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Isolation of Endophytic Bacteria from *Palaquium Maingayi* and their Ability to Promote Plant Growth

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Abstract

Mutual interaction between endophytic bacteria and their plant host benefits both sides in a sustainable environment. To date, various endophytic bacteria with plant growth-promoting potential have been reported from a wide variety of plants. The present study aims to isolate endophytic bacteria with plant growth-promoting characteristics and potentially become biofertilizers to promote plant growth. These endophytic bacteria were isolated from the roots of Palaquium maingayi inhabiting Universiti Putra Malaysia Campus Bintulu (UPMKB) Borneo Base Forest. In total, 66 endophytic bacteria were isolated and examined for their phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore production and antagonistic characteristics against pathogenic fungi and bacteria. Out of 66 endophytic bacterial isolates, 11 were able to solubilize phosphate, produce IAA and siderophore, and promote plant growth. 6 endophytic bacteria isolates were found to have antagonistic activities against Ganoderma borneensis, Fusarium solani and Ralstonia solanacearum. The results of plant inoculation showed that these bacteria strains significantly promote root and shoot biomass on maize plants compared to uninoculated plants. To our knowledge, this is the first report on isolating endophytic bacterial strains from the Palaquium Maingayi tree with plant growth-promoting potential. The success of isolation and identification of endophytic bacteria from selected trees in the UPMKB Borneo base forest can potentially produce bio-fertilizers to promote plant growth for sustainable agriculture.

Keywords: Fusarium solani, Ganoderma borneensis, Ralstonia solanacearum, maize, 16S rRNA, tricalcium phosphate.

1. INTRODUCTION

Nyatoh Tembaga, a tree species from the *Sapotaceae* family, is native to Southeast Asia and used in the furniture industry due to its durability and appearance. It is also a source of natural latex known as gutta-percha, with various applications. Previous studies have shown that extract from Nyatoh timber has antifungal activities. However, there is still limited information on the tree species.

Endophytic bacteria are microorganisms that associate internally with plants and have plant growth-promoting potential. Mutual interaction between endophytic bacteria and their plant host benefits both sides in a sustainable environment (Ahmad et al., 2023). To date, various endophytic bacteria with plant growth-promoting potential have been reported from various plants (Abu Bakar, F. et. al., 2023).
The present study aims to isolate endophytic bacteria from the roots of *Palaquium maingayi* inhabiting Universiti Putra Malaysia Kampus Bintulu (UPMKB) Borneo Base Forest. The isolated bacteria were then characterized for their potential to stimulate plant growth, such as Indole acetic acid (IAA), siderophore production, and phosphate solubilization ability. Additionally, maize plants were chosen to assess further the effect of the selected endophytic bacteria's ability to promote plant growth. Lastly, bacteria isolates were tested for biocontrol potential against *Fusarium solani, Ganoderma boninense* and *Ralstonia solanacearum.*

2. MATERIALS AND METHODS

Sample collection and isolation of endophytic bacteria

The healthy roots of *Palaquium maingayi* were collected in sterile sampling bags and brought to the laboratory. Each sample was washed and surface sterile using 70% ethanol and 5% sodium hypochlorite. Surface sterile samples were marched in sterile distilled water and plated on Nutrient agar (NA), Luria Broth (LB) Agar, King B (KB) Agar and Tryptic Soy Agar (TSA). After 48 hours of incubation, colonies were selected and streaked on NA to obtain a pure culture.
Phytopathogenic fungi and bacteria were obtained from the Genetic Laboratory of Universiti Putra Malaysia Bintulu Campus. Fungi cultures (*Fusarium solani* and *Ganoderma boninense*) were inoculated on Potato Dextrose Agar (PDA), and bacteria culture (*Ralstonia solanacearum*) was inoculated on Triphenyl Tetrazolium Chloride (TZC).

Inoculum preparation

All selected isolates were tested for their plant growth-promoting characteristics and antagonistic activities against pathogenic fungi and bacteria. Bacteria isolated were cultured in Nutrient Broth (NB) overnight and adjusted to OD600=0.1 for inoculation.

Characterization of endophytic bacteria

Quantitative test of Indole Acetic Acid (IAA) production

Bacterial cultures were inoculated in NB supplemented with 0 mg/mL and 2 mg/mL Tryptophan for 24 hours in the dark on a 120 rpm orbital shaker. The culture was centrifuged the following day, and the supernatant was mixed with the Salkowski reagent. After 30 minutes, the development of red or pink colour that indicates IAA was measured using a UV-VIS spectrometer at OD530 and absorbance was compared with a standard curve of pure IAA.

