Research Article

Evaluation of Biocontrol Agent Bacteria Potential as a Plant Growth Promoting Rhizobacteria

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Abstract | Plant growth-promoting bacteria (PGPB) are usually associated with many plant species such as *Carica papaya* and are commonly present in many environments. The PGPR group was the most widely studied group of PGPB (Bashan and Gonzalez, 1999). This group of bacteria usually colonises the root surfaces and the closely adhering soil interface, the rhizosphere. This study aimed to isolate and screen effective bacteria from the papaya plant's rhizosphere and evaluate their potential as plant growth-promoting rhizobacteria (PGPR). Bacteria were isolated from 12 places in Peninsular Malaysia in which the samples were collected from each place from the papaya tree's rhizosphere soil Four isolates which is BK03, BK04, MKB04 and MKB 10 were chosen from total 12 isolates and undergo 6 test which were antibiotic test, casein hydrolysis test, oxidase test, Indole-3-Acetic Acid (IAA) Production Test, P-solubility Test and Nitrogen Fixing Activity Test. Isolates of MKB04 and MKB 10 were found to be resistant to all antibiotics tested and all four isolates demonstrate the ability to transform nitrogen to fix nitrogen. Isolates MKB04 and MKB10 were identified as *Pseudomonas aeruginosa*, BK03 as *Bacillus cereus* and BK04 as *Bacillus atraphaeus.* Only *Pseudomonas aeruginosa* produces hydrolytic enzymes and stimulate plant growth directly by nitrogen fixation, solubilisation of phosphate, and indole acetic acid (IAA) production compared to *Bacillus cereus and Bacilllus atrophaeus.* In greenhouse study *B. cereus, P. aeruginosa* and consortium consist of the mixture of MKB10, BK03 and BK04. showed a significant seed germination, shoot and root length of *Carica papaya* seedling. Consortium of *Bacillus cereus, Bacillus atraphaeus* and *Pseudomonas aeruginosa* showed a significant number of *Carica papaya* leaves and root length after 1 month in the greenhouse.

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Introduction

Beneficial microorganism application as biological control is a promising disease management strategy that aligns with Integrated Pest Management (IPM) principles and promotes ecological sustainability (Collinge *et al.*, 2022). Plant Growth-Promoting Rhizobacteria (PGPR), naturally

occurring soil bacteria, can help suppress plant diseases by producing antibiotics, inducing systemic resistance, and competing with pathogens for nutrients and space (Lugtenberg and Kamilova, 2009). The study on the potential of bacteria as plant growth in Malaysia was raised recently. Some literature discusses the potential use of bacteria to stimulate plant growth and manage soil and plant health (Ismail *et al*., 2016; Welbaum *et al*., 2009). Plant growth-promoting bacteria (PGPB) are usually associated with many plant species and are commonly present in many environments. The PGPR group was the most widely studied group of PGPB (Bashan and Gonzalez, 1999). This group of bacteria usually colonises the root surfaces and the closely adhering soil interface, the rhizosphere (Kloepper *et al*., 1999). As reported by (Gray and Smith, 2005), some of these PGPR can also enter root tissue and establish as beneficial endophytic bacteria (Gray and Smith, 2005). Many can transcend the endodermis barrier, crossing from the root cortex to the vascular system, and subsequently thrive as endophytes in stems, leaves, tubers, and other organs (Beneduzi *et al*., 2012; Compant *et al*., 2005).

Consequently, intimate associations between bacteria and host plants can be formed without harming the plant (Beneduzi *et al*., 2012; Compant *et al*., 2005). *Actinomycetes, Bacillus, Pseudomonas, Alcaligenes, Trichoderma* and non-pathogenic *Fusarium spp*. are the most crucial group of effective microbes (Compant *et al*. 2005). Beneduzi *et al*. (2012) reported that *Bacillus* species are the most common type of bacteria isolates in soil samples and can account for up to 36% of the bacterial populations.

