BIODIVERSITAS
Volume 25, Number 10, October 2024
Pages: 3775-3788

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d251040

Characterization of endophytic bacterial isolates from oil palm (*Elaeis guineensis*) seedlings and ramets for their plant growth promoting potential

IDA AYU PUTU SURYANTI[1], MARIA INDAH PURNAMASARI[3], CAHYA PRIHATNA[3], IMAN RUSMANA[1], ARIS TRI WAHYUDI[1], ANTONIUS SUWANTO[1,2,♥]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Raya Dramaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622833, ♥email: asuwanto@apps.ipb.ac.id

[2]Research Center for Bioresources and Biotechnology, Institut Pertanian Bogor. Jl. Raya Dramaga, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia

[3]Department of Plant Sciences and Plant Pathology, Montana State University. 303 Plant BioScience Building, Bozeman, MT 59717-3150, USA

Manuscript received: 20 August 2024. Revision accepted: 22 October 2024.

Abstract. *Suryanti IAP, Purnamasari MI, Prihatna C, Rusmana I, Wahyudi AT, Suwanto A. 2024. Characterization of endophytic bacterial isolates from oil palm (*Elaeis guineensis*) seedlings and ramets for their plant growth-promoting potential. Biodiversitas 25: 3775-3788.* Using sterile tissue culture to cultivate oil palm (*Elaeis guineensis* Jacq.) seedlings results in the loss of beneficial endophytic bacteria, which can lead to various growth issues, including abnormal flower development and reduced palm oil production. The study aimed to investigate the potential endophytic bacteria in oil palm seedlings and ramets that serve as plant growth promoters. This research began with isolating and characterizing bacterial isolates with plant growth-promoting traits, then identification using 16S rRNA gene sequencing. In vitro and in vivo growth tests were carried out to assess the prospects of these bacterial isolates. Seventeen bacterial isolates were successfully cultured from oil palm seedlings and ramets (seedlings from tissue culture), with 12 and 5 isolates, respectively, which displayed characteristics of plant growth-promoting bacteria: 88% could produce aminocyclopropane-1-carboxylate (ACC) deaminase, 18% exhibited nitrogen-fixing abilities, 47% demonstrated phosphate solubilization, and 12% were producers of indole-3-acetic acid (IAA) hormone. One of the isolates, designated as 3AK, was indicated as the *Aeromonas* genus, which originates from the environment and has all the plant growth-promoting properties tested qualitatively in this study. In vitro growth testing showed that 3AK isolate had the most significant average lengths for both shoots (4.4±0.4 cm) and roots (8.2±0.5 cm). In the greenhouse experiment (in vivo), the oil palm seedlings treated with bacteria, such as 3AK, exhibited differences in shoot and root dry weight compared to the control group, and the difference was statistically significant. These findings collectively indicate that endophytic bacteria code 3AK is isolated from oil palm root, identified as *Aeromonas taiwanensis* through 16S rRNA gene sequencing, and has the potential as a plant growth promoter. Inoculation of *Aeromonas taiwanensis* strain 3AK and PsJN as *Burkholderia phytofirmans* strain PsJN (already known as a model for promoting plant growth) was carried out for the first time on the growth of oil palm seedlings.

Keywords: Endophytic bacteria, oil palm, plant growth promoting, ramets, seedlings

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) plant is a vital commodity in the global food and biofuel industries. It is obtained from two primary products such as crude palm oil and palm kernel oil (Basiron 2007), and contributes to almost 40% of world oil production (Murphy et al. 2021). However, Indonesia's crude palm oil (CPO) production is reported to have decreased by approximately 0.9% in 2021 (Amanta and Nafisah 2022). It was caused by multiple factors, including basal stem rot (BSR) diseases that affect oil palm plants as a result of the *Ganoderma boninense* attack (Paterson et al. 2020; Jazuli et al. 2022) and the consequences of climate change (Sarkar et al. 2020). Around 60% of oil palm plantations in Malaysia have been infected with this pathogen (Bharudin et al. 2022).

Various efforts have been implemented to address this issue and develop exceptional oil palm seedlings. One was implementing in vitro micropropagation, specifically somaclonal somatic embryogenesis with tissue culture and natural efforts to produce superior oil palm seedlings (Yarra et al. 2019). The proliferation of embryogenic structures in tissue culture typically leads to the development of somaclonal variants, which are the primary cause of abnormality phenotypes in flowering plants (Green et al. 2013; Rival et al. 2013). During the tissue culture process, sterile conditions lead to the loss of the beneficial microbial composition in oil palm ramets, which serve as plant growth promoters.

Another problem in planting oil palms is the excessive use of chemical fertilizers. Like other plants, optimal growth and production yields in oil palm depend heavily on fertilization. However, it can replace the function of microorganisms in promoting plant growth. Bacteria as a microorganism, especially in the rhizosphere, are essential for plant development, nutrition, and stress resistance, with miRNAs as important interaction mediators (Finkel et al. 2020; Middleton et al. 2021). So, applying biological fertilizers is becoming more crucial because chemical fertilizers can harm humans and damage the environment

(Om et al. 2009). A better option for a biofertilizer is to inoculate plants with growth-promoting rhizobacteria since this can lessen the need for chemical fertilizers. It has an encouraging potential for ecosystem restoration (Wang et al. 2020).

Microbial inoculation has been thoroughly studied in previous studies on plant development and fertility, especially in oil palm seedlings. Research has demonstrated that applying *Bacillus cereus* and *Trichoderma asperellum* to oil palm seedlings complements each other in fostering plant growth (Syafiq et al. 2021). Additionally, plants have intricate microbial communities on their surfaces and within their tissues, which contain many genes, surpassing the genetic content of the host plant (Zhang et al. 2021). This community of bacteria in plant tissue, known as endophytic bacteria, resides in plant tissue without harming the host (Wu et al. 2021). Research related to isolating endophytic bacteria from seedlings (from roots and plumule-radicle) and oil palm ramets for plant growth promotion is still very limited, considering that the material, especially ramets, is not widely available.

This study aims to investigate the characteristics of bacterial isolates from oil palm seeds developed naturally in nature (seedlings) and those originating from tissue culture (ramets) as a first step to finding beneficial endophytic bacteria that can be cultured. It can later be used for plant growth promoters and decrease abnormalities in flowering (for subsequent research). Therefore, identifying beneficial endophytic bacteria in oil palm seedlings is essential for improving oil palm plants' health, including seedlings and ramets' growth in the acclimatization phase.

MATERIALS AND METHODS

Sample collection and isolation of bacterial endophytes from oil palm ramets and seedlings

This study utilized samples of oil palm ramets B18 obtained from the tissue culture laboratory at PT. Wilmar Benih Indonesia, Cikarang, Bekasi, and oil palm seedlings (dura x pisifera, PPKS high CPO) sourced from PT. Perkebunan Nusantara IV. The surface sterilization procedure includes washing using distilled water three times and immersing in 3% (v/v) sodium hypochlorite solution for three minutes, followed by immersion in 70% (v/v) ethanol for three minutes (Kumar et al. 2020). Then, ramets and seedlings (root and plumule-radicle) were blended in 50 mL sterile equates. These blends were placed onto modified low-nutrient culture media plates in triplicate and incubated at a temperature of 30°C to facilitate the growth of bacteria. Following an incubation period of 2-7 days, select representative colonies that emerged on the Petri plates were aseptically collected using a sterile loop. Colonies with distinctive morphologies were singled out and subjected to purification through sub-culturing.

The resulting pure cultures were analyzed using morphological, biochemical, and molecular characterization. Each bacterial colony from every isolate was cultured in nutrient broth and stored in 20% sterile glycerol at a temperature of -80°C for further research. The growing medium for bacterial isolates is a modification of the methods from (Hidayati et al. 2014). Isolates of endophytic bacteria were cultivated using modification low nutrient culture media: NA 1% consists of 0.013 g/100 mL NB (Merck, Germany) and 15 g L^{-1} agar; soil extract-nutrient agar 1% consisted of 17.75 g L^{-1} soil extract and nutrient agar 1%; root extract-nutrient agar 1% consists of 4 g L^{-1} dry oil palm root and nutrient agar 1%. Each medium was sterilized in an autoclave (121°C, 15Psi, 15 minutes).

In vitro assays to characterize plant growth-promoting bacteria

To classify bacteria based on their beneficial characteristics for plants, four specific abilities were evaluated for each strain: their production of IAA, their capacity for nitrogen fixation, their 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, and their ability to solubilize phosphates. IAA production was assessed using a modification colorimetric technique (Gordon and Weber 1951). Bacteria isolates were grown in nutrient broth containing 0.1% L-tryptophan, a precursor for IAA. Salkowski's reagent was used to test for the presence of IAA, turning the nutrient broth media from yellow to violet.

To assess the nitrogen-fixing activity of the pure bacterial cultures, an N-free semi-solid malate medium (Nfb) was used. This medium consisted of malic acid (5 g, K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), NaCl (0.1 g), $CaCl_2.2H_2O$ (0.02 g), micronutrient solution ($CuSO_4.5H_2O$ 0.04 g; ZnSO4.$7H_2O$ 0.12 g; H_3BO_3 1.4 g; $Na_2MoO_4.2H_2O$ 1 g; $MnSO_4.H_2O$ 1.175 g in 1000 mL of distilled water) 2 mL; 2 mL bromothymol blue; 4 mL FeEDTA solution; 1 mL vitamin solution KOH 4.5 g; agar 15 g; aquades 1000 mL with pH 6.5. The plates were incubated for seven days at 30±2°C, and a color change from pale green to blue indicated the presence of nitrogen-fixing bacteria (Kuan et al. 2016). ACC deaminase activity was determined by culturing bacteria on the Dworkin and Foster (DF) medium, which consisted of KH_2PO_4 (4 g), Na_2HPO_4 (6 g), $MgSO_4.7H_2O$ (0.2 g), glucose (2 g), gluconic acid (2 g), citric acid (2 g), stock solution 1 ($FeSO_4.7H_2O$ 0.1 g in 10 mL aquades) 0.1 mL, stock solution 2 (H_3BO_3 0.001 g; $MnSO_4.H_2O$ 0.01119 g; $ZnSO_4.7H_2O$ 0.1246 g; $CuSO_4.5H_2O$ 0.07822 g; $Na_2MoO_4.2H_2O$ 0.016 g; distilled water 100 mL) 0.1 mL, and agar (22 g) and distilled water (1000 mL). The medium was supplemented with three mM 1-aminocyclopropane-1-carboxylic acid (ACC) (Ali et al. 2014). The phosphate solubilization capacity was evaluated by observing a clear zone around the bacterial colonies when grown on Pikovskaya's agar medium, which consisted of the following reagents (g/L), 10 g dextrose, 0.5 g yeast extract, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.0001 g $MnSO_4$, 0.0001 g $FeSO_4.7H_2O$ and 15 g bacto agar. Bacteria cultures were incubated at 30°C for five days (He et al. 2022).

