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In Vitro Characterization and Identification of a Plant Growth-Promoting Bacteria Consortium from Malaysian Oil Palm (*Elaeis guineensis*) Rhizosphere as a Potential Sustainable Biofertilizer

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ABSTRACT

Reliance on chemical fertilizer has caused many challenges to soil health. The alternative to chemical fertilizer is biofertilizer that can be developed using beneficial bacteria. Thus, this study aims to characterize the potential plant growth-promoting rhizobacteria (PGPR) based on biochemical tests on bacterial isolates, and to identify the potential PGPR based on the 16S rRNA sequencing molecular method as a potential biofertilizer development. The PGPR strains were isolated from oil palm (*Elaeis guineensis*) rhizosphere in Universiti Teknologi MARA (UiTM) Share farm, Malacca, Malaysia on nutrient media agar. Then, the bacteria isolates were screened for nitrogen fixation, phosphate and potassium solubilization, indole-3-acetic acid (IAA) production, siderophore production and hydrolyzing enzyme production tests. The isolates AR 11 and AR 21 strains have presented the most convincing result across all tests, which showed positive results for nitrogen fixation, solubilized phosphate and potassium, and produced indole acetic acid (IAA), siderophore and hydrolyzing enzyme. The selected strains proceeded for molecular identification based on the 16S rRNA gene sequence using 1492R and 27F universal primers. The isolates AR 11 and AR 21 were identified as *Acinetobacter seifertii* and *Aquitalea pelogenes* respectively. These findings extend the potential of native PGPR as an effective biofertilizer to oil palm, offering a locally adapted solution to enhance soil health and reduce dependency on chemical fertilizer. Thus, the oil palm farming sector stands to gain from the beneficial PGPR isolates that are aligned with sustainable agricultural systems and potential biofertilizer development.

Keywords: Oil Palm Rhizosphere Microbiome; Biochemical Characterization; 16s rRNA identification; Plant Growth-Promoting Bacteria; Microbial Inoculants

INTRODUCTION

Oil palm (*Elaeis guineensis*) is one of the world's most important crops, constituting over a third of global vegetable oil production (Kirkman et al., 2022). Oil palm's greater oil output per hectare than other oil-producing crops makes it an especially desirable crop for the manufacture of biofuel, food, and chemicals (Yusoff et al., 2020; Murphy, 2014). The growth of Malaysia's agricultural sector and economy is significantly influenced by the oil palm industry (Arifin et al., 2023; Kushairi et al., 2018). Through substantial contributions and unwavering dedication, Malaysia is one of the Asian countries that successfully takes advantage of the oil palm industry's benefits and is accountable for its global ascent. Malaysia has maintained its position as one of the world's most productive producers of palm oil for many years (Ahmad et al., 2023; Nambiappan, 2018).

To increase the production of oil palm, oil palm plantations require an optimum and balanced supply of nutrients to support optimized growth and yield production (Sigalingging et al., 2024; Foong et al., 2018; Miransari, 2013). Due to that, fertilizers are crucial elements in modern agricultural practices, especially for oil palm. However, excessive application of chemical fertilizers may have unforeseen negative effects on the ecosystem (Prakash, 2023; Adesemoye et al., 2009), which contribute to water pollution, soil pollution, air pollution and heavy metals content (Keni et al., 2022; Venuste, 2023).

Utilizing bacteria that promote plant development, such as those in the genus *Azospirillum*, may influence the growth and yield of several plants of agronomic and ecological importance. This alternative could be an intriguing approach to chemical fertilisers. Additionally, organic agriculture could help to reverse the ecosystem's degradation and ameliorate the situation. Therefore, bio-fertilizer, a combination of nutrients that are suitable for growth, is something of hope for current production-oriented agriculture that produces crops sustainably while preserving and enhancing the environment (Mingma et al., 2021).

The soil bacteria can be specialized as plant growth-promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria reside in the rhizosphere and aid in the growth and development of plants by secreting a variety of regulatory chemicals. They might be located inside the plant (endosphere), on the roots (rhizosphere), or on the leaves (phyllosphere) (Vocciante et al., 2022). Plant growth could be facilitated by PGPR through their own metabolism by fixing nitrogen, generating hormones, or solubilizing phosphates, direct effects on the plant metabolism which can increase water and mineral uptake. It also can improve the root development, increase plant enzymatic activity, aiding other beneficial microorganisms to increase their action on the plant (Poonam et al., 2022; Vocciante et al., 2022; Pérez-Montaño et al., 2014).