Materials and Methods

Screening for effective bacteria

Bacteria was isolated from 12 places in Peninsular Malaysia as in Table 1. Source of 5 bacteria samples were collected from each place from the papaya tree's rhizosphere soil with 30cm diameter in length. Effective microbes were isolated using the ten-fold serial dilution technique. 1 gram of the soil was added to 90 ml sterile distilled water in 250 ml flasks and rotary shaken for 30 min at 150 rpm (Johnson and Curl, 1972). The plating was carried out using the spread technique, where 0.1 ml suspension was spread over the surface of the nutrient agar media in a Petri disk using an L-shaped glass rod. The plates were incubated at 28 ± 2°C for 24 hours to isolate bacteria, 5-10 days on nutrient agar and 7-14 days for fungi on PDA. Representative colonies growing on plates were selected, isolated and transferred to the same source media. The selected isolates were given codes. The bacterial strains were stored at -20°C in 20% glycerol.

Table 1: *Location of isolated bacteria for screening of effective bacteria as biocontrol agent for controlling fungus*

Sample No.	Location	Sources	Isolates
BK 01 - BK 05	TKPM, Gajah Mati, Pokok Sena, KEDAH	Rhizosphere soils	5
BPP 01 – BPP 03	Sungai Bakau, Nibong Tebal, PULAU PINANG	Rhizosphere soils	3
BPR 01 – BPR 08	Sungai Jambu, Selama, PERAK	Rhizosphere soils	8
BM 01 - BM 07	Paya Lebar, Ramuan Cina Besar, MELAKA	Rhizosphere soils	7
BM 08 – BM 13	Pengkalan Balak, MELAKA	Rhizosphere soils	6
BN 01 – BN 04	Rantau, NEGERI SEMBILAN	Rhizosphere soils	4
BPH 01 - BPH 05	Kuala Lipis, PAHANG	Rhizosphere soils	5
UniSZA 1 – UniSZA 5	Gong Badak, Kuala Terengganu, TERENGGANU	Rhizosphere soils	5
UniSZA 6 - UniSZA 8	Gong Medang, Besut, TERENGGANU	Rhizosphere soils	3
MKB 01 - MKB 05	UPM, Serdang, SELANGOR	Rhizosphere soils	5
MKB 06 – MKB 07	Ijok, Selangor	Rhizosphere soils	2
MKB 06 - MKB 13	Cheras Perdana, KUALA LUMPUR	Rhizosphere soils	8

A preliminary screening for potentially effective microbes against *Corynespora. cassiicola* and showed that from the 61 isolates tested, only 16.67% (10 isolates) showed antagonistic activity towards *C. cassiicola*. The remaining 51 isolated bacteria had no antagonist activity and were low to measure. Then, 10 isolates were done to screen the antagonist test further to get the exact value of percentage of inhibition radial growth (PIRG). There were 4 isolated recorded PIRG values below 50%, BPP3, MKB8, and UniSZA 2; the lowest PIRG value was recorded by BPR04 (8.89%). 2 other isolates recorded PIRG values over 50%, which were BM10 (55.09%) and UniSZA 1 (62.27%). Of the 10 isolates, 4 showed very high antagonistic activity with percentage of inhibition radial growth

(PIRG) values of over 80% and were selected for these study. All the 4 isolates (BK03, BK04, MKB04 and MKB10) were tested with antibiotic test, casein hydrolysis test, oxidase test, indole-3-acetic acid (IAA) production test, P-solubility test and nitrogen fixing activity. The four bacterial isolates were then tested on *Carica papaya* plant to test its (i) Effect of Bacteria on Papaya Seeds Germination and (ii) Effect of Bacteria on Plant Growth (Glasshouse Trial).

Antibiotic test on bacteria

The pure culture of bacteria was aseptically emulsified in the Mueller-Hinton Broth (MHB) until the turbidity of MHB visually matched that of the standard turbidity of the McFarland solution. Sterile cotton swabs were dipped into the broth culture and lawn the bacteria by streaking onto a Mueller-Hinton Agar (MHA) plate. After that, the antibiotic discs were placed onto the surface of the agar by using sterile forceps and incubated at 30˚C for 24 hours. After incubation, the susceptibility results produced by the bacteria were observed, and the diameter of the inhibition zone for each antibiotic was measured using a ruler. The measurement was compared to the standard table to determine the bacterial sensitivity or resistance to the antibiotic tested.