Molecular identification of endophytic bacteria isolates

16S rRNA gene sequencing and phylogenetic analysis partially identified the selected bacterial isolates. A sample of 1 pipette tip of bacteria was mixed in 50 µL of nuclease-free water (NFW). The 16S rRNA gene amplification was performed using the PCR method with a composition consisting of 0.5 µL of genomic DNA, 5 µL Gotaq Green Master Mix (Promega), 1 µL primer 63F (5'-CAGGCCTAACACATGCAAGTC-3') 1 µL primer 1387R (5'-GGGCGGWGTGTACAAGGC-3'), and 2.5 µL NFW with a total volume of 10 µL. The PCR conditions consisted of predenaturation (95°C for 5 minutes), denaturation (95°C for 30 seconds), primer attachment (55°C for 30 seconds), chain elongation (72°C for 1 minute), and post-extension (72°C for 7 minutes) which was carried out for 30 cycles with using the Biorad Thermal PCR machine C1000 cyclist.

The 16S rRNA gene amplification visualization was tested by electrophoresis on agarose gel 1%, using a 1 kb marker. The final purified PCR products were sequenced from both directions using the 16S rRNA universal forward and reverse primers at Macrogene, Inc. (Singapore). The 16S rRNA gene of the bacterial isolates was compared against sequences available in the GenBank by the BLAST nucleotide (BLASTn) using non-redundant (nr) and microbes databases. The phylogenetic analysis of the 16S rRNA gene of bacterial isolates with reference bacterial sequences identified in the BLAST search was carried out using the MEGA 6.0 software package (Tamura et al. 2013). The aligned sequences were generated using the Muscle algorithm that was integrated into the system. The output was then utilized to construct a phylogenetic tree by computing distance matrices for neighbor-joining (NJ) analysis with the Kimura two-parameter model and a bootstrapping analysis with 1000 replicates to assess the stability of internal branches. Furthermore, several 16S rRNA genes of previously identified plant growth-promoting bacteria (PGPB) were incorporated as references in the phylogenetic tree.

Confirmation test using hemolysis test and ERIC-PCR

The blood agar test and enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) aim to confirm the closeness of the 3AK isolate from oil palm seedlings to bacteria isolated from certain places. This isolate was compared with three reference bacteria, including *Aeromonas hydrophila* isolated from a freshwater fish collection of the Aquatic Organisms Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor, *Aeromonas hydrophila* JHL3 isolated from a water source (lipolytic bacterial isolation) collection from PT. Wilmar Benih Indonesia, and *Aeromonas caviae* WS7b isolated from a soil sample obtained from a black-pepper plantation on Bangka Island, Indonesia (Malik et al. 2003). *Escherichia coli* DH5α was used as a negative control, and *Staphylococcus aureus* as a positive control. A loopful of individual bacterial isolates from pure cultures was streaked on blood agar media containing 5% (v/v) fresh goat/sheep blood. After 24-48 hours of incubation at 28ºC, a clear zone or color change around the bacterial colonies indicated a positive reaction for hemolysis. Various colors were used to specify the types of hemolysis reactions, such as a dark or greenish-grey zone around the bacteria indicating partial lysis of the blood agar and a distinct, clear zone referring to complete lysis of the blood agar. No zone or color change around the bacterial colony indicated a negative or γ hemolysis reaction (Gilligan 2013).

The ERIC-PCR method used in this study is a modification of the technique used in previous research (Ayu et al. 2014). Isolate code 3AK and two reference bacteria were used in this experiment. The PCR reaction mixture was comprised of 25 µL, including 12.5 µL of GoTaq Green® Master Mix (Promega), 1 µL of 25 pmol ERIC1R (5'-ATGTAAGCTCCTGGGGATTCA), 1 µL of 25 pmol ERIC2F (5'-AAGTAAGTGACTGGGGTGAGCG-3'), 9.5 µL of nuclease-free water, and 1 µL of DNA template obtained using a sterile toothpick from the isolates. The PCR conditions included an initial denaturation step at 95°C for 7 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 49°C for 1 minute, elongation at 65°C for 1 minute and 30 seconds, and a post-extension step at 65°C for 10 minutes (Applied Biosystems, 2720 Thermal Cycler). The PCR cycle was repeated 30 times. A 5 µL aliquot of the resulting PCR products was then electrophoresed for 60 minutes at 50 V through 1% agarose in 1X TAE buffer. The resulting band profiles were subsequently observed under a UV transilluminator, and the profiles were analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYSpc 2.0).

Microbe-microbe in vitro compatibility test

The assessment conducted in this study evaluates the ability of bacteria to thrive in a mutually beneficial relationship when inoculated together. It was conducted on 17 bacterial strains above and 3 bacterial isolates from the PT. Wilmar Benih Indonesia collection from oil palm roots (R6, PsJN, and GS 4.4). Following the growth of the microorganisms at 28°C for 24-48 hours, three to four isolated colonies were transferred to 4 mL of nutrient broth (NB) medium and incubated overnight at 28°C and 200 rpm. One hundred microliters of the test microorganism, with a cell count of approximately 10^8-10^9 CFU/mL, were spread on the surface of fresh NA plates. Sterilized filter paper discs (5-mm diameter, Whatman number 1) were placed on the spread plate (maximum five discs/plate), and each was inoculated with 10 µL the other bacteria isolates of an overnight NB-grown culture of the microorganism to be tested to assess compatibility. The plates were incubated at 28°C and observed at 24-hour intervals over 4 days. Two microorganisms were considered compatible if they could grow together and overlap beyond the disc border without exhibiting an inhibition zone or overgrowth around the disc (Tabacchioni et al. 2021). Two independent experiments consisting of three replicates were conducted for each bacteria-bacteria combination.

In vitro seedling germination test

This test aims to assess the potential impact of bacterial isolates on seed growth rate using a modified technique

(Gholamalizadeh et al. 2017). Rice seeds were treated with a 5% sodium hypochlorite for 5 minutes, followed by three rinses with sterile distilled water. After that, the seeds were immersed in a mixture of 17 bacterial isolates (sync 1 (17 isolates) and sync 2 (17 isolates, PsJN, R6, GS 4.4)) in sterile distilled water (negative control) or the bacterial isolate PsJN (positive control) with an OD_{600} value of 0.5. The seeds were positioned on moist filter paper inside sealed Petri dishes. Bacteria-inoculated and mock-inoculated seeds were placed in separate Petri dishes. For each treatment, 5 seeds in 3 replications were allocated, so 300 rice seeds were used. The Petri dishes were stored in darkness at room temperature for 7 days of incubation at 28°C. Following this period, the emerging root and shoot lengths were measured.

Inoculation of endophytic bacterial isolates is beneficial for the growth of oil palm seedlings in the greenhouse

The objective of in vivo testing, which involves inoculating each treatment on oil palm seedlings in polybags, is to evaluate the impact of bacterial isolates on plant growth and determine their potential as plant growth-promoting agents. This experiment was conducted in a greenhouse using oil palm seedlings (Dura×Pisifera PPKS high CPO) sourced from PT Perkebunan Nusantara IV, which were planted in polybags (20×20 cm) containing a mixture of soil and sand (1:3). The study employed a completely randomized design, which was duplicated, resulting in a total of 60 oil palm seedlings across three treatment groups and a control group. After germination, approximately two months after planting, with an average height of 18-20 cm, the seedlings were inoculated with bacteria. The treatments included inoculation with strain 3AK, strain PsJN as a positive control, a mixture of multiple bacteria (including strains 3AK, PsJN, R6, and GS4.4), and a negative control group with sterile water added to the soil. The selected isolates were first cultured in nutrient agar (NA) medium for the 3AK strain and King's B medium (mL) for the PsJN, R6, and GS44 strains. A 50 mL suspension of the inoculum (with an OD_{600} =0.5) was introduced into the soil of each polybag (Chen et al. 2022). The seedlings were watered regularly daily, and after 12 weeks of growth, the shoot (above-ground) and root parts of the oil palm seedling were harvested for measurements of dry weights.

Statistical analysis

A completely randomized design was used for growth promotion in vitro. A was implemented in the greenhouse to assess growth promotion. One-way ANOVA was conducted, along with Tukey's test for multiple treatment comparisons. The statistical analyses were executed using R and Rstudio version 4.3.1. The significance level was set at $p<0.05$. The results were visualized using boxplot visualizations through the "ggpubr" R package.

RESULTS AND DISCUSSION

Endophytes bacterial isolates from oil palm ramets and seedlings

In this study, 17 different bacterial isolates were successfully cultured from oil palm ramets and seedlings, with details of 5 isolates coming from ramets and 12 isolates coming from seedlings (Figure 1.A). The highest diversity of endophytic bacterial isolates was found in the plumule-radicle seedling area, with seven isolates (Figure 1.B).