Several previous studies have explored the implementation of PGPR on oil palm, particularly in nursery settings, where inoculation with *Pseudomonas fluorescens* demonstrating their potential to enhance oil palm seedlings plant growth (Mayerni et al., 2019; Hastuti et al., 2022). However, research on native PGPR isolated directly from oil palm plantation soils particularly in Malaysia remains limited. This study addresses this gap by characterizing indigenous PGPR isolates from oil palm rhizosphere based on biochemical assays and 16S rRNA gene sequencing, providing foundational data for future biofertilizer development using locally adapted strains. Therefore, the creation of biofertilizers which include organic and other microbial products can progressively lessen oil palm farms' reliance on chemical fertilizers (Zainuddin et al., 2022; Munda et al., 2018; Bhardwaj et al., 2014). Given the advantages of PGPR as potential biofertilizer, this study proposed two hypotheses. The hypothesis of this study is, PGPR can be isolated and selected based on its plant growth-promoting traits. Thus, the primary objective of this study is to isolate, characterize and identify beneficial PGPR from oil palm rhizosphere as a potential biofertilizer for better crop productivity in the future.

MATERIALS AND METHODS

Site and sample collection

The oil palm rhizosphere samples were collected in February 2024, from the share farm Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Campus Jasin located at coordinate (2.2288740022902047" N ,102.45834756651524" E). The sampling site was located on mineral land (sandy clay loam) with a pH of 5.8 and average temperature of 29°C. Shovel, mini hoe and sterile containers were used to collect and store samples. The oil palm seedlings need to be young and healthy to ensure the selection of effective PGPR strains from the root area are successful. The samples then were collected carefully and brought to the laboratory for further procedure.

Isolation of bacteria from rhizosphere of oil palm cultivation

The isolation of bacteria was done under a sterilized surface in laminar airflow. The oil palm roots were cut and washed moderately with sterile distilled water. Appropriate two grams of root were transferred into a 25 mL conical flask that contained eight mL sterile distilled water and shaken in about 10 minutes. Then, about 0.1 mL of sample rhizosphere was pipetted onto a nutrient agar (NA) plate and spread gently using a sterile spreader. These procedures were carried out up to three times to get a wide range of PGPR possibilities. The inoculated plate was incubated in incubator at 30ºC for a day. After one day of incubation, the potential bacteria were selected and streaked it into new nutrient agar plate to obtain a single colony of the bacteria by using an inoculating loop and repeated several times for different colonies. Then, the pure cultured bacteria obtained were preserved invertedly in the chiller at 4 ºC for further studies.

Nitrogen fixation

Nitrogen-free solid malate medium was used to test the nitrogen fixation activity by the isolates (Döbereiner and Day 1976) with some modifications. About 0.1 mL of an overnight nutrient broth bacteria culture was pipetted onto the nitrogen-free media and incubated at 30 ºC for 24 hours following procedure from Hardy et al. (1968). The formation of blue colour on the green plate specifies the existence of nitrogen fixation activity by the isolates.

Phosphate solubilization ability

The assessment of P-solubilization capacity was conducted as outlined in prior studies utilizing the phosphate growth medium developed by the National Botanical Research Institute (NBRIP) (Nautiyal, 1999). Approximately 0.1 mL of a bacterial culture grown overnight in nutrient broth was transferred onto the NBRIP agar medium and incubated for a duration of 48 hours. Colonies were selected based on their phosphate solubilization capability, which was indicated by the formation of a distinct halo zone (Marwa Amri et al., 2023). The dimensions of the phosphate-solubilizing zone were measured for each colony (Nair et al., 1995) by assessing the diameter of the solubilization halo surrounding the colonies post-inoculation on the NBRIP agar media. The solubilization index (SI) was computed using the formula provided by Mingkwan et al. (2020):

$$Solubilization\ Index\ (SI) = \frac{(CD + HD)}{CD} \times 100\%$$

where CD is the colony diameter, and HD is the halo zone diameter.