Qualitative test of Phosphate solubilizing ability

Bacterial endophytic isolates were tested for phosphate solubilization using Pikovskaya (PVK) medium. Strains that produced a clear zone surrounding the colonies after 5 – 7 days of incubation at room temperature were regarded as positive results of phosphate solubilizing.

Qualitative test of siderophore production

The ability of endophytic bacteria to produce siderophores was evaluated qualitatively using Chrome Azurol S (CAS) agar. A drop of bacteria culture was placed on CAS agar and left to incubate for 5 days. The formation of yellow or orange halozone indicates the production of siderophores.

Effect of endophytic bacteria on the biomass of maize

The four most potent bacteria isolates were inoculated in NB and incubated at 30ºC for 24 h on a shaker at 120 rpm. Seeds of maize were surface sterilized by soaking in 2.5% sodium hypochlorite for 3 minutes and soaking in 70% ethanol for 1 minute. Lastly, the seeds were washed 5 times in sterile distilled water. After that, the surface sterile seeds were placed on wet paper using method described by Abdul Baki, 1957. After 5 days, uniform seedling growth was selected for endophytic bacteria inoculation. Four groups of uniform-growth seedlings were separately incubated in 50 mL aliquots of the selected overnight bacteria culture, and one group was incubated in sterile distilled water as a control. After inoculation, treated seedlings were sown in 1 L plastic pots filled with a sterilized soil-cocopeat mixture. Plants were grown in a greenhouse at 25 – 30 ◦C and irrigated

with tap water as required with fertilization using NPK. A pot experiment was conducted in a completely randomized design with five replicates, each under exposed sunlight.

Molecular identification of endophytic bacteria

The boiling method was used to extract the DNA from a selected isolate described by Freschi et al. (2005). Individual colonies were picked up from the culture plate and inoculated in 10 mL Nutrient Broth (NB). On the following day, the bacteria culture broth was centrifuged (10,000 rpm for 10 minutes) to obtain pellet. The pellet underwent a boiling process for 10 minutes, followed by a subsequent cooling phase at a temperature of -20°C for 5 minutes. After that, it was centrifuged (12,000 rpm for 10 minutes) to recover the DNA supernatant. 16S RNA gene was amplified using universal primers 27F and 1492R. The PCR products were sent for sequencing for validation, and the sequences were identified using the NCBI database.

In vitro antagonistic bioassay

All isolates were selected for antagonistic activities against *Fusarium Solani* using the dual culture technique (Niem et al., 2020). Overnight bacteria cultures were streaked 2 cm on the side of the PDA plate, and a 1 cm disk of *Fusarium solani* was placed 2 cm on another side of the PDA plate. After 7 days, the inhibition zone was measured.

The test used an agar well diffusion assay (Agarwal et al., 2020). An overnight culture of *Ralstonia solanacearum* in TZC broth and isolated endophytic bacteria were prepared in NB. 100 µL *of R. solanacearum* were spread on TZC agar, and 60 µL of bacteria isolated were placed on the plate in the well dig (1 cm diameter). Sterile distilled water was used to inoculate the plate as a control. The inhibition zone around the well showed antagonistic activity of the bacteria isolated.

Data analysis

All data were analysed using Analysis of Variance (ANOVA) in SAS software, and the significant differences of the means were analysed using Duncan's test. Test on plant inoculation was analysed in a completely randomised design (CRD) with 5 replicates for each treatment.

3. RESULTS

Isolation and identification

A total of 66 bacteria were isolated from the roots of *Palaqium maingayi*. Table 1 shows 19 most potent strains of bacteria isolates that were selected for identification and identified as *Bacillus subtilis (6), Bacillus safensis (1), Bacillus sp. (1), Pantoea rwandensis* (3), *Klebsiella variicola* (1), *Enterobacter soli* (3), *Enterobacter lignolyticus* (1), *Enterobacteriaceae bacterium* (3). Phylogenetic analysis from 19 isolates classified into 2 phyla: *Bacillota* and *Pseudomonadota*. The majority of the isolates belonged to *Pseudomonadota,* but only phylum *Bacillota* show to obtain antagonistic traits against fungi and bacterial phytopathogens except for C2 and I1 (Figure 1).

