Front. Agr. Sci. Eng. 2024, 11(3): 499–513 https://doi.org/10.15302/J-FASE-2024559
RESEARCH ARTICLE

Isolation, characterization and identification of plant growth-promoting rhizobacteria from the rhizosphere of *Acacia mangium* at sandy BRIS soil

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KEYWORDS

Biofertilizer, corn, organic molasses medium, PGP characteristic, phytohormone, potassium nitrate, siderophores

HIGHLIGHTS

- Isolation of potential PGPR from rhizosphere sandy BRIS soil of *Acacia mangium*.
- The isolated rhizobacteria showed significantly varied growth in organic molasses medium supplemented with KNO_3.
- The ability to fix atmospheric N_2, solubilize P and K, produce IAA and siderophores varied differently for single and mixed strains of the isolated rhizobacteria.
- The single or mixed strains of rhizobacteria had a significant effect on corn phenology, growth and yield.
- Identification of the isolated rhizobacteria at the molecular level.

Received October 5, 2023;
Accepted March 11, 2024.

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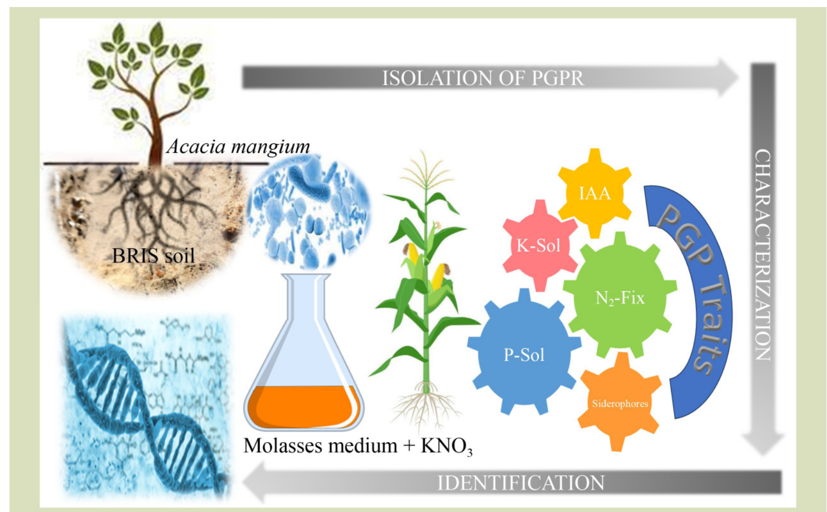
GRAPHICAL ABSTRACT



ABSTRACT

This study has isolated, characterized, and identified potential plant growth-promoting rhizobacteria (PGPR) with multiple PGP characteristics (N_2-fixation, P- and K-solubilization, IAA, and siderophores production) from the rhizosphere BRIS soil of *Acacia mangium*. A total of 24 pure colonies were isolated and only 8 colonies were selected for further evaluation of the growth rate in 5% organic molasses medium supplemented with 2% KNO_3. Based on the biochemical, potential PGP characteristics and growth performance, 3 superior PGPR strains were selected and identified as *Paraburkholderia unamae* (UA1), *Bacillus amyloliquefaciens* (UA6), and *Enterobacter asburiae* (UAA2) by partial sequencing of the 16S rRNA gene. The selected bacterial

strains either in single or mixed (UA1 + UA6 + UAA2) cultures have shown a significant biochemical estimation of the PGP characteristics. Each strain has its own PGPR traits superiority with UA1 showing the best PGP characteristic followed by UA6 and UAA2. The use of mixed bacterial strains was beneficial as it showed the best performance in N_2-fixation, siderophores production, and significant effect on corn phenology, growth and yield compared to using a single strain. These types of microbes showed potential to be used as biofertilizer and should be exploited more.