Potassium solubilization ability

Bacterial isolates were cultivated on a modified Aleksandrov agar medium to assess their capacity for potassium solubilization (Hu et al., 2006). A volume of approximately 0.1 mL of fresh bacterial suspension was applied to the medium and incubated at 30 ºC for a duration of 48 hours. The formation of a clear halo around the colony on the agar plate suggested the potential dissolution of mica as a source of insoluble potassium. The diameters of the potassium solubilization zones (D) and the colonies (d) were measured to determine their solubilization efficiency (KE) (Khanghahi et al., 2017), where:

$$KE = \frac{D}{d}$$

Indole compounds production

Approximately one milliliter of a one-day-old potential bacterial culture was introduced into a 100-milliliter nutrient broth, supplemented with five milliliters of L-Tryptophan as a precursor for IAA. Concurrently, a nutrient broth without bacterial inoculation was utilized as a control. Subsequently, about 1.5 milliliters of the bacterial culture was placed into a sterile microcentrifuge tube and centrifuged at 7000 rpm for seven minutes. One milliliter of the resulting supernatant was combined with two milliliters of Salkowski reagent, following the protocol established by Gordon and Weber (1951). After a settling period of 30 minutes, the solution exhibited a pink coloration, signifying the production of IAA. Absorbance readings were recorded at a wavelength of 535 nm using a spectrophotometer.

Siderophore production

The siderophores were detected using Chrome Azurol S (CAS) agar, which is comparable to the agar described by Schwyn and Neilands (1987). Each of the doable strains was added to CAS agar plates, which were then incubated for 72 hours at 30 °C. Positive siderophore results are indicated by the formation of an orange halo zone surrounding the colony.

Molecular identification of PGPR

The PCR products were prepared in a five µL volume for a single primer amplification using the same universal primers 1492R (5'-TACGGTTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Lane, 1991) for separate reactions of each primer with the bacterial full-length 16S gene, ~ 1500 bp, The PCR was run in the following order: initially denaturing at 94°C for two minutes, followed by denaturation, annealing, and extension at 98°C for 10 seconds, 53°C for 30 seconds, and 68°C for one minute. The standard PCR clean-up procedure was used to purify the PCR products. A Basic Local Alignment Search Tool (BLAST) analysis was performed on the closely similar bacterial 16S rRNA sequences, and the sequences were identified by referencing the NCBI GenBank nucleotide database.

Statistical analysis

All the experiments were performed in three replicates and the average values of three replicates. The data from characterization activities by microbes was analyzed by One-Way ANOVA using the software IBM SPSS Statistics 27.0 (SPSS, USA). Mean values will be compared using the Tukey-b test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and morphology of bacteria from oil palm rhizosphere

In the present study, around six bacteria isolates were positively obtained from the rhizospheres portion of oil palm cultivated at Share farm at Universiti Teknologi MARA (UiTM), Malacca. The significance of bacterial isolation at this site is underscored by the increased likelihood of bacterial interactions with the roots of the oil palm. It has been demonstrated in earlier research that oil palm plantations contain advantageous microbes that may promote plant development (Ariyani et al., 2021). In similar findings, Hasan et al. (2024) stated that, a dynamic and ever-changing network of relationships helped the interaction between microbes and plants. This phenomenon is likely attributed to the crucial roles played by Plant Growth-Promoting Rhizobacteria (PGPRs), particularly in providing nutrients to plants in soils with low fertilization (de Andrade et al., 2023). Plants engage in photosynthesis, allocating approximately 10 to 40% of their photosynthetic metabolites to the rhizosphere through a process known as rhizodeposition. This assertion is corroborated by research conducted by Vetterlein et al. (2020) and Hassan et al. (2019), which indicates that the rhizosphere soil becomes enriched with nutrients, amino acids, and organic energy-rich compounds such as carbohydrates. After one day of incubation, rhizosphere bacteria were seen to have grown completely on a nutrient agar (NA) plate. The rhizosphere bacteria have exhibited a large population, surpassing 300 colony-forming units (CFU). On the incubated NA plates, the morphology of the colony was mostly shown to be gummy, milky-white or white with varying diameters and margins.