Table 1. Molecular identification of bacteria isolates using 16s rRNA

No.	Isolate	BlastN NCBI Results		
		A close match in GenBank	Similarity%	GenBank Accession
1	B10	*Bacillus subtilis*	99.24%	CP116773.1
2	C2	*Bacillus safensis*	98.99%	CP116774.1
3	D3	*Bacillus subtilis*	98.52%	CP116773.1
4	D7	*Klebsiella variicola*	99.93%	CP054254.1
5	E1	*Bacillus subtilis*	98.36%	CP116773.1
6	F7	*Bacillus subtilis*	98.65%	CP116773.1
7	F9	*Bacillus subtilis*	97.77%	CP116773.1
8	I1	*Bacillus sp.*	100%	MT5146449.1
9	J1	*Pantoea rwandensis*	99.39%	CP009454.1
10	J2	*Pantoea rwandensis*	99.44%	CP009454.1
11	J4	*Enterobacter lignolyticus*	98.88%	CP002272.1
12	J8	*Pantoea rwandensis*	99.37%	CP009454.1
13	J10	*Bacillus subtilis*	97.29%	CP116773.1
14	K2	*Enterobacter soli*	97.52%	CP003026.1
15	K3	*Enterobacter soli*	99.58%	CP003026.1
16	K4	*Enterobacteriaceae bacterium*	99.93%	MT386163.1
17	K5	*Enterobacter soli*	97.15%	CP003026.1
18	K7	*Enterobacteriaceae bacterium*	98.15%	CP003938.1
19	L1	*Enterobacteriaceae bacterium*	99.55%	KM021074.1

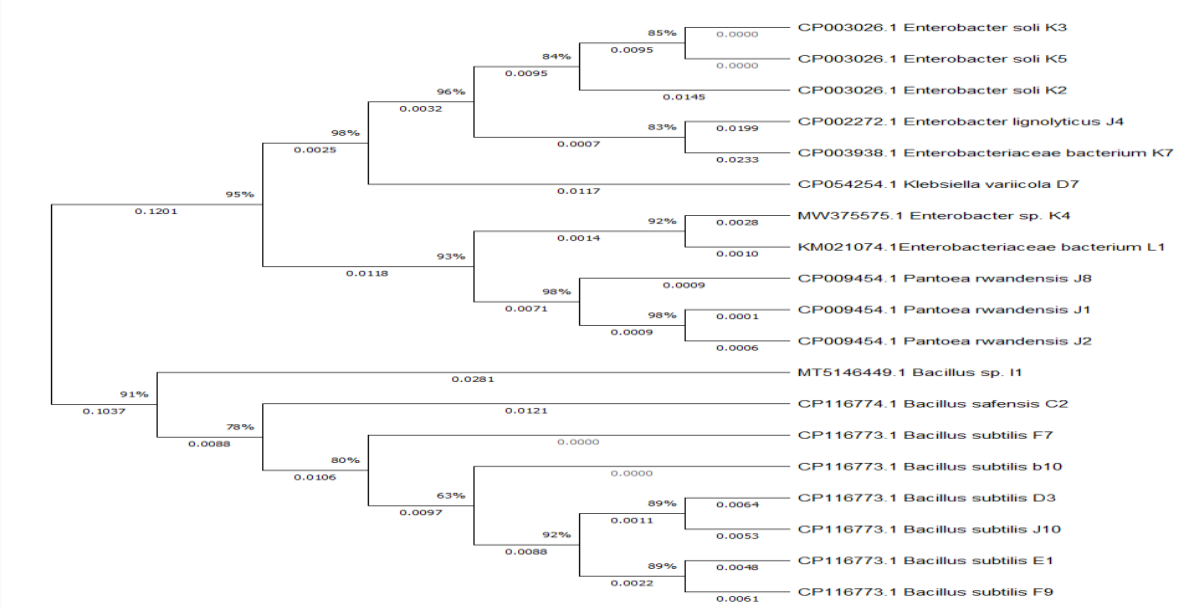

Figure 1. Phylogenetic tree of 16S rRNA gene sequence of endophytic bacterial isolates determined by the neighbour-joining method using Mega 11. Bootstrap values obtained with 1000 replicates are shown at nodes (> 50%). Reference sequences were obtained from GenBank with accession numbers in parentheses.

Plant growth promoting characteristics

Supernatant of 23 (34.84%) isolates developed red colour which indicates production of IAA at a range of 9.04 ± 0.92 – 222.60 ± 1.17 µg/mL within 24 hours in the presence of TRP (Figure 2a). 16 (69.57%) of bacteria produced IAA in the absence of TRP at the range of 1.77 ± 0.53 – 22.98 ± 0.43 µg/mL (Figure 2b). The IAA produced by these bacteria were compared with the IAA standard curve, and the most potent bacteria strain in producing IAA was found in bacteria strains J1 and J2 ranging from 220.92 ± 0.82 – 222.60 ± 1.17 µg/mL at the presence of TRP (Figure 2c).