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1 Introduction

BRIS (Beach Ridges Interspersed with Swales) soil is the sandy soil that develops wide ridges plains along the east coast states of Terengganu, Pahang and Kelantan of Peninsular Malaysia. There are several types of coastal sandy soil area in the world like in Andhra Pradesh (India), and the coastal central provinces of Vietnam. The soil has the same characteristic as BRIS soil in term of soil properties having more than 90% sand, low soil electrical conductivity, low in soil organic content, low in micro and macronutrients, low water holding capacity, low pH and others[1,2]. The physicochemical properties of BRIS soil, the high temperature, high drainage, low water retention capacity and nutrient deficiency have made this soil infertile with limited ability to support plant growth[2].

Since Soil is a complex habitat for many types of microorganisms, various types of beneficial microbes including plant growth-promoting rhizobacteria (PGPR) have been reported to be isolated from many different types of soil around the world except for the BRIS soil area. Interest in PGPR with various beneficial functions on plant growth and yield has increased recently due to their potential as biofertilizer to reduce agro-chemical use and support eco-friendly sustainable food production. Their beneficial effects on plants have been attributed to their plant growth-promoting (PGP) characteristics such as the ability to fix atmospheric nitrogen (N_2), to solubilize mineral phosphorus (P) and potassium (K), and other mechanisms such as the production of phytohormones and competitive suppression of pathogens.

The potential of PGPR in agriculture has steadily increased as one of the most studied groups of beneficial microbes to replace the use of chemical fertilizers, pesticides and other supplements. Isolation of native and local PGPR strains with multiple beneficial characteristics is important for biofertilizer production as native microbes have advantages over the introduced inoculant in terms of adaptation toward the environment[3]. Since BRIS soil is considered problematic, PGPR in this area might be hardy and have superior quality to be used as a potential biofertilizer. Moreover, the isolated PGPR can be also used in other places with the same or different soil and environmental characteristics because microbial populations can adapt to the new ecological opportunities by altering their physiologic, standing genetic variation and evolving via new beneficial mutations[3,4]. This ability of microbes to constantly adjust in response to changing environmental conditions and experience positive selection to adapt to new conditions gives more potential for BRIS soil PGPR to be exploited.

This study was conducted to isolate the BRIS soil PGPR with multiple beneficial characteristics from the rhizospheric soil of *Acacia mangium* which was observed as a dominant tree growing in this area. PGPR with multiple abilities to fix N_2, solubilize P and K, produce siderophores and phytohormone such as Indole Acetic Acid (IAA) and grow in organic molasses medium enriched with KNO_3 were isolated, morphologically and biochemically characterized and identified at the molecular level. The ability to propagate in molasses medium with KNO_3 is one other important factor that could be used to determine the potential of these microbes in biofertilizer production. The usage of such pure or mixed media at an industrial scale would be costly, thus industrial application of microbes needs to use a more economical nutrient source for microbial growth[5]. Molasses is an important cheap agro-industrial byproduct containing high sugar contents (48%–50%) and was the basic raw material used for many microbiological processes[6]. Meanwhile, KNO_3 was added to the fertilizer mixture for the plant's healthy growth and formation of flowers and fruits. KNO_3 is also an effective N_2 source in the fermentation medium for bacterial growth and function[7]. With the fact that the use of mixed strain culture would likely be more effective and last longer than the use of single strain culture due to its synergistic effect[8], biochemical characteristics of the selected BRIS soil PGPR were estimated in single and mixed strain conditions.

2 Materials and methods

2.1 Sample collection and isolation of N_2-fixation and P-solubilization PGPR

The study site is located in three different locations with three different criteria representing the area of BRIS soil in Tembila, Besut Terengganu. The soil samples were collected from the rhizospheric area of selected *Acacia* tree from Point 1: the young BRIS coastal forest (5°45′26.4″ N, 102°37′21.3″ E), Point 2: the agricultural land (5°45′ 43.7″ N, 102°37′ 29.7″ E), and Point 3: the housing land (5°45′32.1″ N, 102°8′18.4″ E) for total bacterial enumeration.

Five random rhizospheric soil samples were withdrawn from 10 to 15 cm depth, mixed into a single composite sample and brought to the Microbiology Laboratory of UniSZA Isolation of rhizospheric bacteria that have both abilities to fix N_2 and solubilize phosphate was done by suspending 10 g of soil in 90 mL sterile saline water, shaking at 150 $r \cdot min^{-1}$ on an orbital shaker for one hour and serially diluted. An amount of 1 mL of serially diluted BRIS soil sample was suspended in 99 mL Burk's N-free broth medium.