The analysis of endophytic bacterial isolates from oil palm ramets and seedlings displayed divergent outcomes when examined for colony shape, microscopic appearance, margin characteristics, elevation, and color of colonies growing in the media (refer to Table 1). All bacteria isolated from ramets exhibited a coccus shape under the microscope, whereas three isolates from seedlings, namely 3AK, 12bPR, and 19PR, had a rod shape. Among the eight isolates of endophytic bacteria classified as Gram-positive, three were obtained from ramets, and 5 were obtained from seedlings. Meanwhile, there were two Gram-negative isolates in ramets and seven isolates in seedlings. 47.1% of the bacterial isolates were Gram-positive, and 52.9% were Gram-negative.

Plant growth promoting characterization of endophytic bacteria isolated from oil palm ramets and seedlings

Plant growth-promoting bacteria (PGPB) have several characteristics, including the ability to produce deaminase, fix nitrogen, dissolve phosphate, and produce the growth hormone indole-3-acetic acid (IAA). These characteristics were demonstrated by 17 isolates of endophytic bacteria from oil palm ramets and seedlings, as shown in Table 2. Bacterial isolate 3AK, originating from oil palm seedling roots, exhibited all plant growth-promoting bacteria capabilities tested in this study. A high percentage of the bacterial isolates originating from oil palm ramets and seedlings could produce ACC-deaminase, with 88% of the isolates possessing this characteristic (Figure 2.A).

All bacterial isolates from oil palm ramets (100%) can produce the hormone ACC-deaminase and about 80% dissolved phosphate. In seedlings, as much as 83% of bacterial isolates can produce ACC-deaminase, while 33% can solubilize phosphate, 25% can fix nitrogen, and 17% can produce the hormone IAA (Figure 2.B). Most bacterial isolates tested had at least one plant growth-promoting characteristic, except for the bacterial isolate 17PR, which did not have it.

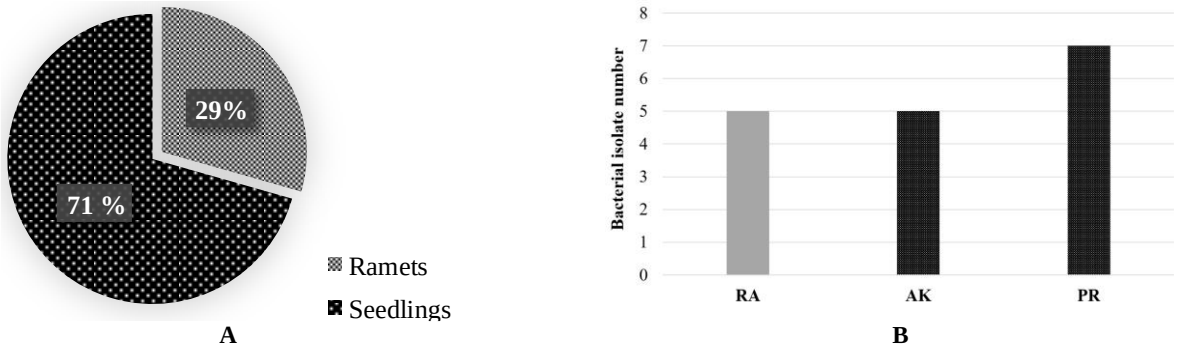

Figure 1. The diversity of endophytic bacterial isolates from oil palm ramets and seedlings that can be cultured in media. A. Percentage of endophytic bacterial isolates; B. The number of endophytic bacterial isolates found in each oil palm: RA: All parts of ramet; AK: Root of seedling; PR: Plumule-radicle of seedling

Table 1. Characterization of colonies of endophytic bacterial isolated from palm oil ramets and seedlings

Isolate code	Shape	Form	Margin	Elevation	Colony on media	Cell shape Gram's reaction
5RA	Coccus shaped	Circular	Erose	Convex	Bright yellow	Positive
7RA	Coccus shaped	Irregular	Entire	Flat	Greyish white with a broken white middle	Positive
9RA	Coccus shaped	Irregular	Entire	Flat	White with a broken white dot in the middle	Negative
16RA	Coccus shaped	Irregular	Undulate	Raised	Yellow to orange with white edges	Positive
21RA	Coccus shaped	Irregular	Lobate	Raised	Greyish white with a dark center	Negative
1AK	Coccus shaped	Circular	Undulate	Raised	Clear white purplish	Negative
2AK	Coccus shaped	Circular	Undulate	Convex	Greyish white with a white in the middle	Negative
3AK	Rod-shaped	Irregular	Entire	Convex	Pale brownish white	Negative
4AK	Coccus shaped	Irregular	Erose	Raised	Greyish white	Negative
RD2B	Coccus shaped	Circular	Entire	Raised	Broken white	Positive
12aPR	Coccus shaped	Irregular	Undulate	Convex	Broken white with white and dark in the middle	Negative
12bPR	Rod-shaped	Punctiform	Entire	Raised	Shiny grayish white	Positive
15PR	Coccus shaped	Irregular	Entire	Raised	Pale yellowish white	Positive
17PR	Coccus shaped	Punctiform	Entire	Raised	Clear white	Negative
18PR	Coccus shaped	Circular	Undulate	Raised	Broken white	Negative
19PR	Rod-shaped	Irregular	Entire	Raised	Broken white brownish	Positive
22PR	Coccus shaped	Irregular	Undulate	Raised	Yellowish white	Positive

Table 2. Characterization of plant growth-promoting bacterial endophytes from oil palm seeds

Sample code	Source	ACC- deaminase production	Nitrogen fixation	Phosphate solubilization	IAA production
5RA	Ramet	+	-	+	-
7RA	Ramet	+	-	+	-
9RA	Ramet	+	-	-	-
16RA	Ramet	+	-	+	-
21RA	Ramet	+	-	+	-
RD2B	Seedling (root)	+	-	+	-
1AK	Seedling (root)	+	-	-	-
2AK	Seedling (root)	+	+	-	+
3AK	Seedling (root)	+	+	+	+
4AK	Seedling (root)	+	+	-	-
19PR	Seedling (plumule-radicle)	+	-	+	-
22PR	Seedling (plumule-radicle)	+	-	-	-
12aPR	Seedling (plumule-radicle)	+	-	-	-
12bPR	Seedling (plumule-radicle)	+	-	+	-
15PR	Seedling (plumule-radicle)	-	-	+	-
17PR	Seedling (plumule-radicle)	-	-	-	-
18PR	Seedling (plumule-radicle)	+	-	-	-

Notes: (+): positive activity, (-): negative activity

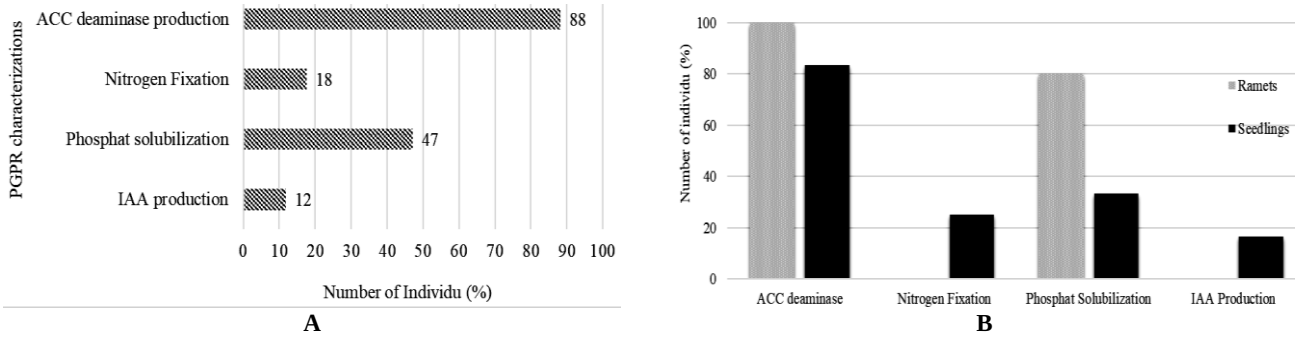

Figure 2. The percentage of endophytic bacterial isolates from oil palm ramets and seedlings that exhibit plant growth-promoting bacteria (PGPB) characteristics; A. Percentage of all endophytic bacterial isolates that have PGPR characteristics; B. Percentage of endophytic bacterial isolates for each characterization of PGPB isolated from oil palm ramets seedlings

Identification and confirmation of beneficial endophytic bacteria isolated from oil palm seedlings as plant growth promoter

The 3AK strain (Figure 3) isolated from oil palm seedling roots exhibited all plant growth-promoting bacteria characteristics examined in this study. The results indicated that the 3AK strain displayed 99.69% similarity to *Aeromonas taiwanensis* strain A2-50, 99.45% with *Aeromonas dhakensis* strain P21, 99.38% with *Aeromonas hydrophila* strain DSM 30187 and *Aeromonas caviae* strain ATCC 15468, and 99.30% with *Aeromonas sanarelli* strain A2-67. The similarity percentage shows that the 3AK isolate is similar to *Aeromonas taiwanensis* strain A2-50 (Query Cover: 100%; E-value 0.00; Accession Number: NR 116585.1). The outgroup used was *Cronobacter dublinensis* strain DES187. A phylogenetic tree was generated using the neighbor-joining method. The partial 16S rRNA gene of the putative endophytic bacterial isolates and representative bacterial-type strains of related taxa (Figure 4). The *Aeromonas taiwanensis* strain 3AK sequence has been submitted to the NCBI GenBank database under accession number PQ510448.

The hemolysis tests the pathogenic ability of 3AK isolate, and the three *Aeromonas* bacteria reference *Aeromonas hydrophila*, *Aeromonas hydrophila* JHL3, and *Aeromonas caviae* WS7b. Pathogenic bacteria can hemolyze erythrocytes, forming hemolysis zones around the colonies on blood agar plate media. Hemolysis, an exoprotein with enzymatic and toxin activity, is a characteristic of pathogenic bacteria (Hajar et al. 2015). The results of the hemolysis test demonstrated that the three bacterial isolates, including the 3AK isolate, exhibited alpha (α) hemolysis, the same as *Aeromonas hydrophila* JHL3 and *Aeromonas caviae* WS7b. However, the *Aeromonas hydrophila* bacteria isolated from freshwater fish showed beta hemolysis, similar to the positive control (*S. aureus*). The study revealed that all bacterial isolates with positive hemolysis were beta (β). The transparent clear zone around the bacterial colony is a characteristic of beta hemolysis, formed due to the medium's lysis of red blood cells.