Among the 66 bacterial isolates, 42 (63.63%) isolates were able to solubilise phosphate, indicated by the distinct development of a halo zone growing around each colony on Pikovskaya's medium (Figure 3a). Bacterial strains with the highest phosphate solubilising efficiency were found in bacterial strains J1, J2, K4 and L1 with a solubilising phosphate index of >3 (Figure 3b).

A total of 19 (28.78%) isolates were positive for siderophore production, indicated by the development of an orange/yellow halozone around the bacteria colony (Figure 4a). Strains E1 and J10, identified as *Bacillus subtillis* exhibited the highest SI of 3.00 ± 0.00, as shown in Figure 4b.

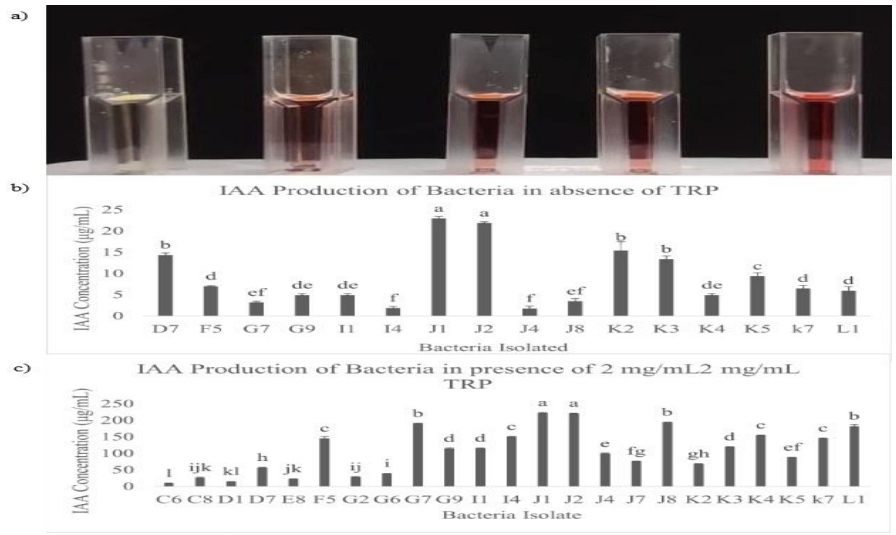

Figure 2. a) Red/pink colour development of supernatant mixture indicate IAA presence in the sample. b) IAA concentration produced by isolated bacteria without TRP. c) IAA concentration produced by isolated bacteria with 2 mg/mL TRP.

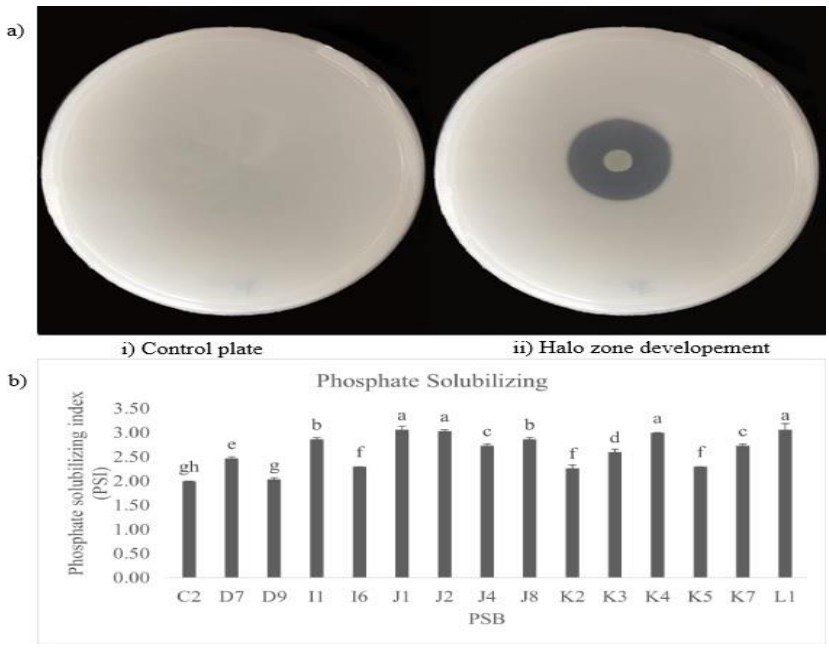

Figure 3. a) Clear halo zone developed around bacteria colony after 5 days on PVK agar. b) Phosphate solubilising index (PSI) of medium score PSB.