Bacterial incubation was done at a room temperature (28 ± 2 °C) for both aerobic and anaerobic conditions. After 2 days in the Burk's medium, 20 µL of the culture was aliquots and transferred to Pikovskaya (PVK) agar medium for six days incubation period. The colonies surrounded by a halo zone that appeared on the PVK agar were considered bacteria that have both abilities to fix N_2 and solubilize P, and the viable bacterial cell count was expressed as colony forming units (CFU).

The point that showed the highest CFU will be selected as the sampling point for isolation of potential superior PGPR. Each colony with different morphological appearances was subsequently re-streaked onto a new plate using the nutrient agar medium until pure colonies were obtained. The selected pure colony of bacterial isolates were streaked onto nutrient agar slants and kept in the chiller (4 °C) for further studies.

2.2 Morphological and biochemical characterization of bacterial isolates

The isolated bacterial pure colonies were subjected to morphological and biochemical characterization according to the methods described[9]. The Gram reaction, catalase test, nitrate reduction, urease, ammonia production, and indole test were performed according to standard methods.

2.3 *In vitro* screening and estimation of bacterial isolates for PGP traits

Estimation of PGP characteristics of selected BRIS soil PGPR isolates was done in single and mixed strain conditions.

2.3.1 Biological nitrogen fixation (BNF)

The nitrogenase activity of the isolates was determined by the growth on a N-free medium, the Burks Agar (HiMedia). Pure bacterial colonies were streaked on the Burks Agar and incubated for 5 days at room temperature. The appearance of the bacterial colonies indicated a positive test.

The bacterial BNF was estimated using the acetylene reduction assay method (ARA) adapted from the methods described[10]. The overnight culture of pure isolated PGPR inoculum in Nutrient Broth (NB) medium was transferred into a 10 mL vacutainer containing 4 mL of N-free solid malate medium. Single strain BNF ability determination used 50 µL volume of overnight culture in NB broth medium while mixed strains used 20 µL of each strain and mixed in the vacutainer. A gas-tight syringe was used to replace 10% of the air in the vacutainer with pure acetylene gas (99.8%). The inoculate was incubated for 1 h in that vacutainer.

Finally, 1 mL of the air sample was injected into the gas chromatography (7820A) that was equipped with a hydrogen flame ionization detector (HID) and stainless-steel column Porapak T. The amount of nitrogenase activity was calculated using the formula[11];

$$N = \frac{hx \times C \times V}{hs \times 24.9 \times t} \tag{1}$$

where, N is the concentration of C_2H_4 ($nmol \cdot mL^{-1} \cdot h^{-1}$), hx is the peak value of the sample, C is concentration of standard C_2H_4 ($nmol \cdot mL^{-1} \cdot h^{-1}$), V is volume of the vial, hs is peak value of C_2H_4, t is the time taken to complete a reaction (h).

2.3.2 Phosphate solubilization

The phosphate-solubilizing bacteria were isolated using PVK medium (HiMedia)[12] incubated at room temperature for six days. The colonies that appeared and were surrounded with a halo zone on the PVK agar medium were considered P-solubilizer bacteria. Meanwhile, inorganic phosphate solubilization was quantified using the modified vanadomolybdophosphoric acid method[13].

A volume of 0.5 mL (for single strain test) and 0.2 mL (for mixed strain test) of each pure overnight culture in NB broth

medium was aliquoted into 50 mL PVK broth medium in a 100-mL conical flask containing 0.5% $Ca_3(PO_4)_2$ that was prepared with the final pH 6 before autoclave. The culture was incubated at room temperature and shaken at 150 $r·min^{-1}$ for 12 days. The uninoculated medium served as a control and was also kept under similar conditions.