Characterization of plant-growth promoting rhizobacteria activities

All six plant growth-promoting rhizobacteria (PGPR) were subjected to a comprehensive assessment of their biochemical properties (Table 1). The isolates underwent qualitative analysis to determine their ability for nitrogen fixation in a nitrogen-free solid malate medium, which included bromothymol blue as an indicator. Each isolate demonstrated a positive result in the nitrogen fixation assessment (Figure 1a) which has been proven by shifting the media colour from green to blue on nitrogen-free media. The green colour indicates the neutral pH (pH 7) while the blue colour indicates the alkaline pH. This does occur due to the PGPRs have been producing ammonia as a byproduct of their activity. Ammonia is a basic compound that can cause an increase of pH surrounding the medium. The consistent shifting green to blue colour on nitrogen-free medium indicates active biological nitrogen fixation, highlighting their key mechanisms by which PGPR can enrich soil nitrogen through atmospheric nitrogen fixation. The results are per previous studies of Mir et al. (2021) and Kuan et al. (2016) which have found that PGPR inoculation can biologically fix atmospheric nitrogen and provide an alternative technique to reduce the fertiliser-N input. Similar studies have been reported that several PGPRs exert a beneficial effect on the plant growth of many crops, such as sugarcane, rice and oil palm (Zakry et al., 2012; de Andrade et al., 2023; Peng et al., 2023). Nitrogen is a necessary component of chlorophyll, which facilitates photosynthesis, and protein, which is made up of amino acids and serves to catalyze chemical reactions and transport electrons. It provides plants a dark green colour and stimulates the growth and development within their leaves, stems, and other vegetative parts (Shah et al., 2016). Thus, biological N_2 fixation is recognized as an important component of the nitrogen cycle in a range of ecosystems, including several extreme environments.

Table 1. Nitrogen fixation, phosphate and potassium solubilizations test results on PGPRs

Characteristics	**Bacteria Strain**					
	AR 1	**AR 7**	**AR 11**	**AR 13**	**AR 14**	**AR 21**
Nitrogen fixation	+	+	+	+	+	+
Phosphate solubilization (%)	114.3 ± 0.040^{c}	200.0 ± 0.100^{b}	333.3 ± 0.168^{a}	113.3 ± 0.238^{c}	141.7 ± 0.105^{bc}	337.5 ± 0.093^{a}
Potassium solubilization (%)	181.8 ± 0.074^{c}	288.9 ± 0.072^{b}	312.5 ± 0.074^{b}	166.7 ± 0.108^{c}	166.7 ± 0.157^{c}	281.8 ± 0.079^{a}

Values are means ± standard deviation. Values with the same letters in each characteristics column among the bacterial strains are not significantly different according to Tukey-b at $p<0.05$

Furthermore, the phosphate solubilization percentage from all strains ranged from 113.3% to 337.5%. All isolates formed a clear zone on P-solubilization agar plates (Figure 1b). The phosphate solubilization percentage indicated that isolate AR 21 exhibited the highest phosphate solubilization activity among all isolates followed by AR 11, AR 7 and AR 14 over a two-day incubation period, corresponding to a 337.5%, 333.3%, 200.0%, and 141.7% respectively. While the other isolates including AR 13 and AR 1, represent the strains with low to intermediate percentages (over 113.3%). The result showed that there was a 224.2% variation in results between the highest and lowest phosphate solubilization percent. Phosphorus is the most limited nutrient for plant growth. It is plentiful in many agricultural soils, but it is inert in plants due to low levels of soluble phosphate. In plants, phosphorus is crucial for regulating physiological reactions and strengthening durability against abiotic stressors including heat, salinity, drought, waterlogging, high carbon dioxide levels, and heavy metal toxicity (Lambers, 2022; Hawkesford et al., 2023). In this study, the phosphate solubilization test has revealed that isolate AR 21 is the highest phosphate solubilizing ability among the other isolates to solubilize the insoluble phosphate from NBRIP media followed by AR 11. These results demonstrate that the isolates can dissolve insoluble phosphate, making it accessible to plants which is a valuable trait for reducing dependency on chemical phosphate fertilizer. This happens through the process that involves the formation of organic and inorganic acids through acidification, chelation, and exchange processes. These processes can lower the pH of the media and increase the phosphorus solubility and able to release it (Srilakshmi et al., 2021; Khan et al., 2009; Bhattacharyya and Jha 2011; Seshachala et al., 2012; Alori et al., 2017). The region surrounding the PGPRs colony on media will exhibit a halo zone as the process of the breakdown and solubilisation of insoluble phosphate. Apart from AR 21 ability to produce polyhydroxyalkanoate (PHA), this isolate's capability to solubilise phosphate is not well understood. Based on the results of this research, each isolate displays a distinct ability to solubilize phosphate from the tested plate which may be categorized as moderate to high levels. The potential of phosphate solubilizing rhizobacteria to solubilize insoluble phosphates can improve plant development and yield by enhancing the availability of phosphorus to the plant (Mir et al., 2021). In other words, oil palm rhizosphere has presented a diverse population of phosphate-solubilizing microorganisms which three bacteria of the genera *Acinetobacter*, *Klebsiella*, *Staphylococcus*, and *Burkholderia* were associated with the solubilization of calcium phosphate with high soluble P yields (Acevedo et al., 2014).