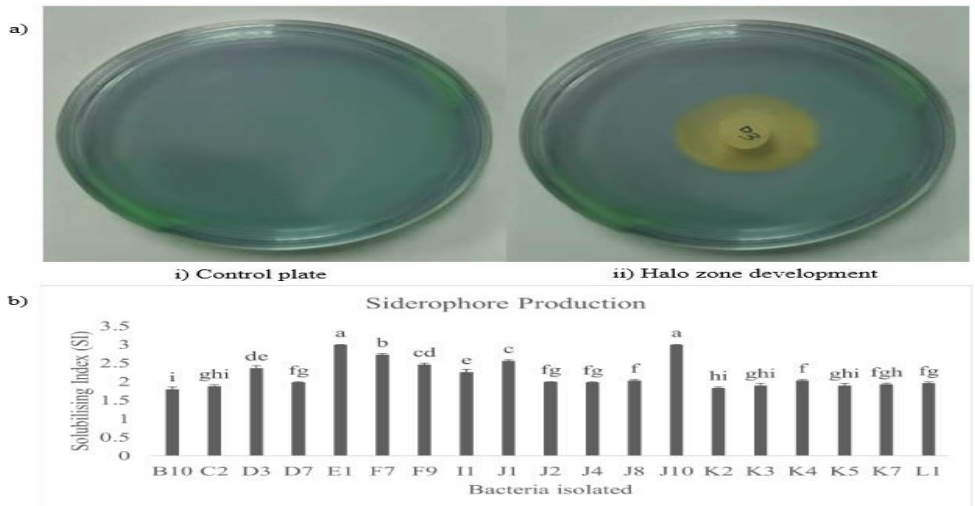

Figure 4. a) Development of orange halozone around bacteria colony on CAS agar. b) Graph of siderophore production of endophytic bacteria.

Table 2 demonstrates the 4 most potent bacteria strains (D7, K7, K3 and C2) that were selected to test their ability to promote the biomass of maize plants. Treatments with D7 and K7 resulted in the most substantial dry weight increase, with K3 following closely in rank. However, no significant differences were observed between treatment with C2 and the control group (Figure 5a). These findings suggest that bacterial isolates D7 and K7 have the most potent PGP traits among the tested isolates, which can significantly enhance maize growth (Figure 5b).

Table 2. Plant growth promoting characteristic of selected endophytic bacteria isolates

Plant Growth Promoting on Maize Plant				
Isolate	**Molecular Identification**	**IAA (µg/mL)**	**Phosphate**	**Siderophore**
D7	*Klebsiella variicola*	56	Medium	Low
K7	*Enterobacteriaceae bacterium*	145	Medium	Low
K3	*Enterobacter soli*	99	Medium	Low
C2	*Bacillus safensis*	0	Medium	Low

a)

Treatment	Plant height (means ± stderr) (cm)	Shoot dry weight (means ± stderr) (g)	Root dry weight (means ± stderr) (g)
D7	44.78 ± 0.23a	4.08 ± 0.18a	0.24 ± 0.06a
K7	44.08 ± 0.47a	4.05 ± 0.22a	0.24 ± 0.09a
K3	41.58 ± 0.32b	2.52 ± 0.17b	0.15 ± 0.05b
C2	32.62 ± 0.24c	1.58 ± 0.14c	0.11 ± 0.08bc
Control	31.98 ± 0.27c	1.38 ± 0.13cd	0.09 ± 0.03c

b)

Figure 5. a) Table of stem and root weight of maize plants after 6 weeks of incubation in greenhouse conditions. b) Plant growth enhancing properties of endophytic bacteria on maize plants in a pot experiment. D7, K7, and K3 were maize treated with IAA-producing bacteria that also had multiple PGP traits, whereas C2 was maize treated with multiple PGP trait bacteria that did not produce IAA.