On days 6 and 12 of incubation, the pH of the culture medium for each culture was recorded. An amount of 25 mL of each culture medium was transferred to 50 mL centrifuge tubes after 6 days of inoculation. The cultures were centrifuged at 10,000 $r·min^{-1}$ for 10 min. A volume of 2.5 mL supernatant was transferred into a 50-mL beaker. Then, 20 mL of dH_2O was added into the beaker followed by 2.5 mL of Barton's reagent. The mixing was incubated for 10 min. The absorbance was read at 430 nm after 10 min centrifugation in UV-Visible spectrophotometer (UV-Vis, Shimadzu UVmini-1240). The same procedures were repeated after 12 days incubation period. Total soluble P was calculated from the standard curve of KH_2PO_4. The standard solution was prepared by mixing KH_2PO_4 with 5 mL of concentrated H_2SO_4 and diluted to 1 L with dH_2O.

2.3.3 Potassium solubilization

Potassium solubilization of bacterial isolates was studied by the spot test method on Aleksandrov medium (HiMedia) plates containing muscovite mica as an insoluble form of K and the pH of the medium was adjusted to 7.2 by using sodium hydroxide prior to sterilization[14]. A loopful of 24-h culture of purified bacterial strains was spotted on the Aleksandrov agar plates and incubated at room temperature for 3 days. A positive result was indicated by the formation of a solubilization zone around the bacterial colony on the agar plates.

The quantitative measurement of K-solubilization was a modified method based on the bacterial abilities to release K from muscovite mica in the medium[14]. A volume of 0.5 mL (for single bacterial strain test) and 0.2 mL each (for mixed bacteria strains test) overnight bacterial culture in NB broth medium was inoculated into 25 mL Aleksandrov broth medium in 50 mL Falcon tube. The inoculated and uninoculated (control) tubes were shakenly (120 $r·min^{-1}$) incubated at room temperature for 5 days. After that, the broth cultures were centrifuged at 10,000 $r·min^{-1}$ for 10 min and 1 mL of supernatant was aliquoted into a 50 mL volumetric flask. The liquid volume was made to 50 mL using distilled water and mixed thoroughly. The K content in the supernatant was determined spectrometrically using an atomic absorption spectrometer (Perkin Elmer, Analyst 400 AAS).

2.3.4 Colorimetric estimation of indole-3-acetic acid (IAA)

Estimation of indole-related compound (IRC) particularly IAA was colorimetrically determined using Triptic Soy Broth (TSB) medium (HiMedia)[15]. A loopful of pure fresh bacterial culture was inoculated in 100 mL TSB medium in the 200 mL conical flask with the addition of 5 mL L-tryptophan as the precursor of IAA. TSB without any bacterial inoculation served as a control. The cultures were incubated at room temperature and shaken at 120 $r·min^{-1}$ for 5 days.

After 5 days, the bacterial culture was centrifuged at 7000 $r·min^{-1}$ for 7 min. The IAA estimation in the supernatant was done using Salkowsky's reagent and colorimetric assay. 1 mL of supernatant was mixed with 2 mL Salkowsky reagent and incubated for 25 min to allow the development of a pink color which indicates the production of IRCs (including IAA). The absorbance was read at 535 nm using the UV-Vis. The total IRCs content, especially IAA production was calculated from the standard curve of pure IAA stock.

2.3.5 Siderophore production

The ability of bacteria to produce siderophore was assayed using the Chrome Azurol S (CAS) agar[16]. Formation of Fe-CAS dye complex causes the blue color of the medium. All bacterial strains were streaked on CAS agar and incubated for 24 h at 33 °C. The development of an orange halo zone around the colony indicates a positive result because the presence of siderophores had removed Fe from the Fe-CAS dye complex.

The estimation of siderophore production was measured based on the modified method[17,18]. An amount of 0.5 mL (for single bacteria test) and 0.2 mL (for mixed strains test) of fresh overnight bacterial inoculum in NB medium was inoculated into 40 mL of CAS broth medium in 100 mL conical flask. The CAS broth medium without bacterial inoculation served as a control. The cultures were incubated at room temperature and shaken at 120 $r·min^{-1}$ for 5 days. The bacterial broth cultures and control were centrifuged at 10,000 $r·min^{-1}$ for 10 min. The amount of siderophores in the supernatant was determined spectrometrically at 630 nm using the UV-Vis. The total percentage of siderophores unit was calculated from the formula[18]:

$$\text{Siderophore unit} = (1 - \text{As}/\text{Ar}) \times 100\%$$

where, Ar is the absorbance of reference and As is the absorbance of sample.