This lysis is caused by the toxic hemolysin produced by bacteria to destroy red blood cells, resulting in the denaturation of hemoglobin and forming a colorless product (Gilligan 2013). Table 3 shows that the bacterial isolate 3AK experienced the same alpha (α) or partial hemolysis as *Aeromonas hydrophila* JHL3, which was isolated from a water source (lipolytic bacterial isolation), and *Aeromonas caviae* WS7b, which was isolated from a soil sample obtained from a black-pepper plantation on Bangka Island, Indonesia. *Aeromonas hydrophila* bacteria isolated from freshwater fish from the Department of Aquaculture, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor collection underwent beta (β) hemolysis. ERIC-PCR method assessed the genetic similarities between bacterial strains (Alsultan and Elhadi 2022).

Figure 3. Characteristic of A. Endophyte bacteria isolate code 3AK colony, which has the potential as plant growth promoting; B. 3AK Gram staining showed red cells indicating gram-negative bacteria

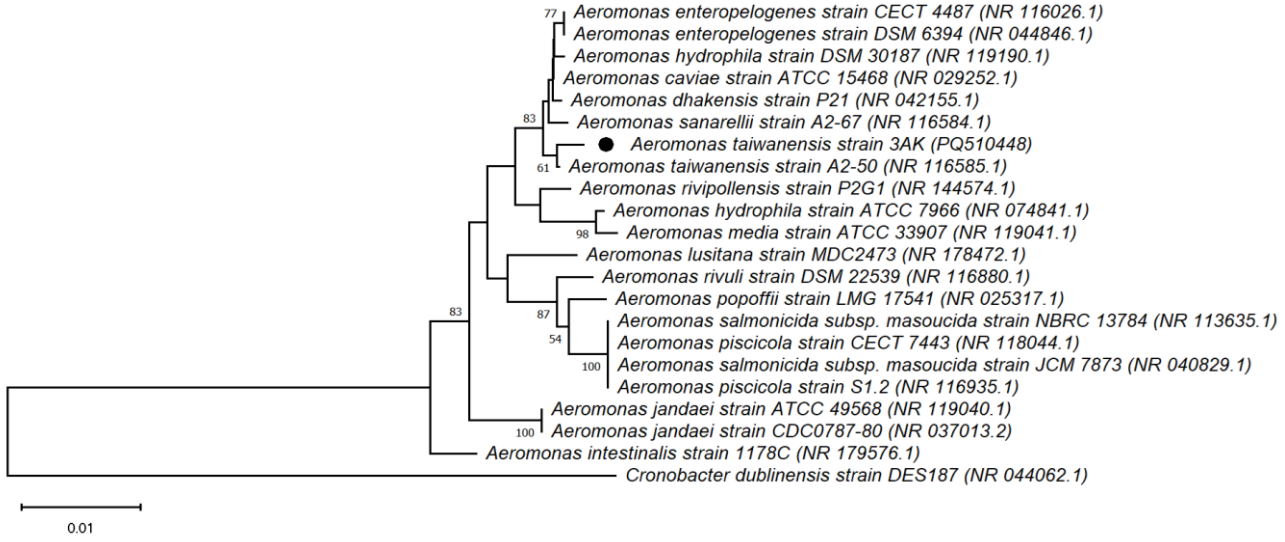

Figure 4. The phylogenetic tree of *Aeromonas taiwanensis* strain 3AK compared to their closest relative species based on the 16S rRNA gene construct with the neighbor-joining method with 1000x bootstraps value. *Cronobacter dublinensis* strain DES187 was used as an out-group. Notes: The data on the type strains of related species were from the GenBank database (the accession numbers are given after the species name), and the black circle sign is the location of *A. taiwanensis* strain 3AK

Table 3. Hemolysis test on blood base agar of bacterial isolate code 3AK

Bacteria	Hemolysis alpha (α)	Hemolysis beta (β)	Hemolysis gamma (γ)
E. coli DH5α (negative control)			√
Staphylococcus aureus (positive control)		√	
3AK	√		
Aeromonas hydrophila JHL3	√		
Aeromonas hydrophila		√	
Aeromonas caviae WS7b	√		

Notes: α: partial hemolysis; β: complete hemolysis; and γ: no hemolysis

The study found that the ERIC primers were reliable for determining the genetic relationships between 3AK strains and *Aeronomas* originating from diverse sources. The electrophoresis results are in Figures 5.A and Figure 5.B indicate that the 3AK bacterial isolate has some bands parallel to *Aeromonas hydrophila* JHL3. The *Aeromonas hydrophila* bacteria isolated from freshwater fish has a band parallel to *Aeromonas hydrophila* JHL3 at 1000bp. Therefore, the 3AK bacterial isolate is closely related to *Aeromonas hydrophila* JHL3, which comes from the environment based on the NTSYS. Grouping between *Aeromonas hydrophyla* originating from the environment (JHL3) and pathogens occurs at a coefficient of 0.3 (Figure 5.C).

The plant growth promoting compatibility of endophytic bacterial isolates from oil palm ramets and seedlings

Compatibility is a crucial factor in formulating bioinoculants that promote plant growth. It is essential to determine the compatibility between bacterial inoculants to increase plant growth efficiency, particularly in combined inoculations containing compatible bacteria (Santiago et al. 2017). Compatibility tests aim to identify whether these bacterial isolates can collaborate and synergize with other bacteria when isolated simultaneously, ensuring they are efficient and do not inhibit each other (Sondang et al. 2023). Out of these isolates, 73.8% exhibited compatible interactions with other bacteria. Bacterial isolate 3AK showed compatibility with isolates GS 4.4, R6, and PsJN (Table 4). Microbial interactions that produce clear zones indicate that these bacterial isolates can inhibit each other. If a clear zone is not produced, the two bacterial isolates are compatible and can be inoculated simultaneously without inhibiting each other (Figure 6).

Inoculation of endophytic bacterial isolates from oil palm seeds increases plant growth in media (in vitro)

In vitro testing of plant growth on rice seeds is typically conducted using petri dishes to rapidly assess the impact of beneficial bacterial isolates applied to rice seeds within seven days. The results showed that all shoot lengths of rice seeds that were inoculated with bacteria were higher compared to the negative control (2.2±0.6 cm), except for the RD2B bacterial isolate treatment (1.9±0.6 cm) (Figure 7.A). The inoculation of the 3AK bacterial isolate showed the highest shoot length of 4.4±0.4 cm, significantly different from the negative control and different but not significant from the PsJN isolate as a positive control,

3.4±1.1 cm. The 3AK bacterial isolate also showed no significant differences from almost all isolates.

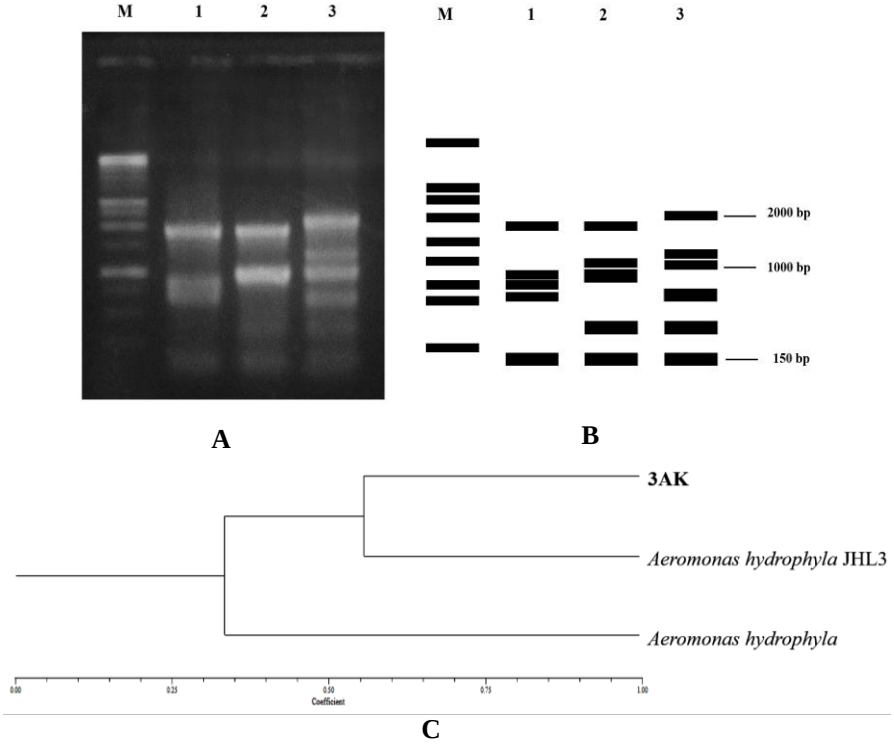

Figure 5. ERIC-PCR profiles bacteria isolate code 3AK with *Aeromonas hydrophila* JHL3 from the environment and *Aeromonas hydrophila* from the fish pathogen. A. ERIC-PCR Profiles; B. Illustration of ERIC-PCR profiles by MS. Excel and gene analyzer, i.e., M: Molecular markers; 1: 3AK isolate; 2: *Aeromonas hydrophila* JHL3 (from the environment); and 3: *Aeromonas hydrophila* (from the pathogen in fish); C. Dendogram generated from ERIC-PCR profiles of 3AK isolate