Bio-agent control against phytopathogens

There are 6 bacteria isolated that showed antagonistic activity against both fungi and bacteria phytopathogens (Table 3). All of these bacteria were identified as genera bacillus as shown in Table 1, which were commonly known for their antagonistic traits against pathogens. Bacteria strain F7 and E1 show the highest mycelia inhibition of *Ganoderma boninense,* which is 60.68% and 59.83% (Figure 6a.1). Meanwhile, only strain F7 show the highest mycelia inhibition of *Fusarium solani* by 60.53% (Figure 6a.2). Moreover, the biocontrol strains were also effective against bacterial phytopathogenic known as *Ralstonia solanacearum*, with inhibition diameter zones ranging from 0.93 ± 0.07 to 2.12 ± 0.06 cm. Strain J10 exhibited the highest inhibition diameter zone, as indicated in Table 3. These findings suggest that the six *isolated Bacillus subtillis* possess significant biocontrol potential against various phytopathogens and could be utilized as natural alternatives to synthetic pesticides in agriculture.

Table 3. Relative Index of percentage suppression by bacteria isolates against phytopathogens and clear zone inhibiting pathogenic bacterial growth.

Strains	Mycilia inhibition percentage %		Inhibition zone diameter (cm)
	Fusarium solani	*Ganoderma boninense*	*R. solanacearum*
B10	47.37 ± 1.33c	51.28 ± 1.92b	1.57 ± 0.07b
D3	37.89 ± 0.67d	51.28 ± 0.85b	1.27 ± 0.03c
E1	54.21 ± 1.96b	59.83 ± 1.48a	0.93 ± 0.07d
F7	60.53 ± 1.08a	60.68 ± 0.85a	1.37 ± 0.03c
F9	46.84 ± 0.97c	49.57 ± 0.85b	1.40 ± 0.06bc
J10	49.47 ± 0.00c	53.85 ± 1.48b	2.12 ± 0.06a

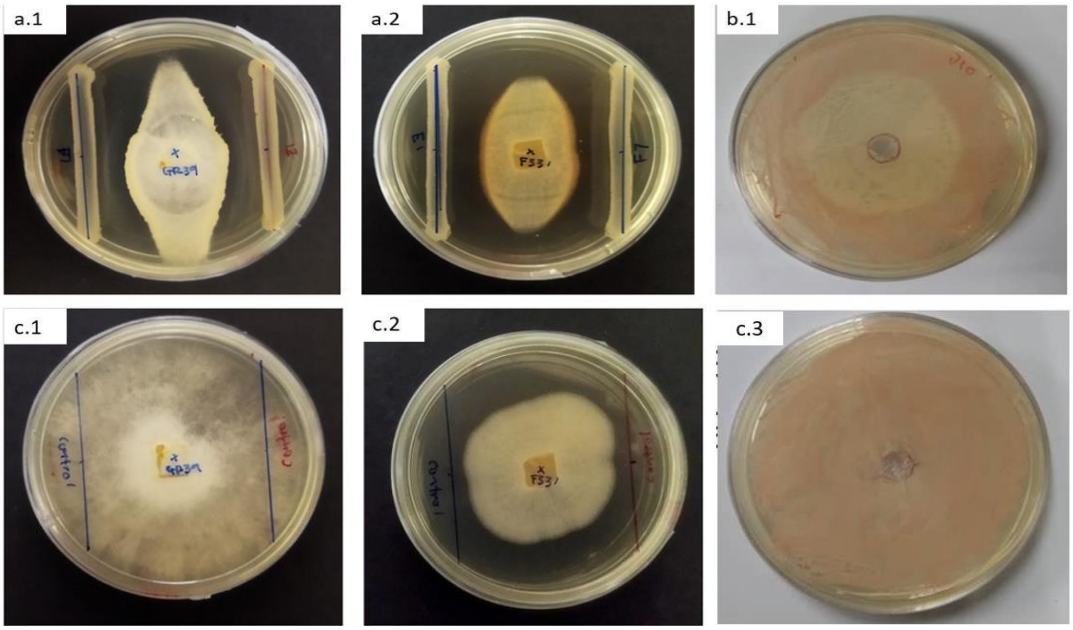

Figure 6. Dual culture of bacteria isolates and Phytopathogenic Fungi (a.1) *Ganoderma borneensis* and (a.2) *Fusarium solani* and control plate of (c.1) *Ganoderma borneensis* and (c.2) *Fusarium solani* after 7 days. (b.1) showed agar well diffusion assay for antagonistic activity against *R. solanacearum* and (c.3) control plate of antagonistic against R. *solanacearum.*