Casein hydrolysis test on bacteria

The bacterial strains were prepared for overnight culture in nutrient agar plates at four quadrants and incubated at 30˚C for 24 hours. Casein media were autoclaved. Then, the media were dispensed in a petri dish, and after the media solidified, the bacteria overnight culture was streaked at the centre of the plate in a straight line. The plate was incubated at 30˚C for 24 hours. Each bacteria tested was observed for hollow and transparent zones around the colony.

Oxidase test

The production of a purple colour within 10 seconds was recorded as positive, its development in 10-60 seconds was delayed positive, and the absence of colouration or its later development was negative. The filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. Then, moisten the paper with sterile distilled water. Pick the colony to be tested with a loop and smear it onto the filter paper. The observation was done on the inoculated area of the paper. Production of a purple colour within 10 seconds was recorded as positive, its development in 10-60 seconds as delayed positive, and the absence of colouration or its still later development as negative (Steel, 1961).

Indole-3-acetic acid (IAA) production test

Indole-3-Acetic Acid (IAA) activity was determined by centrifuging 72-hour-old cultures at 3,000 rpm for 30 minutes. Subsequently, 2 mL of supernatant was mixed with two drops of 0.1 ml orthophosphoric acid and 4 ml of Salkowski reagent (the mixture of 50 mL of 35% sulphuric acid, H_2SO_4 and 1 ml of 5M Iron (III) chloride, $FeCl_3$ solution). Then, the mixtures were incubated in the dark for 30 minutes. IAA production was indicated by the development of pink colour (Ismail *et al.*, 2016; Mat Amin *et al.*, 2010).

P-solubilising activity

The P-solubilising activity was determined by spot inoculated the respective isolates on National Botanical Research Institute's Phosphate (NBRIP) agar medium (g/l): glucose: 10g; tricalcium phosphate: 10g; $MgCl_2.6H_2O$: 5g; $MgSO_4.7H_2O$: 0.25g; KCl: 0.2g; $(NH_4)2SO_4$: 0.1; bacto agar: 15g.) incubated for 5-10 days at 28±2˚C. The formation of a clear zone indicates that the P-solubilising activity is positive (Ismail *et al.*, 2016; Mat Amin *et al.*, 2010).

Nitrogen-fixing activity (Qualitative Assay)

Nitrogen-fixing (qualitative assay) was identified using the following methods (Park *et al.*, 2005). The Burk's nitrogen-free agar media (10 g glucose, 0.41 g KH_2PO_4, 0.52 g K_2HPO_4, 0.05 g Na_2SO_4, 0.2 g $CaCl_2$, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.005 g $FeSO_4{\cdot}7H_2O$, 0.0025 g $Na_2MoO_4{\cdot}2H_2O$, 1.8 g agar) were prepared (all per litre distilled water) for semi-solid, and 15 g agar for solid medium was used throughout the study (Wilson and Wisniewski, 1989). Adjust the pH of the media to 7.0±0.1. Autoclave at 121˚C for 15 minutes. Pour the media into a test tube and let it cool. Grow the isolate into the agar by using a sterilised inoculation loop. Incubate at 28±2˚C for 24-72 hours. The isolates will be characterised as nitrogen-fixing bacteria based on pellicle formation.

Effect of bacteria on papaya seeds germination

The bacteria were tested on papaya seedlings to determine plant growth-promoting potential after inoculation. The papaya seeds were soaked in 95% ethanol for 5 minutes and then soaked in 5.25% sodium hypochlorite for 30-60 minutes, followed by 6 times washed with sterile distilled water for surface