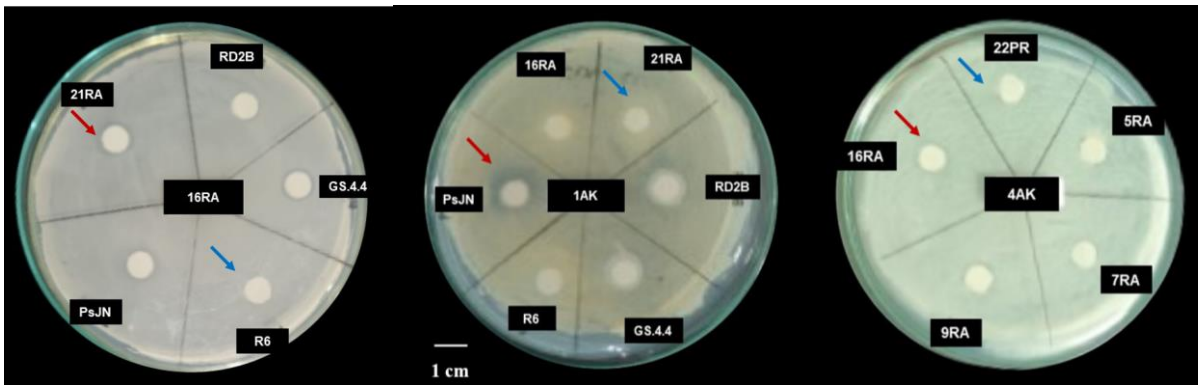

Figure 6. Test to determine compatibility between two bacterial strains using the agar diffusion. Notes: Incompatible interactions (-) result in a clear zone of inhibition, while compatible interactions (+) lead to the coexistence and mixing of the two organisms. Red arrows indicate two incompatible bacterial isolates (-), and blue arrows indicate no inhibition zone or compatibility (+)

The bacterial isolate 3AK displayed the most extended root length among other treatments, measuring 8.2±0.5 cm (Figure 7.B). While the inoculation of 3AK was different but not significant from the negative control, positive control (PsJN isolate), and all treatments, the root length inoculation of the PsJN isolate (positive control) was 7.2±0.8 cm. In general, the 3AK bacterial isolate inoculated on rice seeds and tested in vitro demonstrated the highest shoot and root lengths of all treatments. It was almost significantly different from all treatments with $p<0.05$. In vitro test results suggested that the 3AK bacterial strain had the potential to function as a plant growth stimulant (Figure 7.C). Hence, testing was carried out by inoculating the 3AK isolates on oil palm seedlings for in vivo testing in the greenhouse.

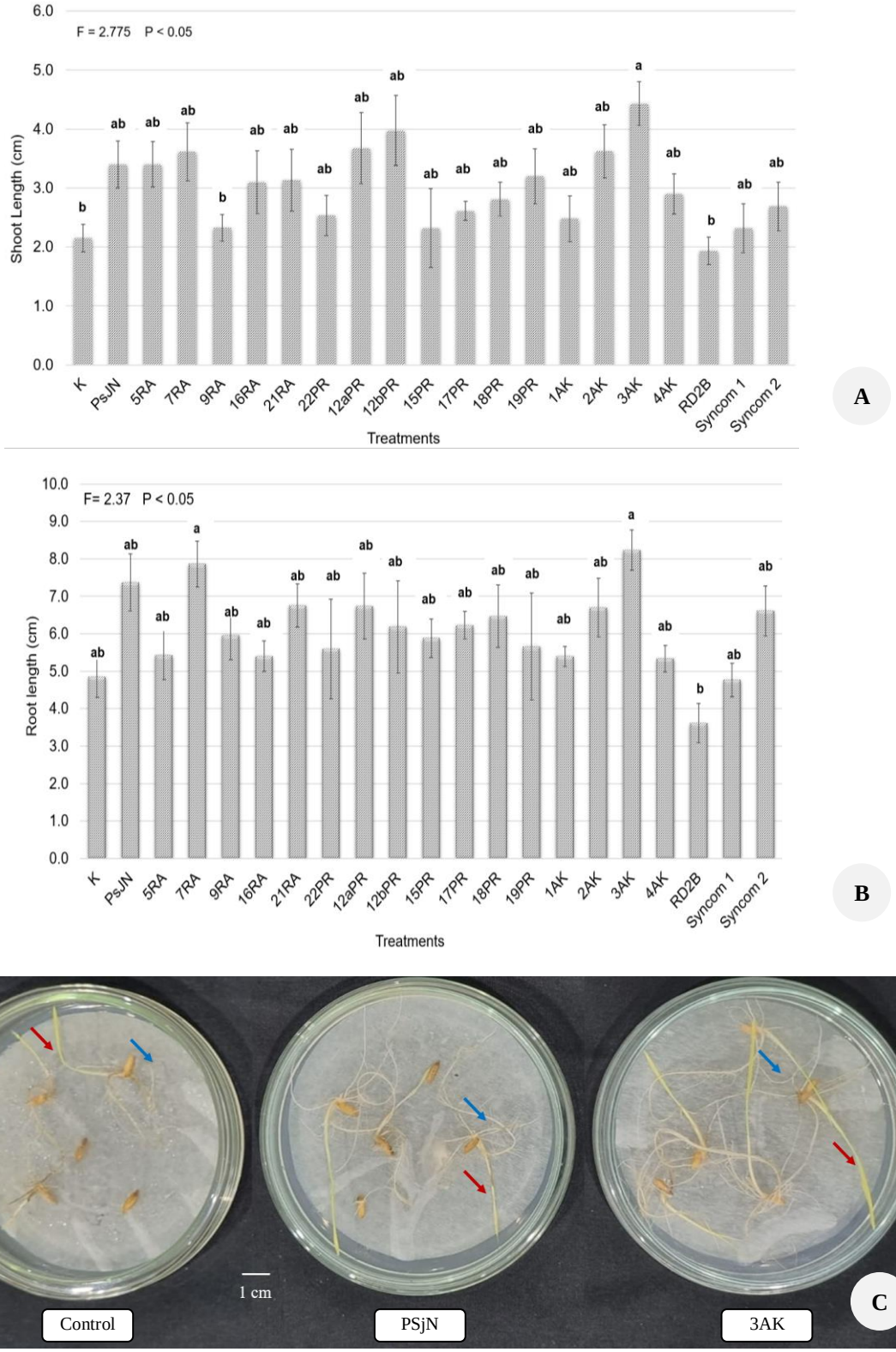

Figure 7. In vitro test outcomes for beneficial bacterial isolates derived from oil palm ramets and seedlings. A. The shoot length results from the beneficial bacterial isolate after inoculation; B. The root length results from the beneficial bacterial isolate after inoculation; C. Morphology of rice seeds from the control group (-), PsJN isolate as positive control, and the 3AK bacterial isolate after 7 days. Different letters signify substantial differences in the average values of root and shoot lengths, with a statistical significance level of $P<0.05$, as determined by ANOVA and Tukey's test. The error bars represent the standard deviations. The red arrow is the shoot, and the blue arrow is the root

Table 4. Compatibility test between endophyte bacteria isolates from oil palm ramets and seedlings

	1AK	2AK	3AK	4AK	12aPR	12bPR	15PR	17PR	18PR	19PR	22PR	5RA	7RA	9RA	16RA	21RA	RD2B	GS 4.4	R6
1AK																			
2AK	+																		
3AK	+	+																	
4AK	+	+	+																
12aPR	-	+	+	-															
12bPR	+	+	+	-	+														
15PR	+	+	-	-	+	+													
17PR	+	+	+	-	+	+	+												
18PR	-	+	+	+	+	+	+	-											
19PR	+	+	-	-	+	+	+	+	+										
22PR	+	+	-	+	+	+	+	+	+	+									
5RA	+	+	-	+	+	+	+	+	+	+	+								
7RA	+	+	-	+	+	+	+	+	+	+	+	-							
9RA	+	+	-	-	+	+	+	+	+	+	+	-	+						
16RA	+	+	+	-	+	+	+	+	+	+	-	+	+	+					
21RA	+	+	+	+	-	+	+	+	+	+	+	-	+	+	-				
RD2B	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-			
GS 4.4	-	+	+	-	-	+	+	+	+	+	+	+	-	+	-	+	+		
R6	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
PsJN	-	+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+

Notes: (+): isolates synergism with each other; and (-): isolates are not synergistic

In vivo growth experiment in a glasshouse

After measuring the in vitro growth, in vivo testing was performed on oil palm seedlings using the inoculation treatment with PsJN bacterial isolates, which indicated as *Parabulkhoderia phytofirmans* strain PsJN, which has been widely used as a plant-growth-promoting bacteria as a positive control (C+), the negative control (C-) with distilled water or uninoculated, bacterial isolate 3AK and mixed bacterial isolates (3AK, PsJN, R6, and GS 4.4) treatments. PsJNT isolate was reported to have high 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) activity, reducing ethylene levels in developing or stressed plants (Sessitsch et al. 2005). The inoculation of PsJN isolates on grapevines resulted in the production of phenolic metabolites. This allowed for identifying additional biocontrol mechanisms developed by this PGPR to induce plant resistance to pathogens (Miotto-Vilanova et al. 2019). The highest shoot dry weight of oil palm seedlings was observed in the PsJN isolate inoculated group, which amounted to 2.3±0.5 g. This value was significantly higher than the control group's 1.7±0.8 g and not significantly different from the isolate 3 AK and Mix isolate groups, which had 2.0±0.5 g and 1.8±0.4 g of shoot dry weight, respectively (Figure 8.A). The roots of oil palm seedlings had a dry weight pattern similar to the shoot dry weight pattern, with the highest treatment for the PsJN isolate being 0.7±0.2 g. However, the treatments had no significant differences (Figure 8.B). The dry weight of the seedlings inoculated with the 3AK bacterial isolate was higher than that of the negative control group. The morphology of oil palm seedlings inoculated with beneficial bacterial isolates after three months can be seen in Figure 8.C. Inoculation can be carried out in the nursery phase of the oil palm seedlings to observe a more evident difference in growth.