4. DISCUSSION

According to Liu et al. (2020), plants attract beneficial microorganisms from the environment to survive in unfavorable conditions. In the forest environments where *Palaquium maingayi* grows without human disturbance or fertilizer application, microorganisms accumulate naturally, which supports the growth needs of *P. maingayi*. Bacterial identification in this study employed standard bacteriological methods, including molecular and biochemical characterization commonly used in similar studies. The study aimed to identify endophytic bacteria in the roots of *P. maingayi* molecularly. The results revealed that the dominant bacterial groups were *Pseudomonadota,* followed by *Bacillota.* This finding aligns with Etminani and Harighi's (2018) report on the prevalence of endophytic bacteria in wild Pistachio Trees. The identified genera included *Pantoea, Pseudomonas, Bacillus, Klebsiella,* and *Stenotrophomonas*. The most dominant phyla in the endophytic bacteria community were *Proteobacteria, Firmicutes, Actinobacteria*, and *Bacteroidetes*. However, recent studies (Zhang et al., 2019 and Panda et al., 2022) have revised the most common phyla, with Proteobacteria and Firmicutes now referred to as *Pseudomonadota* and *Bacillota*.

Indole acetic acid (IAA) is an Auxin phytohormone responsible for several functions in plant vegetative development. According to Mano and Nemoto (2012), the production of IAA from endophytic bacteria can be divided into two important pathways: tryptophan (Trp)- independent and Trp-dependent pathways. The bacterial isolates strain J1 and J2, identified as *Pantoea rwandensis* have been observed to exhibit the greatest production of IAA in the presence of Tryptophan with a recorded value of 222.60 ± 1.17 and 220.92 ± 0.82 µg/mL, respectively. In a recent study conducted by Fouda et al. (2021), Trp-independent of *B. cereus* and *B. subtilis*, which were isolated from *Pulicaria incisa*, exhibited maximum IAA production of 117 ± 6 and 108 ± 4.6 µg/mL, respectively, when supplemented with 5 mg/mL tryptophan. However, the study conducted by Zhang et al. (2021) demonstrated that TRP-dependent IAA-producing bacteria exhibited a remarkable ability to synthesise IAA, with concentrations reaching as high as 3477 µg/mL and 3378 µg/mL. In this study, Trp-dependent IAA-producing bacteria were not able to produce a high concentration of IAA.

Phosphate solubilization traits of Phosphate solubilizing bacteria (PSB) are one of the most important traits that benefit host plants. Isolates J1 and J2 show the highest clear zone formation on Pikovskaya agar, indicating solubilizing of Tricalcium phosphate source. These isolates were identified as *Pantoea rwandensis* which showed similarity of 99.39% and 99.44%, respectively. According to Chen and Liu (2019), PSB mainly belongs to *Bacillus, Pseudomonas, Arthrobacter, Agrobacterium, Enterobacter, Serratia, Rhizobium,* and *Burkholderia. Pantoea rwandensis* also has been reported as PSB obtained from Passionflower (Cueva-Yesquén et al. 2021). Compared to other studies, it has been observed that other PSB exhibit superior ability to solubilize phosphate.

Besides phosphate, iron is one of the most important elements for organisms, including plants and microorganisms. Under iron stress, microorganisms can produce low-molecular-mass compounds with high affinity for ferric ions, termed siderophores, to provide available iron for the host plant. Other than providing an available iron source to host plants, the ability to solubilize iron also indirectly help in the plant defense, which allows the PGPB to compete iron from pathogen under iron stress condition. In this study, all antagonistic bacteria strains were able to produce siderophore, and strains E1 and F7, which identified as *Bacillus* subtilis show the highest orange halo zone on CAS agar with a Solubilizing Index of 3, indicating medium/middle efficiency in solubilizing iron. A similar report by Kumar et al. 2020, siderophore-producing endophytic bacteria isolated from *Oryza sativa* L. were dominated by the genus *Bacillus*.

Bacillus and *Pseudomonas* were commonly known for their antagonistic traits against pathogenic fungi and bacteria (Lacava et al., 2022). Similarly, in this study, all antagonistic bacteria belonged to the Bacillus genera, which have antagonistic activity against fungi and bacteria pathogens. Bacteria isolates strain isolates F7 and E1 were recorded to suppress over 54% to 60% of *Fusarium solani* and *Ganoderma boninense.* However, bacteria strain J10 show strong inhibition against bacterial wilt *Ralstonia solanacearum,* a common disease in the *Solanaceae* family. All of the antagonistic bacteria strains were identified as *Bacillus subtilis*. However, further tests on the planta inhibition assay of *F. solani*, *G. boninense*, and *R. solanacearum* were needed to confirm the effectiveness of these antagonistic strains.