sterilisation before seed inoculation. (Ismail *et al.*, 2016; Mat Amin *et al.*, 2010). The inoculation treatments were as follows: Uninoculated control (soaked in sterile distilled water and served as a control), Isolate MKB04, Isolate MKB10, Isolate BK03, Isolate BK04 and consortium (Mix of MKB04 + MKB10 + BK3 + BK4). Plant bioassay screening was established using the growth pouch method (GPM). To help break seed dormancy, the seeds were immersed in warm (about 40°C) sterile distilled water for 2 hours. Subsequently, the seeds were placed between 2-layer tissue paper and moistened with sterile distilled water for germination. After 9 days, the germinated seeds were undergone for inoculation. The inoculation was made by soaking the germinated seeds for 2 hours in respective bacterial inoculants, each isolate with more than 10^8 colony-forming units (CFU) per ml. Growth pouches were held upright. Each growth pouch contained 2 layers of paper towel, which were exposed to UV light before insertion into the growth pouch. The germinated seeds with respective treatments were transplanted into the growth pouches and arranged in a completely randomised design with three replications under laboratory conditions (Ismail *et al.*, 2016; Mat Amin *et al.*, 2010).Daily monitoring and watering of seeds in growth pouches were conducted. After 26 days of inoculation, harvesting and data collection were conducted. Data collections were root length (cm), root dry weight (mg), shoot length (cm), shoot dry weight (mg). Data on plant growth parameters were analysed for variance, and a mean comparison was made using Duncan's Multiple Range Test at a 95% significance level. The statistical analysis used the Statistical Analysis System (SAS) version 9.3.

Effect of bacteria on plant growth (Glasshouse Trial)

Ten replicates for each treatment of 3 weeks of homogenous papaya seedlings were planted in the polybag (12' x 8') containing a 3:2:1 soil mixture. 5 ml of bacteria solution for each treatment with 5g of NPK green was poured into the seedlings at a second. Followed by 5 ml bacteria solution with 5g of NPK green on day 7th and 3rd application on day 14th. For treatment 1 is bacteria solution (*P. aeruginosa*), treatment 2 (*B. cereus*), treatment 3 (*B. atrophaeus*) and treatment 4 is consortium, which is mix of all 3 bacteria solution (*P. aeruginosa* + *B. cereus* + *B. atrophaeus*). As for control the papaya tree were just fertilized with 5g of NPK Green with nutrient broth. The seedlings were watered daily and left in the glasshouse for 1 months. The leaf number and the height of the seedlings were measured every week. The polybags were arranged randomly in the greenhouse. An experiment was conducted on CRD experimental design. After 1 months, the seedlings were cut into specific part which is leaf, stem and roots. The soil was removed by soaking the polybag in the water to loosen the soil from the roots. All parts were then dried in the oven with 60 °C of temperature for 60 hours. The dry weight of all parts was recorded and analysis.

Results and Discussion

Screening of plant growth-promoting properties of four effective bacteria

Antibiotic test on bacteria

All 4 effective bacteria tested on six antibiotics that were CEC30, D10, ENR5, CN10, TE30 and AMC30 (Table 2). Bacteria BK04 was resistant to the CEC30 but susceptible to all other antibiotics. Meanwhile, BK03 were resistant to CEC30 and D10 and susceptible to ENR05, CN10 and AMC30. MKB10 and MKB04 show the same result, which is resistant to all antibiotics except ENR5 and CN10.

Table 2: *Test of several antibiotic resistant on four effective bacteria*

	CEC 30	D 10	ENR 5	CN 10	TE 30	AMC 30
BK04	R	S	S	S	S	S
BK03	R	R	S	S	R	S
MKB10	R	R	S	S	R	R
MKB04	R	R	S	S	R	R

**R = Resistant, S = Susceptible*

Chemical test on bacteria

Several chemical tests were done to screen the bacteria's potential as a plant growth promoter ability. MKB04 and MKB10 were rod-shaped bacteria, with both being gram-negative. Both also appeared with yellow-green bacteria without odour and were considered very fast-growing bacteria; meanwhile, BK03 and BK04. For the casein hydrolysis test, all 4 bacteria, MKB04, MKB10, BK03, and BK04, showed positive results (Table 3) in exoenzyme cases. It was proved by producing a zone of proteolysis surrounding the colony on skim milk agar (Figure 1).