Besides, all isolates showed a convincing result based on their potassium efficiency ability values (Figure 1c). The strains were inoculated in modified an Aleksandrov agar plate to quantitatively measure its potassium solubilization capabilities. The K-solubilization efficiency by these bacterial strains ranged from 312.5 to 166.7%, with AR 11 able to significantly solubilize the highest K, followed by AR 7 and AR 21. In contrast, the other strains including AR 1, AR 13, and AR 14 were moderate K-solubilized which is equivalent to 181.8, 166.7 and 166.7% respectively. The most prevalent inorganic cation is potassium (K), which is crucial for optimum development of plants (Xu et al., 2020; White et al., 2010). It has a role in multiple physiological functions, including photosynthesis, translocation, enzyme activation within the plant, and regulating stomata opening and closing (Rawat et al., 2022). This trait enables the isolates to contribute to long-term soil fertility by releasing K from mineral reserves, making them valuable candidates for biofertilizer development in K-limited agroecosystmes. According to the current investigation, all of the isolates were able to solubilize potassium, with AR 11 (*Acinetobacter seifertii* sp.) being the most significant. It aligns with previous research reported that several microorganisms can solubilize insoluble forms of K-bearing minerals such as feldspar, mica, illite, and orthoclase, by secreting organic acids that either directly decompose K or chelate the silicon ion to release K in solution (Toba et al., 1991; Bennett et al., 2011). This causes the formation of a zone of clearance around the spots which indicates the potassium solubilization (Kumar et al., 2015). This finding also is in line with the previous studies (Ashfaq et al., 2020), which reported *Acinetobacter* sp. can solubilize potassium to support plant growth and plant promoting bacteria.

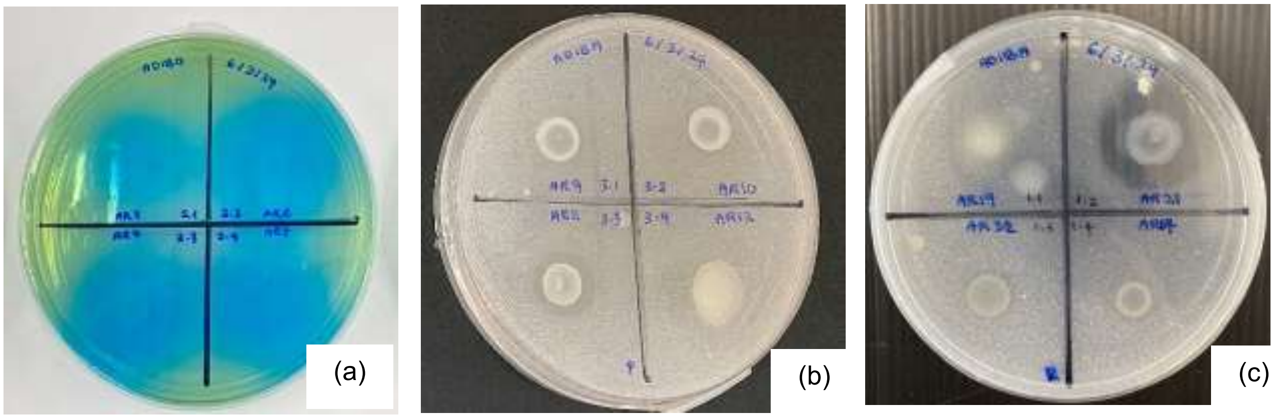

Fig. 1. (a) Nitrogen-free (b) P-solubilization (c) K-solubilization agar plate after inoculation with bacterial isolates. Positive indications are shown by the change in colour from green to blue in (a) and and formation of halo zone around the colony in (b) and (c).