2.4 Growth of BRIS soil bacteria in 5% molasses medium and 2% KNO_3

Molasses medium (5% molasses in 150 mL dH_2O) supplemented with 2% KNO_3 was prepared and autoclaved at 121 °C for 15 min. The molasses medium was based from sugarcane molasses obtained from the local market. One fresh overnight loopfull of pure BRIS soil bacteria isolates that have both abilities to fix N_2 and solubilize P were grown in a molasses medium. The content was shaken at 150 $r \cdot min^{-1}$ for 5 days at room temperature. After 5 days, bacterial growth and viable cell count were determined by the spread plate method on nutrient agar.

2.5 Effects of the isolated PGPR on the growth and yield of corn (*Zea mays* L.)

The field experiment was undertaken at the Faculty of Bioresources and Food Industry farm (5°45′ 36.5″ N 102°37′ 18.0″ E), Universiti Sultan Zainal Abidin, Besut Campus, Terengganu, Malaysia. The field experiment took 70 days from March to May 2022 in tropical weather at an average temperature ranging between 28 and 36 °C. The total rainfall for the growing year and the growing period were 4573 mm and 510 mm, respectively.

Preparation of selected bacterial inoculum was done by growing in 6% molasses medium for three days and kept at 4 °C in the chiller for further use. The optical density of the cell suspension was adjusted to 0.4 A at 600 nm using UV-Vis (approximately 3×10^7–4×10^7 $cells \cdot mL^{-1}$). Corn variety namely Manis Madu was used as a test crop, which is well-adapted and commonly used by farmers of the study area. The experiment consists of 5 treatments (T_1 = control, T_2 = UA1, T_3 = UA6, T_4 = UAA2, and T_5 = mixed strain of UA1, UA6, and UAA2). Each treatment was given with 500 g per plant of organic material (chicken dung) 1 week before planting and 50 g per plant of NPK fertilizer (Nitrophoska® Blue fertilizer, Behn Meyer) respectively at 9 and 30 days after planting and Urea (46% N) at 25 g per plant at 15 and 45 days after planting. Inoculation was done by respectively pouring 200 mL of single bacterial solution and 70 mL of each bacterial solution for mixed culture treatment (T_5) on the soil around the plant base at 1 week after sowing and repeated at every week interval until 9 weeks of planting.

The experiment was laid out in randomized complete block design (RCBD), with three replications of each treatment. The experimental area was cleaned, ploughed and prepared well, and each plot was levelled up to 30 cm. An area of 55.25 m^2 (11.4 m × 3.25 m) was used as an experimental unit (plot), accommodating 6 rows of each 11.4 m length. Spacing between plants in a row was 0.6 m and 0.45 m between rows. All other agronomic practices like weeding, pest, and disease control were kept similar manner to all the plots during the experimental period.

Crop phenology data (days to emergence, tasseling, and physiological maturity) were recorded when 50% of the plants in a plot emerged and flowered, and 80% of the plants attained yellow coloration, respectively. The growth parameters: leaf number per plant, cob length, and plant height were measured. Plant height (cm) was measured from the base of the plant to the uppermost leaves. The number of functional leaves per plant was determined by visual count when the plant attained the silking stage. The number of cobs per plant, the total number of rows per cob and the number of grains per row were counted and the cob length (cm) was measured during harvesting the crop. Grain yield was measured using an electronic balance and then adjusted to 12.5% moisture content. Harvest index (%) was calculated as the ratio of grain yield to total biomass yield.