Discussion

To assess the potential benefits of the isolate, the qualitative properties of plant growth-promoting bacteria (PGPB) were tested, including the ability to produce deaminase, fix nitrogen, dissolve phosphate, and synthesize indole-3-acetic acid (IAA). Out of the 17 strains isolated from oil palm ramets and seedlings (Figure 1), most exhibited at least one of the PGPB traits tested in this study, except bacterial isolate 17PR, which showed no such traits (Table 2). The majority of the bacterial isolates were capable of producing aminocyclopropane-1-carboxylate (ACC)-deaminase, with 88% showing this ability (Figure 2.A). ACC-deaminase is beneficial for promoting plant growth and mitigating stress (Gupta and Pandey 2019). ACC deaminase functions by reducing plant ethylene levels. An increase in plant hormone ethylene can impede plant growth and development, and if the levels rise beyond a certain point, it could also result in plant death. By decreasing ethylene levels, ACC deaminase helps plants cope with various stresses, including salinity, drought, and heavy metals (Zarei et al. 2020). Oil palm seeds, whether originating from tissue culture propagation (ramets) or developed normally (seedlings) in the acclimatization phase, must adapt to their environment so that a lot of ACC-deaminase is produced. Seedlings have bacterial isolates with more plant growth-promoting characteristics than ramets. Seedlings arise from sexual reproduction, which leads to genetic diversity among offspring, thereby increasing the potential for beneficial PGPR colonization (Nwigwe et al. 2023).

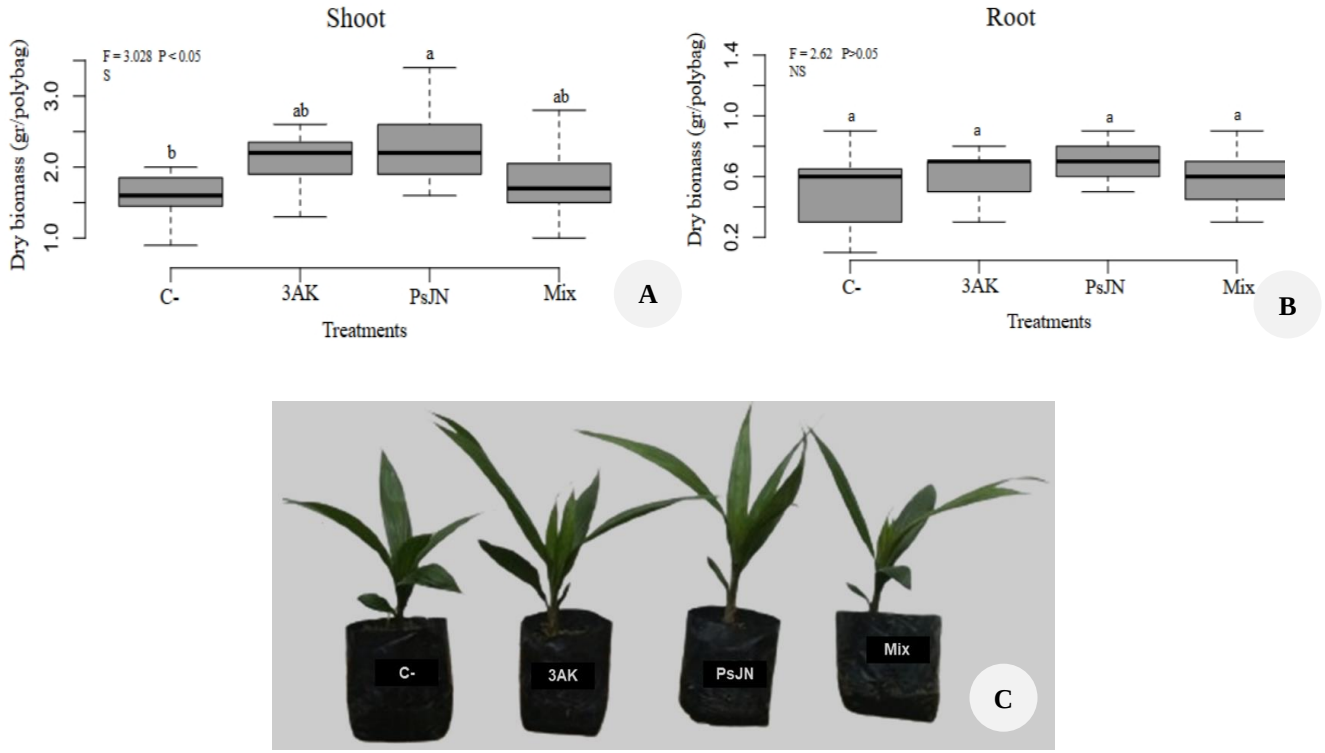

Figure 8. Effects of beneficial bacteria isolates inoculation on the growth of oil palm seedlings after 3 months of incubation in the greenhouse. On average, A. Dry shoot weight; B. Dry root weight; C. Representative images of seedlings growing on control or non-treated and inoculated seedlings. Note: C-: negative control; PsJN: bacterial isolate strain PsJN as positive control; 3AK: bacterial isolate 3AK; and Mix: bacterial isolates consisting of PsJN, 3AK, R6, and GS 4.4

However, the ability of the PGPR to produce IAA, another vital growth hormone, was relatively low, with only 12 % (Figure 2.A). This may be due to the scarcity of nutrients, particularly tryptophan, which acts as a precursor for IAA, as well as other factors such as low pH levels, salinity, and suboptimal temperatures (Lebrazi et al. 2020).

Bacterial isolates derived from ramets (clonal) only exhibited two characteristics of PGPB, namely ACC-deaminase and phosphate solubilization. In contrast, isolates from oil palm seedlings displayed all of the PGPB characteristics (Figures 2B-2C). Clonal propagation (ramets) lacks genetic diversity, particularly in the roots, which can result in fewer opportunities for bacteria with plant growth-promoting traits to colonize (Barrett 2015; Freschet et al. 2021). Additionally, clonal propagation can cause selective pressure, whereby only bacteria with specific traits necessary for the growth of the ramet can survive and thrive (Fasusi et al. 2021).

This study reported that one of the endophytic bacterial isolates, coded as 3AK, a gram-negative bacterium extracted from the roots of oil palm seedlings, displayed all the characteristics of plant growth-promoting bacteria tested in the study qualitatively (Figure 3). This meant that the presence or absence of these traits was determined (as shown in Table 2). The results of the 16S rRNA gene identification and phylogenetic tree constructed from the sequence analysis (revealed that strain 3AK belonged to the genus *Aeromonas*, which shared 99.69 similarity with *Aeromonas taiwanensis* strain A2-50 (Figure 4). *Aeromonas* is a genus of bacterial species that belong to the gamma subclass of *Proteobacteria* and are Gram-negative, facultatively anaerobic, rod-shaped bacteria that resemble members of the Enterobacteriaceae family. This genus is widely distributed in aquatic environments and is known to infect humans, fish, and other animals (Sime-Ngando 2015; Barger et al. 2021). The species *Aeromonas taiwanensis* sp. nov. (strain type A2-50T5CECT 7403T 5LMG 24683T) and *Aeromonas sanarellii* sp. nov. (strain type A2-67T 5CECT 7402T 5LMG 24682T) that were found in the wounds of two patients in Taiwan were 99.6-99.8% similar to *Aeromonas caviae* strains (Alperi et al. 2010). Although the information on the presence of plants in the *Aeromonas* genus, especially *Aeromonas taiwanensis* in plants, is currently limited, some studies have revealed that these organisms can be detected in various sources, including plants, and possess plant growth-promoting properties. *Aeromonas taiwanensis* has been reported to be isolated from the rhizosphere of water hyacinth (Kabeer et al. 2018) and from soil samples obtained from areas of wood decay (Gupta et al. 2024). Furthermore, *Aeromonas hydrophila* was reported as a biocontrol agent for controlling fusarium wilt disease in several sweet sorghum varieties (*Sorghum bicolor* (L.) (Ariawan et al. 2015). Additionally, an *Aeromonas* spp. consortium has been studied as a producer of salt-tolerant Acyl-homoserine lactones (AHL) in wheat plants (Nawaz et al. 2020). In *Arabidopsis* roots, members of the Aeromonadaceae family can increase H_2O_2 accumulation in dehydrated guard cells, causing stomata to

close and save water. Furthermore, this group of bacteria can induce synergistic jasmonic acid-related regulators that control metabolic activity, reproduction, and defense against diseases (He et al. 2022). The confirmation test of bacterial isolate 3AK showed the same alpha (α) or partial hemolysis as *Aeromonas hydrophila* JHL3 and *Aeromonas caviae* WS7b (Table 3). The result of dendogram enterobacterial repetitive intergenic consensus (ERIC)-PCR profiles showed that bacterial isolate 3AK is in one group to *Aeromonas hydrophila* JHL3 (Figures 5A-5.C). Thus, bacterial isolate 3AK is indicated as a genus *Aeromonas* similar to *Aeromonas hydrophila* JHL3 originating from the environment and not from freshwater fish that cause disease. The identification and characterization of endophytic bacterial isolate 3AK, which is indicated as the *Aeromonas taiwanensis* strain 3AK originating from the roots of oil palm seedlings, is very interesting because of the limited sources of information on bacteria that have pathogenic properties but have the ability of plant growth-promoting bacteria (PGPB) and this is being reported for the first time. For further research, identification with whole genome sequencing and metabolomic analysis are needed to confirm the species and explore the potential of bacterial isolate 3AK more deeply.