An oxidase test was conducted, and again MKB04 and

MKB10 were positive, which produced cytochrome oxidase, and BK03 and BK04 were negative. Bacteria colonies with clear zones on NBRIP agar medium plates indicate their ability to solubilise phosphate (Figure 2). The result of this test shows that MKB04 and MKB10 were positive, while BK03 and BK04 were negative. The nitrogen-fixing test proved the ability of all four bacteria to transform nitrogen into fixed nitrogen.

Table 3: *Chemical tests on 4 effective bacteria*

Tests	MKB04 P. aeruginosa	MKB10 P. aeruginosa	BK04 B. atrophaeus	BK03 B. cereus
Cell Shape	Rod	Rod	Rod	Rod
Gram reaction	-Ve	-Ve	+Ve	+Ve
Colour	Yellow-green	Yellow-Green	White	White
Odour	No	No	Odour	Odour
Grow	Very fast	Very fast	Very fast	Very fast
Casein hydrolysis	+ve	+ve	+ve	+ve
Oxidase	+ve	+ve	-ve	-ve
IAA	-ve	-ve	-ve	-ve
Solubilise P	+ve	+ve	-ve	-ve
N Fixing	+ve	+ve	+ve	+ve

Positive reaction: +ve. Negative reaction: -ve R: Resistant S: Susceptible [a]Very fast colonies appeared after 24 hours incubation. Fast colonies appeared after 48 hours of incubation. Slow colonies appeared after 72 hours of incubation. + = positive; - = negative

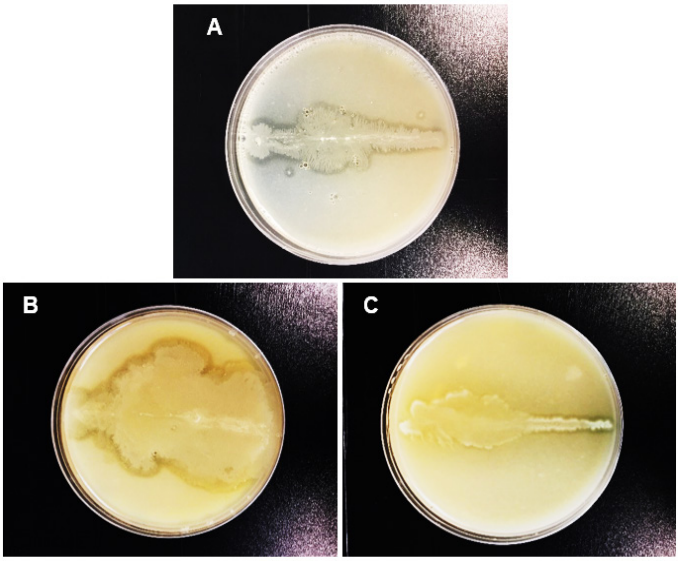

Figure 1: *Casein hydrolysis among (A) BK03, (B) BK04, (C) MKB04 and MKB10 on Skimmed milk agar incubated at room temperature.*

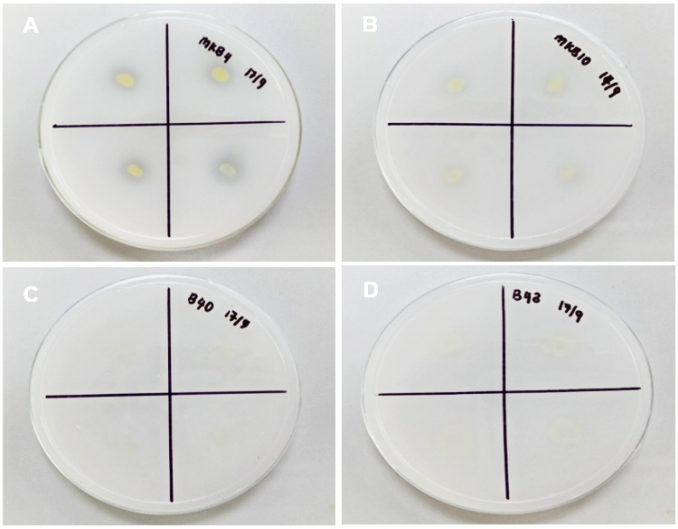

Figure 2: *Qualitative assay for P-solubilizing bacteria. The clear zone indicated a positive reaction. (A) MKB04, (B) MKB10, (C) BK04 and (D) BK03.*