2.6 Molecular identification of the isolated BRIS soil bacteria

The genomic DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega) according to the protocol described by manufacturers. The Polymerase Chain Reaction (PCR) was used to amplify the 16S gene from the extracted DNA sample by using universal forward (27f, AGAGTTTGATCMTGGCTCAG) and reverse (1492r, GGTTACCTTGTTACGACTT) primers. A final volume of 45 µL master mix was prepared by mixing 3 µL of 25 $\mu mol \cdot L^{-1}$ $MgCl_2$, 5 µL of 10X PCR reaction buffer, 1 µL of 10 $\mu mol \cdot L^{-1}$ dNTPs mix, 0.5 µL of 5 μL^{-1} Taq polymerase, 0.75 µL of 10 $\mu mol \cdot L^{-1}$ forward and reverse primer respectively into 34 µL ultra-pure water. A volume of 5 µL DNA sample was mixed with the mixture in a 0.2 mL PCR tube. PCR was run using the cycling conditions: initial denaturation of 5 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C, and final extension at 72 °C for 5 min.

The DNA band formation was observed by mixing 5 µL DNA sample with 2 µL DNA loading dye and loaded into respective wells of 1% agarose gel. The sample was electrophoresed in 1 × TAE buffer at 80 mV for 50 min. The gel was viewed under a UV Transilluminator. The DNA band was checked with the 1 kb DNA ladder. The viewed band with the expected size was excised from the agarose gels and DNA gel purification was

performed using the Wizard® PCR Preps DNA Purification System protocol (Promega, USA) according to manufacturer instructions.

The PCR product was sent to First Base Laboratory, Selangor, Malaysia for sequencing. The closely related bacterial 16S rRNA sequences were subjected to Basic Local Alignment Search Tool (BLAST) analysis and the sequences were identified by referring to the nucleotide database from NCBI GenBank database. The phylogenetic analyses were done accordingly[19]. The ClustalW algorithm was used to align and compare the sequences. The evolutionary distances were computed based on the neighbor joining (Unrooted Tree) by NCBI BLAST Tree Method.

2.7 Experiment design and statistical analysis

The experiments were arranged in a completely randomized design with 3 replicates except for the field experiment with corn that used RCBD. Data were analyzed using Analysis of Variance from SPSS version 21. Multiple comparisons were done using Tukey's multiple comparison.

3 Results

3.1 Enumeration, isolation, characterization and selection of BRIS soil PGPR

Enumeration results for dual function (N_2-fixation and P-solubilization) rhizobacteria found that there were a large number of bacteria colonies existing in the rhizosphere of the *A. mangium* at BRIS soil area (Table 1). The results showed that the CFU number for anaerobic bacteria is higher than aerobic bacteria in Point 1 (young BRIS coastal forest) and Point 2 (agricultural land). Meanwhile, Point 3 (housing land) showed the lowest and vice versa CFU count compared to Points 1 and 2. Nevertheless, the aerobic and anaerobic bacterial count at Point 1 was significantly different to Point 3. While the agricultural land (Point 2) showed not much different to the young BRIS coastal forest (Point 1). The young BRIS coastal forest (Point 1) showed the highest count for aerobic and anaerobic bacteria compared to Points 2 and 3. Thus, the rhizospheric soil of *Acacia* from Point 1 was selected as the isolation point to isolate potential superior PGPR from BRIS soil for further studies.

After repeated streaking on the nutrient agar and based on the difference in bacterial colonies' color and size, a total of 24 bacterial isolates were successfully isolated and purified. Upon screening and evaluation of further morphological characteristics and Gram-staining test, these 24 bacterial isolates showed almost the same results and identical characteristics of cell shape and size and Gram's reaction. Therefore, from the total of 24 bacterial isolates that were isolated, only 8 were selected as typical species and labeled as UA1 to UA6 for aerobic bacteria and UAA1 and UAA2 for facultative anaerobic bacteria.

The significant difference between the eight colonies is their size. There were four distinct sizes (pinpoint, small, moderate and large) of colonies and not much difference between the color and shape of the colonies was recorded (Table 2). Most of the colonies appeared in creamy white except for UAA2 which is in red. Meanwhile, the shape of the colonies was mostly rod except for UA1 and UA2 which were round. Biochemical characterization of the bacterial isolates showed positive reactions to catalase, nitrate reduction, urease, ammonia production and indole test except for UA3 and UAA1 which showed negative results in the ammonia test. The facultative anaerobic UAA1 and UAA2 also showed a negative reaction to indole and urease tests.