Selected bacteria isolates were identified as *Enterobacteriaceae bacterium* (K7), *Enterobacter soli* (K2*), Klebsiella variicola* (D7), and *Bacillus safensis* (C2). The bacteria selected in this test were used to show the effect of different IAA concentrations produced by bacteria isolated toward maize growth. The results from the pot experiment showed the potential of IAA-producing bacteria to promote maize growth when compared to non-IAA-producing bacteria and control treatment plants. Smyth et al. (2011) suggested that the effectiveness of the candidate bacteria strain performance to promote plant growth cannot be determined by their PGP traits in vitro only but based on the environmental conditions and plant-microbe interactions. Significant differences in maize growth suggest the versatility of bacteria to colonize in other environments from their native. IAA produced by the bacteria isolates helps in the root development of maize. Other than that, maize treated with select bacteria strains shows their potential to increase the effectiveness of fertilizer used during pot experiments compared to control treatment plants.

Plant growth-promoting endophytic bacteria (PGPE) from *P. maingayi* have been found to regulate phytohormone levels, enhance nutrient use efficiency, enhance plant growth, inhibit the growth of pathogens in vitro, and establish symbiotic associations. These findings suggest that although endophytic bacteria isolated have the same PGP trait, IAA concentrations produced by endophytic bacteria play a major role in promoting maize growth. A future research direction is by analyzing further parameters of the PGP traits of endophytic bacteria and pot experiments such as nitrogen fixation, Potassium solubilizing, ACC (1-aminocyclopropane-1-carboxylate) deaminase and NPK soil content of pot experiment prove the potential of these endophytic bacteria as plant growth promoting agent.

5. CONCLUSION

To our knowledge, this is the first report on isolating endophytic bacterial strains from the *Palaquium Maingayi* tree that can potentially promote plant growth. Selected isolates improved plant growth in maize plants, indicating that the bacteria have the potential to be used to produce bio-fertilizers in the future to promote plant growth for sustainable agriculture.

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REFERENCES

1. Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria 1. Crop science, 13(6), 630-633.
2. Abu Bakar, F., Iskandar Pakasa, M. H., Sharaman, F. E., Wong, S. C. & Lepun, P. Exploration of Plant Growth-Promoting Endophytic Bacteria from the Roots of Native Plant *Uncaria Borneensis* in Sarawak, Borneo. European Chemical Bulletin, 12(4), 12545-12554.
3. Afzal, I., Shinwari, Z. K., Sikandar, S., & Shahzad, S. (2019). Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiological research, 221, 36-49.

4. AHMAD, K. I., MAULANA, M., MAGIMAN, Z. R., SEEMAB, A., & Faridah-hanum, I. (2023). Diversity and utilization of ethnomedicinal plants in Sarawak, Borneo. *The Malaysian Forester*, *86*(1), 125-152.
5. Brady, C. L., Cleenwerck, I., Van der Westhuizen, L., Venter, S. N., Coutinho, T. A., & De Vos, P. (2012). Pantoea rodasii sp. nov., Pantoea rwandensis sp. nov. and Pantoea wallisii sp. nov., isolated from Eucalyptus. International journal of systematic and evolutionary microbiology, 62(Pt_7), 1457-1464.
6. Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. Plant physiology, 26(1), 192.
7. Jasim, B., Jimtha, C. J., Jyothis, M., & Radhakrishnan, E. K. (2013). Plant growth promoting potential of endophytic bacteria isolated from Piper nigrum. Plant growth regulation, 71(1), 1-11.
8. Kumar, V., Jain, L., Jain, S. K., Chaturvedi, S., & Kaushal, P. (2020). Bacterial endophytes of rice (Oryza sativa L.) and their potential for plant growth promotion and antagonistic activities. South African Journal of Botany, 134, 50-63.
9. Pingale, S. S., & Virkar, P. S. (2013). Study of influence of phosphate dissolving microorganisms on yield and phosphate uptake by crops. Euro J Exp Biol, 3(2), 191-193.
10. Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., & Glick, B.R. (2016). Plant growth-promoting bacterial endophytes. Microbiological research, 183, 92-99.
11. Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. Analytical biochemistry, 160(1), 47-56
12. Senthilkumar, M., Anandham, R., Madhaiyan, M., Venkateswaran, V., & Sa, T. (2011). Endophytic bacteria: perspectives and applications in agricultural crop production. In Bacteria in agrobiology: crop ecosystems (pp. 61-96). Springer, Berlin, Heidelberg.