Test on P-Solubilise and N-Fixing were important characters for that purpose. In N-Fixing tests, all bacteria tested were favourable to this test. It was a good sign of the potential of MKB10, MKB04, BK03 and BK04 as plant PGPB (Figure 3). It was consistent with the report by (Banerjee *et al.*, 2014), which mentioned several free-living bacteria can also fix nitrogen and provide it to plants. One PGPB element was fulfilled. Inoculation of plants with PGPB enhances the assimilation of essential nutrients and plant-associated biological nitrogen fixation (Bashan *et al.*, 2014). Using nitrogen-fixing microorganisms and plant growth-promoting bacteria (PGPB) is a necessary alternative to chemical fertilisers for the cultivation of agricultural plants (Katiyar, 2016; Premachandra *et al.*, 2016). Nitrogen is one of the three macronutrients required for high crop yields. Three-quarters of our atmosphere consists of nitrogen gas. Elemental nitrogen must be transformed into usable forms before it is available for plant uptake. Nitrogen is the major component of chlorophyll, which plays a vital role in photosynthesis (Premachandra *et al.*, 2016; Katiyar *et al.*, 2016). Certain microbes can convert atmospheric nitrogen into utilisable forms of nitrogen for themselves and plants via biological fixation (J. Kim and Rees, 1994). As the second most important plant growth-limiting nutrient after nitrogen, Phosphorus (P) is a crucial element for the survival of most organisms, including plants (Premachandra *et al.*, 2016). The amount of phosphorus in the soil is generally relatively high, but most of this is insoluble, so it is not available to support plant growth (Bianco and Defez, 2010). Two

(MKB04 and MKB10) out of four bacteria tested showed potential as P-solubilise bacteria, and two others showed negative results. Much of the soluble inorganic phosphorus applied as chemical fertiliser is immobilised, then unavailable to plants and wasted (Bianco and Defez, 2010; Khan *et al.*, 2007). The insoluble phosphorus is present as an inorganic mineral such as apatite or as one of several organic forms, including inositol phosphate (soil phytate), phosphomonoester, or phosphodiester (Khan *et al.*, 2007). Much of the soluble inorganic phosphorus used as chemical fertiliser is immobilised soon after it is applied, making it unavailable to plants and waste. It has been suggested that PGPB may help plants overcome abiotic stresses by providing them with IAA (Katiyar, 2016). Meanwhile, all tested bacteria (MKB10, MKB04, BK03 and BK04) didn't produce IAA or were too low to measure (Figure 4). After all, plant cells secreted, taken, and transported the bacterially produced IAA. Together with the plant's pool of IAA, it stimulates an auxin signal transduction pathway, including various auxin response factors. Consequently, plant cells grow and proliferate. A glass house study proved that MKB10, MKB04, BK04, BK03 and Consortium show the potential to increase papaya's growth rate, except for *B. atrophaeus*. With a 30% growth rate compared to control on consortium, there was a significant increment in plant growth.

