo 2020). It could carry more pollen with very good quality (germination rate 92.7%) than other species, such as *Elaeidobius plagiatus, Elaeidobius subvitattus,* and *Microporum* spp. (Kouakou *et al.* 2014). Its population tended to be stable throughout the year because *E. kamerunicus* was not suppressed during the rainfall season (Wahid and Kamarudin 1997).

E. kamerunicus should be fit and active to pollinate oil palm. The high population of *E. kamerunicus* will increase pollination frequency to reach high fruit set value. *E. kamerunicus* fitness, activity, and population growth can be maintained through host-microbiome interaction. Microbes can supply essential materials such as proteins, vitamins, and minerals to support host health and development. A study in honey bees (*Apis mellifera*) has shown that microbes alter expression levels of nutrient metabolism-related genes. Bees that fed on sterile pollen led to growth delay and high mortality rates compared to those that fed on raw pollen (Duan *et al.* 2021). Bees (*Apis mellifera*) that were fed on sterile pollen grew 1.5 times slower to reach the pre-pupal stage, 66% smaller and had high mortality rates compared with those that were fed on raw pollen (Dharampal *et al.* 2019). A similar result has been seen in weevil (*Rhynchophorus ferrugineus*), an introduction of intestinal bacteria to germ-free weevil significantly increased the levels of hemolymph protein, glucose, and triglyceride compared to germ-free weevil without intestine bacteria (Habineza *et al.* 2019).

A previous study on *E. kamerunicus* had reported that the pupal stage was dominated by *Serratia marcescens* and *Enterobacter cloacae* (Hussein *et al.* 1995). However, no data on bacteria associated with the imago phase of *E. kamerunicus* has been reported until now. Our study showed that the bacteria associated with *E. kamerunicus* in the imago phase are *Bacillus cereus, Bacillus megaterium, Lysinibacillus fusiformis, Staphylococcus sciuri, Bacillus wiedmannii, Lysinibacillus macroides, Bacillus toyonensis, Bacillus subtilis*, and *Bacillus nealsonii*.

This study aimed to evaluate the bacteria associated with *E. kamerunicus* and compare the bacteria community in two different populations and fruit sets. The result from the unculturable approach employing T-RFLP analysis showed that some T-RFs were specific to either the AMP or GMP weevil population (Figure 1). However, these two populations also share the same bacteria community structure, which was assumed to be core bacteria in *E. kamerunicus* weevil. Moreover, *in silico* analysis of T-RF using MiCA III tool revealed that the dominant bacteria of *E. kamerunicus* from AMP oil palm plantation were *Bacillota* and *Pseudomonadota*, while GMP oil palm plantation was dominated by *Bacteroidota* and *Actinomycetota* (Figure 2). The unculturable bacteria were also more abundant in the AMP weevil population compared with GMP weevil population.

The similarity index was calculated using Jaccard and Sorensen similarity indexes to verify the T-RF data. The value of the similarity index is in the range of 0 and 1. The value of 1 implies that both community structures are identical, while 0 means no shared species between the two samples (Hao *et al.* 2019). The result of the similarity index value in this study was less than 1, indicating that the bacteria community structure of AMP and GMP weevil populations was not identical. A further method was conducted to identify the bacteria community structure of AMP and GMP weevil populations using a culturable approach.

The result from the culturable approach revealed that bacteria were found in both AMP and GMP weevil populations (Figure 3). Bacteria shared among two or more samples from a particular host or environment are also known as the core microbiome. It represented the characteristics of a host or environment (Neu *et al.* 2021). For example, a core microbiome of *Tyria jacobaeae* taken from three different habitats was *Ralstonia* (Gomes *et al.* 2020). This study showed that the core microbiome of *E. kamerunicus* was *B. cereus, B. megaterium*, and *L. fusiformis* because it was found in both oil palm plantations with high relative abundance value, and high similarity of DNA sequence based on phylogenetic tree (Figure 4).

Furthermore, our study also showed that unique bacteria were only found in specific weevil populations (Figure 3). Bacteria only found in the AMP weevil population might play an important role in increasing *E. kamerunicus* fitness as the fruit set value in the AMP oil palm plantation was higher than the GMP oil palm plantation. This high fruit set value was attributed to the high effectivity pollination performed by *E. kamerunicus* as an oil palm pollinator. The unique bacteria include *S. sciuri, B. wiedmannii, L. macroides*, and *B. toyonensis*. Recent studies have shown that those bacteria benefit insects, increasing their fitness as hosts.

B. toyonensis, which has been consumed as a probiotic under the name Toyocerin and has been shown to improve the fitness of rabbits (Matusevicius *et al.* 2011), piglet (Kantas *et al.* 2015), and chicken (Bushwereb *et al.* 2020). Moreover, *B. wiedmannii* was reported to reduce the pathogenicity of nematode in *Amphimallon solstitiale* (Skowronek *et al.* 2021). The presence of this bacteria in *E. kamerunicus* might decrease *Elaeolenchus parthenonema* pathogenicity as a nematode that infected *E. kamerunicus*, but further study must be conducted to evaluate this.

In addition, *L. macroides* and *S. sciuri* were also reported to have the ability for insecticide degradation. *L. macroides* isolated from *Spodoptera litura* was able to degrade Imidacloprid (Yalashetti *et al.* 2017), while *S. sciuri* isolated from *Spodoptera frugiperda* also able to degrade *Lufenuron* (De Almeida *et al.* 2017). The ability of insecticide degradation in *S. sciuri* was lower than in *L. macroides*. Insecticides such as Permethrin and Cypermethrin have been a significant cause the decline of *E. kamerunicus* population in the field (Hasibuan *et al.* 2002; Yusdayanti and Hamid 2015). Therefore, the presence of these bacteria might help *E. kamerunicus* to degrade insecticide and improve its fitness.

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