These isolates were also tested for other biochemical tests such as indole compound, siderophore and hydrolyzing enzyme production (Table 2). From six isolates selected, about one isolate, AR 21 have proven their ability to produce a significant IAA production that over 200 µg/mL which was 260.3 µg/mL. Indole-3-acetic acid is one of the most important plant hormones that affect plant cell division, extension, and differentiation, stimulates seed germination, initiates lateral, adventitious root formation, and responses to light and gravity (Tang et al., 2023; Teale et al., 2006). It has been documented that the production of IAA by bacteria may vary between species and strains and additionally be influenced by growth stage, culture conditions, and substrate availability (Lata et al., 2024; Gyanendra et al., 2020). Among six isolates, AR 11 was one of the isolates that produced the most significant indole acetic acid compound, with over 200 µg/mL of IAA production. It is due to some microorganisms producing auxins in the presence of a suitable precursor such as L-tryptophan (Noor et al., 2023). It is also reported that tryptophan increases the production of IAA showed in *Acinetobacter baumannii* which consider it can produce auxins when exposed to tryptophan (Lin et al., 2018). From the results, the synthesis of indole-3-acetic acid indicates their ability to stimulate root initiation and elongation, faciliting improved water and nutrient absorption. Thus, the ability to synthesise IAA is believed to be an advantageous technique for identifying beneficial microorganisms, indicating that bacteria that produce IAA have a significant impact on plant development (Ratnaningsih et al., 2023).

Table 2. Indole compound, siderophore and hydrolyzing enzyme production of selected rhizobia isolates.

Characteristics	**Bacteria Strain**					
	AR 1	**AR 7**	**AR 11**	**AR 13**	**AR 14**	**AR 21**
Indole compounds (µg/mL)	146.47± 0.742[bc]	163.24± 0.072[b]	260.3 ± 0.074[b]	163.24± 0.180[b]	118.53± 0.157[c]	157.65 ± 0.079[a]
Siderophore production	+	-	+	-	+	+
Hydrolyzing enzyme production	+	+	+	+	-	+

Values are means ± standard deviation. Values with the same letters in each characteristics column among the bacterial strains are not significantly different according to Tukey-b at p<0.05.

Also, the siderophore production test indicated that all isolates were able to acquire iron from the CAS plate test by has produced a substantial formation of orange colour around the colonies for about seven days of incubation except for isolate AR 7 and AR 13 (Figure 2a). According to the current study, four out of six isolates confirmed positive for siderophores metabolites production on the CAS plate test by observing the converting colour of blue to orange. The basis of this test is the competition for iron between a chelator (siderophore) produced by isolates and the ferric complex of an indicator dye, Chrome Azurol S (CAS). Due to its apparent greater affinity for iron (III), the siderophore takes the iron from CAS. When a positive reaction occurs, the CAS reagent's colour changes, from blue to orange (Milagres et al., 1999). The presence of shifting from blue to orange reflect the isolates' potential to improve plant iron nutrition and contribute to induced systemic resistance against fungal and bacterial diseases. This result is consistent with earlier studies that identified *Brevibacillus* sp. and *Burkholderia* sp. as the bacteria that produce siderophores throughout the rhizosphere of oil palm plantations (Nor, 2020). The study has proven that their isolates have the potential to be the most effective strain for biofertilizers due to the multitasking activity ability. Microbes and some plants are mainly secretors of siderophore. It is capable of producing trivalent iron chelates and has a high affinity for trivalent iron ions (Sheng et al., 2024). Siderophores are among the effective iron transport systems that microbes have developed for dealing with iron-limited environments. These tiny molecular productions are required for taking in and transferring iron ions from the soil (Liu et al., 2022).

For the hydrolyzing enzyme production test, all the tested strains showed positive results except for AR 14 (Figure 2b). Moreover, all isolates from the current study were able to produce hydrolyzing enzyme except for AR 14. The secretion of hydrolytic enzymes from these isolates facilitate organic matter decomposition and nutrient release and also contributes to biological control that can enable them to thrive in complex soil environments and supports sustainable soil health. Cellulase-positive colonies were identified by the presence of a light-yellow zone surrounding the colony on a red background (Dogan and Taskin 2021). In addition to penetrating through wounds and holes, bacteria use hydrolytic enzymes which include cellulase to rapidly colonize plant tissues. Enzymes that break down cell walls, such as cellulases, xylanases, and pectinases, are believed to be in charge of plant-microbe interactions and root intercellular colonization (Verma, 2001; Kandel et al., 2017). This is because the cell wall is mainly composed of cellulose while the middle lamella between cell walls contains mainly pectin (Haas et al., 2021). However, the significance of this research arises from knowing that this potential has not been well investigated in diazotrophic endophytes (Reinhold-Hurek and Hurek, 1998).