Two experiments were conducted to assess the potential of 3AK isolate for promoting plant growth. The first experiment involved rice seeds in a petri dish (in vitro), and the second experiment involved oil palm seedlings in a glasshouse (in vivo). Both tests were designed to evaluate the effects of 3AK isolate on plant growth in different environments. The experiment began with a compatibility test that showed 17 endophytic bacterial isolates, including 3AK isolate and beneficial bacterial isolates codes GS 4.4, R6, and PsJN, as seen in Table 4. 3AK isolate was compatible with two bacterial isolates derived from ramets, 6 from seedlings, and isolates GS 4.4, R6, and PsJN. So that this isolate can be used for further testing. The 3AK bacterial isolate, which was inoculated on rice seeds and grown on agar media in Petri dishes as part of an in vitro test, showed the longest shoot and root lengths out of all the treatments, which included the negative control, PsJN bacterial isolate (as the positive control), 3AK bacterial isolate, and a mix of bacteria isolates (3AK, PsJN, R6, and GS 4.4). Moreover, the result showed that the 3AK isolate significantly differed from the negative control, with a p-value less than 0.05 for the shoot lengths (Figure 7). Additionally, the bacterial inoculation testing in the greenhouse revealed that the 3AK isolate exhibited similar characteristics to the PsJN bacterial isolate, which served as the positive control for plant growth promotion for all measured variables, such as shoot and root dry biomass (as illustrated in Figure 8). *Burkholderia phytofirmans*, especially the PsJN strain, has been widely used as an agent for plant growth-promoting bacteria. Another strain of *Burkholderia* is CP01, isolated from the rhizosphere of oil palm, which also has an ability as an antifungal (Prihatna et al. 2022). Previous research has demonstrated the efficacy of using bacterial isolates to enhance the growth of oil palm seedlings. Studies have shown that oil palm plantlets inoculated with R12 (*Acetobacter diazotrophicus*) and Sp7 (*Azospirillum brasilense*) exhibited better growth than control plants at 280 days of age (Om et al. 2009). Additionally, two indigenous endophytic bacteria isolates, A2.2.1 and D5.2.1, from the root system of oil palm were found to improve the growth of oil palm (*Elaeis guineensis* Jacq.) seedlings in the pre-nursery, including plant height, total leaf area, fresh weight of seedlings and roots (Mayerni et al. 2019).

Furthermore, *Bacillus cereus* bacteria (UPM15) have been shown to contribute to the root growth of oil palm seedlings for up to six months after planting (Syafiq et al. 2021). Inoculated endophyte bacteria, *Burkholderia cenocepacia*, *Pseudomonas fluorescens*, and arbuscular mycorrhizae can increase the height and diameter of the stem of oil palm seedlings (Kalbuadi et al. 2024). The results of wheat research grown in a greenhouse with sterile soil media showed an increase in root length and rhizosheath soil mass of wheat seedlings. Sterile soil causes the proportion of exopolysaccharide producers to be able to bind water during storage to maintain a soft texture on gel products. The bacterial population was higher in sterilized than unsterilized treatments (Mahmood et al. 2014). Additionally, the scarcity of nutrients in sterile soil, which lacks or has few soil microbes, results in longer plant roots. The scarcity of essential nutrients forces plants to expend most of their energy on root growth to find necessary nutrients (Yan and Ma 2021). The data from this study demonstrated that 3AK isolate could promote the growth of oil palm seedlings, as shown by the outcomes obtained from in vitro tests conducted on petri plates and in vivo tests carried out in a greenhouse utilizing polybags. These findings indicated that 3AK isolate had the potential to function as a plant growth-promoting agent.

In conclusion, the endophytic bacterial isolate 3AK was indicated to the *Aeromonas taiwanenesis* strain 3AK based on 16S rRNA gene testing, suggesting its potential as a plant growth-promoting agent, especially during the early stages or acclimatization phase of oil palm growth. This 3AK strain has only been reported as a plant growth promoter for the first time. Most of the *Aeromonas* genus are often reported as pathogens, but isolate 3AK has similarities to this genus, which comes from the environment. Besides, *Burkholderia phytofirmans* strain PsJN, known as a PGPR model, was tested for the first time on the growth of oil palm seedlings. To determine the mechanism by which 3AK bacterial isolates enhance plant growth, further testing on other plants and molecular analyses, such as metabolomics, are required.

ACKNOWLEDGEMENTS

The author would like to thank the Center for Higher Education Fund (*Balai Pembiayaan Pendidikan Tinggi*) and the Indonesia Endowment Fund for Education Agency (*Lembaga Pengelola Dana Pendidikan*) from the Indonesian Ministry of Education, Culture, Research, and Technology for the scholarship (ID Number: 202101121287) which have funding this research. The author also thanks the Research and Development Centre for Biotechnology of

PT. Wilmar Benih Indonesia for their technical support throughout this study.

REFERENCES

Ali SZ, Sandhya V, Venkateswar Rao L. 2014. Isolation and characterization of drought-tolerant ACC deaminase and exopolysaccharide-producing fluorescent *Pseudomonas* sp. Ann Microbiol 64 (2): 493-502. DOI: 10.1007/s13213-013-0680-3.

Alperi A, Martínez-Murcia AJ, Ko W-C, Monera A, Saavedra MJ, Figueras MJ. 2010. *Aeromonas taiwanensis* sp. nov. and *Aeromonas sanarellii* sp. nov., clinical species from Taiwan. Intl J Syst Evol Microbiol 60 (9): 2048-2055. DOI: 10.1099/ijs.0.014621-0.

Alsultan A, Elhadi N. 2022. Evaluation of ERIC-PCR method for determining genetic diversity among *Escherichia coli* isolated from human and retail imported frozen shrimp and beef. Intl J Food Contam 9 (1): 12. DOI: 10.1186/s40550-022-00098-1.

Amanta F, Nafisah N. 2022. Produktivitas kelapa sawit tetap terbatas seiring melonjaknya harga minyak goreng di Indonesia https://repository.cips-indonesia.org/media/publications/355798-produktivitas-kelapa-sawit-tetap-terbata-00cfbc21.pdf. [Indonesian]

Ariawan IWG, Suprapta DN, Suniti NW. 2015. Pemanfaatan *Aeromonas hydrophila* untuk mengendalikan penyakit layu *Fusarium* pada beberapa varietas sorgum manis (*Sorghum bicolor* (L.) Moench). Agroteknologi Tropika 4 (2): 81-92. [Indonesian]

Ayu E, Suwanto A, Barus T. 2014. *Klebsiella pneumoniae* from Indonesian tempeh were genetically different from that of pathogenic isolates. Microbiol Indonesia 8 (1): 9-15. DOI: 10.5454/mi.8.1.2.

Barger PC, Liles MR, Beck BH, Newton JC. 2021. Correction to: Differential production and secretion of potentially toxigenic extracellular proteins from hypervirulent *Aeromonas hydrophila* under biofilm and planktonic culture. BMC Microbiol 21 (1): 33. DOI: 10.1186/s12866-021-02088-3.

Barrett SCH. 2015. Influences of clonality on plant sexual reproduction. Proc Natl Acad Sci USA 112 (29): 8859-8866. DOI: 10.1073/pnas.1501712112.

Basiron Y. 2007. Palm oil production through sustainable plantations. Eur J Lipid Sci Technol 109 (4): 289-295. DOI: 10.1002/ejlt.200600223.

Bharudin I, Ab Wahab AFF, Abd Samad MA, Xin Yie N, Zairun MA, Abu Bakar FD, Abdul Murad AM. 2022. Review update on the life cycle, plant-microbe interaction, genomics, detection, and control strategies of the oil palm pathogen *Ganoderma boninense*. Biology 11 (2): 251. DOI: 10.3390/biology11020251.

Chen C, Wang M, Zhu J, Tang Y, Zhang H, Zhao Q, Jing M, Chen Y, Xu X, Jiang J, Shen Z. 2022. Long-term effect of epigenetic modification in plant-microbe interactions: modification of DNA methylation induced by plant growth-promoting bacteria mediates promotion process. Microbiome10 (1): 36. DOI: 10.1186/s40168-022-01236-9.

Fasusi OA, Amoo AE, Babalola OO. 2021. Characterization of plant growth-promoting rhizobacterial isolates associated with food plants in South Africa. Antonie van Leeuwenhoek 114 (10): 1683-1708. DOI: 10.1007/s10482-021-01633-4.

Finkel OM, Salas-González I, Castrillo G, Conway JM, Law TF, Teixeira PJPL, Wilson ED, Fitzpatrick CR, Jones CD, Dangl JL. 2020. A single bacterial genus maintains root growth in a complex microbiome. Nature 587 (7832): 103-108. DOI: 10.1038/s41586-020-2778-7.

Freschet GT, Pagès L, Iversen CM, Comas LH, Rewald B, Roumet C, Klimešová J, Zadworny M, Poorter H, Postma JA, et al. 2021. A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. New Phytol 232: 973-1122. DOI: 10.1111/nph.17572.

Gholamalizadeh R, Khodakaramian G, Ebadi AA. 2017. Assessment of rice-associated bacterial ability to enhance rice seed germination and rice growth promotion. Braz Arch Biol Technol 60: e17160410. DOI: 10.1590/1678-4324-2017160410.

Gilligan PH. 2013. Identification of pathogens by classical clinical tests. In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (eds). The Prokaryotes. Springer, Berlin, Heidelberg. DOI: 10.1007/978-3-642-30144-5_90.

Green M, Lima WAA, Figueiredo AFD, Atroch AL, Lopes R, Cunha RNVD, Teixeira PC. 2013. Heat-treatment and germination of oil palm seeds (*Elaeis guineensis* Jacq.). J Seed Sci 35 (3): 296-301. DOI: 10.1590/S2317-15372013000300004.

Gordon SA, Weber RP. 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol 26 (1): 192-195. DOI: 10.1104/pp.26.1.192.

Gupta S, Pandey S. 2019. ACC Deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (*Phaseolus vulgaris*) Plants. Front Microbiol 10: 1506. DOI: 10.3389/fmicb.2019.01506.

Gupta S, Tanveer A, Dwivedi S, Yadav K, Morya VK, Yadav D. 2024. Isolation and characterization of *Aeromonas taiwanensis* strain for simultaneous production of cellulase, amylase, pectinase, and protease enzymes. Biosci Biotechnol Res Asia 21 (2): 655-670. DOI: 10.13005/bbra/3254.

Hajar T, Leshem YA, Hanifin JM, Nedorost ST, Lio PA, Paller AS, Block J, Simpson EL. 2015. A systematic review of topical corticosteroid withdrawal (steroid addiction) in patients with atopic dermatitis and other dermatoses. J Am Acad Dermatol 72 (3): 541-549. DOI: 10.1016/j.jaad.2014.11.024.