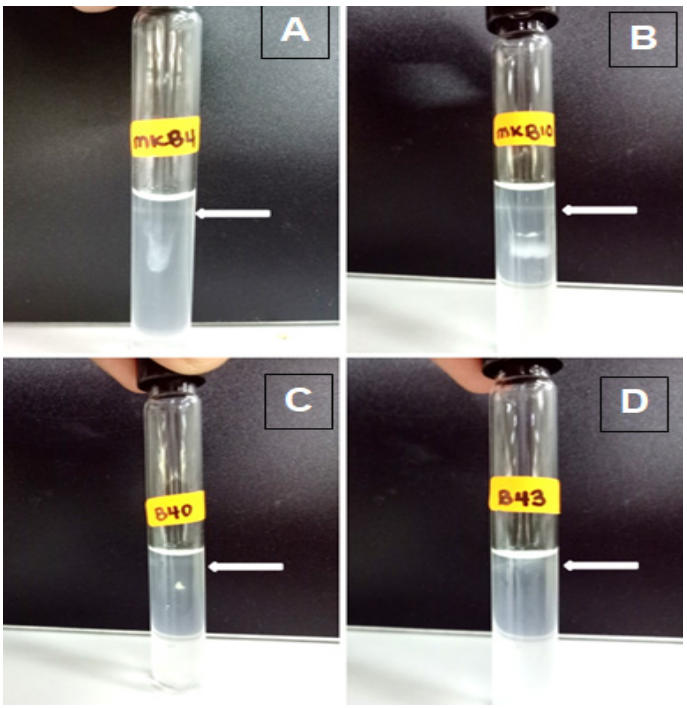

Figure 3: *Identification of Nitrogen-fixing activity (Qualitative Assay). (A) MKB04 (B)MKB10, (C) BK04 and (D) BK03. Forming of pellicle (arrow) indicated the positive result*

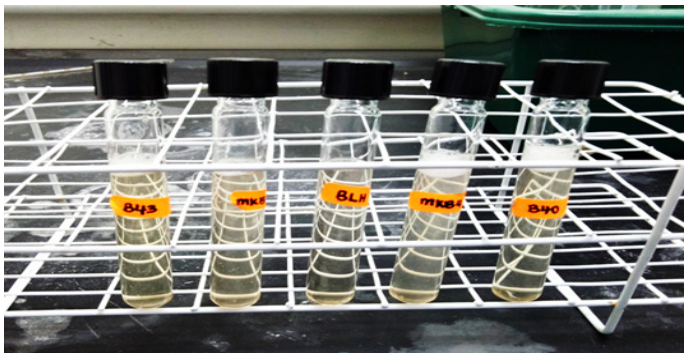

Figure 4: *IAA test on effective bacteria with no changing in colour*

Effect of bacteria on papaya seeds germination and shoot production

The effective bacteria tested have different effects on *C. papaya* seeds. MKB10, BK04 and consortium show an influence on the seed germination process. Consortium shows the most extended shoots followed by MKB10 with 6.98 cm and 6.68 cm, respectively (Table 4), with no significant difference. BK04 then followed it with 6.16 cm, then followed by Control with a significant difference ($p<0.05$). Meanwhile, no seeds were germinated on BK03. Thus, the percentage of germination was higher in the consortium, with 90% germination, followed by control with 85% and then 80% of MKB10. Meanwhile, no germination was recorded on BK04 (Figure 5).

Table 4: *Effect of bacteria on shots and roots length 25th days*

Treatment	Seeds Germination (%)	Shoots Length (cm)	Root Length(cm)
Control	85	1.48^{c}	0.30^{c}
MKB10	80	6.66^{a}	2.92^{a}
BK03	70	6.16^{b}	2.46^{b}
BK04	0	0^{d}	0^{c}
Consortium	90	6.98^{a}	3.20^{a}

Figure 5: *Germination of Carica papaya's seeds on different treatment of bacteria (BK04, Consortium and Control) on wet tissue.*

Table 5: *Effect of bacteria on growth of C. papaya seedlings in different bacteria treatment*

Treatment	Stem	Leaf		Root			Total dry weight (g)
	Height (cm)	Dry weight (g)	Number (mean)	Dry weight (g)	Length (cm)	Dry weight (g)	
Control	23.60^{b}	2.54^{b}	9.20^{c}	2.38^{c}	19.90^{d}	3.44^{b}	8.36^{b}
P. aeruginosa	26.50^{a}	3.30^{a}	10.00^{b}	3.12^{ab}	26.00^{b}	4.90^{a}	10.78^{a}
B. cereus	22.90^{b}	2.60^{b}	10.40^{b}	3.26^{a}	24.40^{bc}	4.78^{a}	10.64^{a}
B. atrophaeus	25.90^{a}	3.24^{a}	10.80^{b}	2.66^{bc}	23.86^{bc}	3.90^{b}	9.8^{b}
Consortium	26.10^{a}	2.72^{b}	11.60^{a}	3.30^{a}	29.80^{a}	4.90^{a}	10.92^{a}