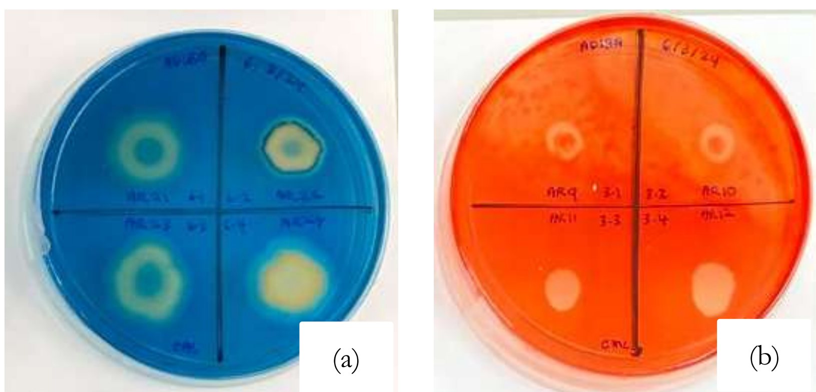

Fig. 2. (a) Chrome Azurol S (CAS) (b) Hydrolyzing enzyme production agar plates after inoculation with selected isolates. Positive results are indicated by the formation of halo zone around the colonies.

Identification of PGPR from oil palm rhizosphere

The selected bacterial isolates were identified via sequencing of its 16S rRNA gene. Isolates AR 11 and AR 21 were viable options because they were excellent in several areas, including nitrogen fixation, siderophore and enzyme synthesis, phosphate and potassium solubilization, and high indole compound production. Isolates AR 11 and AR 21 were tentatively identified as *Acinetobacter* sp. and *Aquitalea* sp. based on their colony and cell morphologies respectively. The 16S rRNA gene sequence analysis revealed the oil palm-associated bacteria based on BLAST comparison searches against the NCBI nucleotide database of the isolate showed 99.6% similarity to such as *Acinetobacter seifertii* strain (NCBI accession no: NR_024640) and 97.2% similarity to closely related species for AR 11 (Figure 3a) and *Aquitalea pelogenes* strain with NCBI accession number of NR_148648 for AR 21 (Figure 3b), confirming species-level identification. The bacterial strain exhibit Zn solubilization on a laboratory scale including *Klebsiella* sp., *Acinetobacter* sp., *Gluconacetobacter* sp., *Burkholderia* sp., *Serratia* sp., *Citrobacter* sp., and *Enterobacter* sp., (Haroon et al., 2021; Ajmal et al., 2021). It was confirmed from previous study that the root length of wheat seedlings increases by 16 – 40% through the inoculation of microbes (Gandhi et al., 2022). Several investigations have found comparable findings for *Acinetobacter seifertii* sp. to those for *Acinetobacter* sp. *Acinetobacter* sp. is found in the rhizosphere of many plants and is abundant in nature. *Acinetobacter* sp. is a significant PGPR since it is known for producing antibiotics, siderophores, gibberellin, IAA, biosurfactants and emulsifiers. It also solubilizes phosphate, potassium, and zinc (Shilpa et al., 2023). According to Moussa Sondo et al. (2023), found in vitro phenotypic study of the isolates' ability to promote plant development showed that *Burkholderia*, *Ralstonia*, *Acinetobacter*, and *Pseudomonas* species were the most abundant in siderophore synthesis and phosphate solubilization. In line with previous reports, *Acinetobacter* species are biochemically diverse microbes of environmental significance that are capable of breaking down hydrocarbons, oil, and halogenated organic pollutants, producing a large range of exopolysaccharides and enzymes, and altering heavy metals (Manna et al., 2021). However, very little research has been done on *Aquitalea* sp. Ishizawa et al. (2017) discovered that the *Aquitalea* sp. strain H3 colonizes plants and stimulates the development of L. minor. It is evident that strain H3 exerts its growth-promoting abilities regardless of the presence of other bacteria.