He D, Singh SK, Peng L, Kaushal R, Vílchez JI, Shao C, Wu X, Zheng S, Morcillo RJL, Paré PW, Zhang H. 2022. Flavonoid-attracted *Aeromonas* sp. from the *Arabidopsis* root microbiome enhances plant dehydration resistance. ISME Multidisp J Microb Ecol 16 (11): 2622-2632. DOI: 10.1038/s41396-022-01288-7.

Hidayati E, Tri Wahyudi A, Suwanto A, Widyastuti R. 2014. Abundance of culturable bacteria isolated from maize rhizosphere soil using four different culture media. Microbiol Indonesia 8 (1): 33-40. DOI: 10.5454/mi.8.1.5.

Jazuli NA, Kamu A, Chong KP, Gabda D, Hassan A, Abu Seman I, Ho CM. 2022. A review of factors affecting *Ganoderma* basal stem rot disease progress in oil palm. Plants 11 (19): 2462. DOI: 10.3390/plants11192462.

Kabeer R, Varghese R, Sylas VP. 2018. Rhizosphere of water hyacinth as a niche for multidrug resistant *Aeromonas taiwanensis* and *Paenibacillus taiwanensis*: A study from a tropical wetland of South India. Rhizosphere 6: 20-22. DOI: 10.1016/j.rhisph.2018.01.007.

Kalbuadi DN, Darmawan, Pulunggono HB, Santi LP. 2024. Effect of beneficial microbes on macronutrient uptake and oil palm seedling growth in peat soil. IOP Conf Ser: Earth Environ Sci 1308 (1): 012029. DOI: 10.1088/1755-1315/1308/1/012029.

Kuan KB, Othman R, Abdul Rahim K, Shamsuddin ZH. 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. PLoS One 11 (3): e0152478. DOI: 10.1371/journal.pone.0152478.

Kumar V, Jain L, Jain SK, Chaturvedi S, Kaushal P. 2020. Bacterial endophytes of rice (*Oryza sativa* L.) and their potential for plant growth promotion and antagonistic activities. S Afr J Bot 134: 50-63. DOI: 10.1016/j.sajb.2020.02.017.

Lebrazi S, Niehaus K, Bednarz H, Fadil M, Chraibi M, Fikri-Benbrahim K. 2020. Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. J Genet Eng Biotechnol 18 (1): 71. DOI: 10.1186/s43141-020-00090-2.

Mahmood T, Mehnaz S, Fleischmann F, Ali R, Hashmi ZH, Iqbal Z. 2014. Soil sterilization effects on root growth and formation of rhizosheaths in wheat seedlings. Pedobiologia 57 (3): 123-130. DOI: 10.1016/j.pedobi.2013.12.005.

Malik A, Wenuganen S, Suwanto A, Tjahjono B. 2003. Cloning, DNA sequence, and expression of *Aeromonas caviae* WS7b chitinase gene. Mol Biotechnol 23 (1): 1-10. DOI: 10.1385/MB:23:1:1.

Mayerni R, Warnita, Yuliatri, Rukmana S, Okta Sagita Chan. 2019. Effect of indigenous endophytic bacteria on growth of palm oil seedlings (*Elaeis guineensis* Jacq) in the nursery. IOP Conf Ser: Earth Environ Sci 347: 012026. DOI: 10.1088/1755-1315/347/1/012026.

Middleton H, Yergeau É, Monard C, Combier J-P, El Amrani A. 2021. Rhizospheric plant-microbe interactions: miRNAs as a key mediator. Trends Plant Sci 26: 132-141. DOI: 10.1016/j.tplants.2020.09.005.

Miotto-Vilanova L, Courteaux B, Padilla R, Rabenoelina F, Jacquard C, Clément C, Comte G, Lavire C, Ait Barka E, Kerzaon I, Sanchez L. 2019. Impact of *Paraburkholderia phytofirmans* PsJN on grapevine phenolic metabolism. Intl J Mol Sci 20 (22): 5775. DOI: 10.3390/ijms20225775.

Murphy DJ, Goggin K, Paterson RRM. 2021. Oil palm in the 2020s and beyond: Challenges and solutions. CABI Agric Biosci 2 (1): 39. DOI: 10.1186/s43170-021-00058-3.

Nawaz MS, Arshad A, Rajput L, Fatima K, Ullah S, Ahmad M, Imran A. 2020. Growth-stimulatory effect of quorum sensing signal molecule

n-acyl-homoserine lactone-producing multi-trait *Aeromonas* spp. on wheat genotypes under salt stress. Front Microbiol 11: 553621. DOI: 10.3389/fmicb.2020.553621.

Nwigwe C, Fossey A, De Smidt O. 2023. Effects of plant growth promoting rhizospheric bacteria (PGPR) on survival, growth and rooting architecture of Eucalyptus hybrid clones. Forests 14 (9): 1848. DOI: 10.3390/f14091848.

Om AC, Ghazali AHA, Keng CL, Ishak Z. 2009. Microbial inoculation improves growth of oil palm plants (*Elaeis guineensis* Jacq.). Trop Life Sci Res 20 (2): 71-77.

Paterson RRM. 2020. Depletion of Indonesian oil palm plantations implied from modeling oil palm mortality and *Ganoderma boninense* rot under future climate. AIMS Environ Sci 7 (5): 366-379. DOI: 10.3934/environsci.2020024.

Prihatna C, Pramudito TE, Arifin AR, Nguyen TKN, Purnamasari MI, Suwanto A. 2022. Antifungal peptides from a *Burkholderia* strain suppress basal stem rot disease of oil palm. Phytopathology 112 (2): 238-248. DOI: 10.1094/PHYTO-11-20-0529-R.

Rival A, Ilbert P, Labeyrie A, Torres E, Doulbeau S, Personne A, Dussert S, Beulé T, Durand-Gasselin T, Tregear JW, et al. 2013. Variations in genomic DNA methylation during the long-term in vitro proliferation of oil palm embryogenic suspension cultures. Plant Cell Rep 32 (3): 359-368. DOI: 10.1007/s00299-012-1369-y.

Santiago CD, Yagi S, Ijima M, Nashimoto T, Sawada M, Ikeda S, Asano K, Orikasa Y, Ohwada T. 2017. Bacterial compatibility in combined inoculations enhances the growth of potato seedlings. Microb Environ 32 (1): 14-23. DOI: 10.1264/jsme2.ME16127.

Sarkar MdSK, Begum RA, Pereira JJ. 2020. Impacts of climate change on oil palm production in Malaysia. Environ Sci Pollut Res 27 (9): 9760-9770. DOI: 10.1007/s11356-020-07601-1.

Sessitsch A, Coenye T, Sturz AV, Vandamme P, Barka EA, Salles JF, Van Elsas JD, Faure D, Reiter B, Glick BR, Wang-Pruski G, Nowak J. 2005. *Burkholderia phytofirmans* sp. nov., a novel plant-associated bacterium with plant-beneficial properties. Intl J Syst Evol Microbiol 55 (3): 1187-1192. DOI: 10.1099/ijs.0.63149-0.

Silambarasan S, Abraham J. 2020. Biodegradation of carbendazim by a potent novel *Chryseobacterium* sp. JAS14 and plant growth promoting *Aeromonas caviae* JAS15 with subsequent toxicity analysis. 3 Biotech 10 (7): 326. DOI: 10.1007/s13205-020-02319-w.

Sime-Ngando T. 2015. *Aeromonas*: A book review. Front Microbiol 6: 106. DOI: 10.3389/fmicb.2015.00106.

Sondang Y, Muflihayati, Anty K, Siregar R. 2023. The effect of functional bacterial consortium on nutrient content of liquid organic. IOP Conf Ser: Earth Environ Sci 1160 (1): 012079. DOI: 10.1088/1755-1315/1160/1/012079.

Syafiq TH, Nusaibah SA, Rafii MY. 2021. Effectiveness of bioinoculants *Bacillus cereus* and *Trichoderma asperellum* as oil palm seedlings growth promoters. Pertanika J Trop Agric Sci 44 (1): 157-170. DOI: 10.47836/pjtas.44.1.09.

Tabacchioni S, Passato S, Ambrosino P, et al. 2021. Identification of beneficial microbial consortia and bioactive compounds with potential as plant biostimulants for a sustainable agriculture. Microorganisms 9 (2): 426. DOI: 10.3390/microorganisms9020426.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30 (12): 2725-2729. DOI: 10.1093/molbev/mst197.

Wang J, Li R, Zhang H, Wei G, Li Z. 2020. Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC Microbiol 20 (1): 38. DOI: 10.1186/s12866-020-1708-z.

Wu W, Chen W, Liu S, Wu J, Zhu Y, Qin L, Zhu B. 2021. Beneficial relationships between endophytic bacteria and medicinal plants. Front Plant Sci 12: 646146. DOI: 10.3389/fpls.2021.646146.

Yan X-L, Ma X. 2021. Responses of root morphology and seedling growth in three tree species to heterogeneous supplies of ammonium and nitrate. For Ecol Manag 479: 118538. DOI: 10.1016/j.foreco.2020.118538.

Yarra R, Jin L, Zhao Z, Cao H. 2019. Progress in tissue culture and genetic transformation of oil palm: An overview. Intl J Mol Sci 20 (21): 5353. DOI: 10.3390/ijms20215353.

Zarei T, Moradi A, Kazemeini SA, Akhgar A, Rahi AA. 2020. The role of ACC deaminase producing bacteria in improving sweet corn (*Zea mays* L. var *saccharata*) productivity under limited availability of irrigation water. Sci Rep 10 (1): 20361. DOI: 10.1038/s41598-020-77305-6.

Zhang J, Cook J, Nearing JT, Zhang Junzeng, Raudonis R, Glick BR, Langille MGI, Cheng Z. 2021. Harnessing the plant microbiome to promote the growth of agricultural crops. Microbiol Res 245: 126690. DOI: 10.1016/j.micres.2020.126690.