**Consortium = Mixture of MKB10, BK03 and BK04.*

Effect of bacteria on papaya seedling growth

Effective bacteria were confirmed to influence the growth of *Carica papaya* seedlings. Three treatments show the ability of the bacteria to increase plant growth (stem high) with significant differences ($p<0.05$) compared to the control (Table 5). The highest was recorded on *P. aeruginosa* with 26.50 cm, followed by the consortium and *B. cereus*. The lowest was on *B. aethropus* with only 22.90 cm. Meanwhile, on dry stem weight, *P. aeruginosa* recorded the highest with 3.30g and the weakest on control with 2.54.

The number of leaves on the treated plant was significantly higher than the control. The consortium recorded the greatest number of leaves with significant differences ($p<0.05$) to three other treatments, which were *P. aeruginosa, B. atrophaeus* and *B. cereus*. Consortium shows the highest number of leaves, with 11.60, but it is insignificant for *P. aeruginosa* and *B. cereus*. Regarding the dry weight of leaves, the three treatments significantly differ from the control. Consortium recorded the highest with 3.30 g, followed by *B. cereus* with 3.26g with no significant difference ($p>0.05$). The lowest was control, followed by *B. atrophaeus*, the second lowest with no significant difference. The influence of effective bacteria on root boosters was also tested. On root length, all 4 treatments were significantly different from the control. The longest was recorded at consortium with 29.80 cm. *B. atrophaeus* recorded the shortest length among treatments at 23.86 cm and *B. cereus* at 24.40 cm, with no significance among them. Regarding the dry weight of *C. papaya* roots, there is no significant difference between the three treatments: Consortium, *P. aeruginosa* and *B. cereus* as root boosters. Thus, *B. atrophaeus* seemed to have no effect of plant booster on *C. papaya* as *B. atrophaeus* has no significant difference compared to the control. Meanwhile, on the total dry weight (stem, leaf, and roots) of *C. papaya*, the best growth performance was shown by Consortium, followed by *P. aeruginosa* and *B. cereus*. Still, there was no significant difference between them (Figure 6).

Figure 6: *Effect of effective bacteria: (A= Control; B= MKB10; C= BK03; D= BK04 and E= Consortium) on C. papaya growth in greenhouse trial*

Conclusions and Recommendations

In conclusion, nitrogen and phosphorus are key elements for the growth and metabolism of plants. All bacteria were N-fixing and P-solubilised. So, *Pseudomonas aeruginosa*, *Bacillus cereus, Bacillus atrophaeus* and its consortium were proven as potential PGPB The i*n vitro* performance of two isolates (MKB04) and (MKB10) were identified as *Pseudomonas aeruginosa*, produces hydrolytic enzymes and stimulate plant growth directly by nitrogen fixation, solubilisation of phosphate, and indole acetic acid (IAA) production compared to *Bacillus cereus and Bacilus atrophaeus. Pseudomonas aeruginosa* and *B. cereus* and consortium were proven as potential PGPB by greenhouse study with significant difference ($p<0.05$) compared to the control.

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Novelty Statement

This study evaluates the potential of plant growth-promoting rhizobacteria (PGPR) of the effective bacteria isolated from the papaya plant's rhizosphere located in 12 isolation point in Peninsular Malaysia . The findings of this research confirmed the *P. aeruginosa* as Plant Growth-Promoting Bacteria (PGPB) as mentioned by Stella Matthews and Halimi bin Mohd Saud, (2011).

Author's Contribution

Mohammad Hailmi Sajili: Conducted the experiment and data collection.
Tajul Afif Abdullah: Checked the statistical analysis.
Zakry Fitri Ab Aziz and Zaiton Sapak: Checked the method for the experiment.
Siti Aishah Abu Bakar and Nor Azi Asminda Johari: Proof read the article.
Muslianie Md Isa: Checked the grammatical errors.

Generative AI or AI assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest.

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