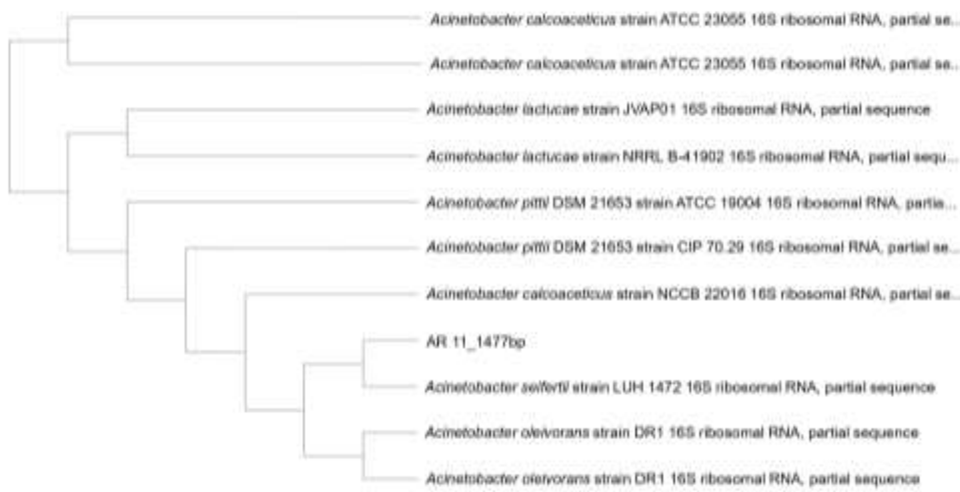

Fig. 3. (a) Phylogenetic tree of isolate AR 11

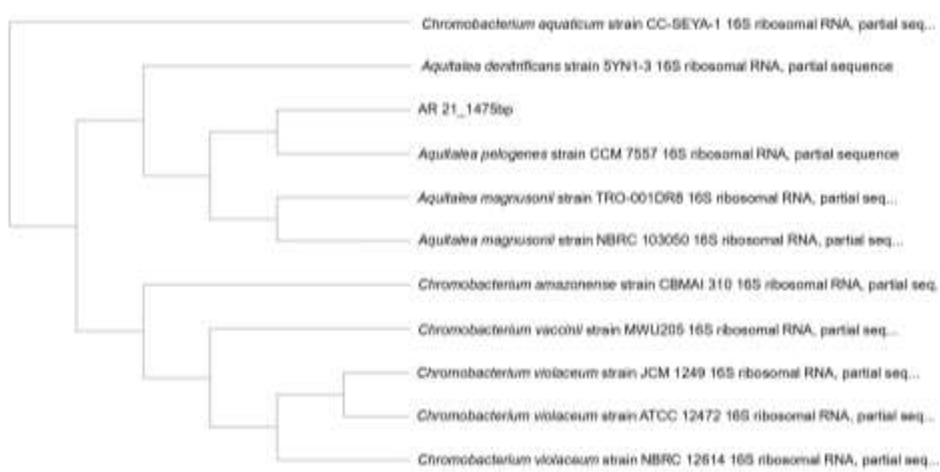

Fig. 3. (b) Phylogenetic tree of isolate AR 21

All characterization tests that were performed on the selected isolates produced positive results. This is due to a varied population of PGPR that can be found in abundance in agricultural soil (Sayyed et al., 2019). PGRR offers the plants several advantages, including enhanced plant nutrition and soil nutrient enrichment as well as the promotion of plant development (Basu et al., 2021). They provide a wide range of advantageous functions for plants, including N_2 fixation, IAA synthesis, siderophore synthesis, and P solubilization (Shaikh et al., 2016; Shaikh et al., 2018). This happens through a variety of mechanisms, such as direct contact with pathogens and mutualists as well as indirect interactions with free-living bacteria that improve the plant's availability of nutrients (Morgan et al., 2005). Thus, plant health, growth, and productivity are significantly impacted by the rhizosphere microbiome (Bennett et al., 2011). Notably, this research reports *Aquitalea* sp. as a potential PGPR for the first time in the oil palm rhizosphere. While previously documented in wastewater systems, its expression of nitrogen fixation, phosphate and potassium solubilization, IAA synthesis, siderophore synthesis and hydrolyzing enzyme production suggests a previously unrecognized role in plant-microbe interactions. However, this study has limitation which is the PGPR was assessed under *in-vitro* conditions, which may not fully reflect bacterial performance in complex soil environments compared to field trials. For future studies, the researcher should evaluate the efficacy of these strains under varying environmental conditions to confirm their agronomic potential.

CONCLUSION

From the current study, the bacterial isolates from oil palm rhizosphere can potentially promote plant growth which could be directly attributed to the beneficial effects of biological N_2 fixation and IAA production and indirectly to phosphate and potassium solubilizations, iron siderophore and hydrolyzing enzyme productions and successful characterized as PGPR. The strains were molecularly identified as *Acinetobacter* sp. and *Aquitalea* sp. These results demonstrate potential and significant for the initial invention of biofertilizers for Malaysian oil palm production industry. These preliminary results are valuable as an initial overview of the mechanisms by PGPR enhance early plant growth stage. Therefore, further field experiments are required to better understand the roles of these PGPR and rhizobia on oil palm yield and lower the application of chemical fertilizer rates.

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