All 8 bacterial isolates are positive to have the PGP characteristics (N_2-fixation, P- and K-solubilization, and siderophores production) (Table 2). All isolates showed the growth of bacterial colonies with the same scores on Burks agar medium meaning that they are positive in N_2-fixation. They

Table 1 Bacterial count from three different rhizospheric soil of *Acacia mangium* at BRIS soil

Location	Coordinate	Colony count ($CFU \cdot g^{-1}$)	
		Aerobic	Anaerobic
Point 1	5°45′26.4″ N 102°37′21.3″ E	$1.06 \times 10^6 \pm 0.88^c$	$1.16 \times 10^6 \pm 3.18^c$
Point 2	5°45′43.7″ N 102°37′29.7″ E	$9.67 \times 10^5 \pm 0.58^{bc}$	$1.01 \times 10^6 \pm 0.88^{bc}$
Point 3	5°45′32.1″ N 102°8′18.4″ E	$8.00 \times 10^5 \pm 1.00^{ab}$	$7.00 \times 10^5 \pm 0.58^a$

Note: Means with the same letters are not significantly different at $p < 0.05$ Tukey's multiple comparison, $n = 3$.

Table 2 Morphological and biochemical characterization of selected eight isolates of BRIS soil rhizobacteria

Isolates	Gram reaction	Size	Shape	Color	Catalase	Nitrate reduction	Indole	Urease	Ammonia	Nitrogen fixation	Phosphate solubilization	Potassium solubilization	Siderophores production
UA1	–	Pinpoint	Round	Creamy white	+	+	+	+	+	+	+++	+++	++
UA2	–	Pinpoint	Round	Creamy white	+	+	+	+	+	+	++	++	++
UA3	+	Moderate	Rod	Creamy white	+	+	+	+	–	+	+	+	++
UA4	+	Large	Rod	Creamy white	+	+	+	+	+	+	+	+	++
UA5	+	Large	Rod	Creamy white	+	+	+	+	+	+	++	++	+++
UA6	+	Large	Rod	Creamy white	+	+	+	+	+	+	++	++	+++
UAA1	–	Small	Rod	Red	+	+	–	–	–	+	+	++	+++
UAA2	–	Small	Rod	Creamy white	+	+	–	–	+	+	++	+++	+++

Note: "+" indicates a positive reaction and "–" indicates a negative reaction; +, least; ++, moderate; +++, strong.

were also capable with different scores to solubilize P and K and produce siderophores. The strains that showed larger halo zones were considered to have higher PGP characteristics in solubilizing P and K and producing siderophores. Based on the production of the halo zone, UA1 showed the highest potential of P- and K-solubilization and siderophores production compared to other aerobic isolates. Among all isolates, UA3 and UA4 showed the weakest ability in P- and K-solubilization. Meanwhile, UAA2 showed a higher ability to solubilize P and K compared to other facultative anaerobic bacteria (UAA1)

Meanwhile, the study of selected bacterial isolate's ability to grow in a 5% molasses medium supplemented with 2% KNO_3 showed positive results. All bacterial strains were able to grow in a 5% molasses medium and withstand 2% KNO_3 concentration (Fig. 1). After 5 days of incubation, the highest bacterial count was recorded by UA6 with lg10.81 $CFU \cdot mL^{-1}$, while UAA1 showed the lowest count with lg9.73 $CFU \cdot mL^{-1}$.

Based on the results of bacterial morphological, biochemical and potential PGP characteristics (Table 2), and their growth performance in molasses medium (Fig. 1), it was concluded that UA1 looks almost similar to UA2 while UA3, UA4, UA5, and UA6 were also similar to each other. Therefore, three bacterial strains namely UA1, UA6, and UAA2 were selected as superior typical BRIS soil PGPR (Fig. S1). The PGP traits for the ability to fix N_2, solubilize P and K, and produce siderophores and IAA were quantitatively estimated and the isolates were further identified at the molecular level. The effects of the selected BRIS soil bacterial isolates on crop phenology, growth, and yield on corn were also evaluated.

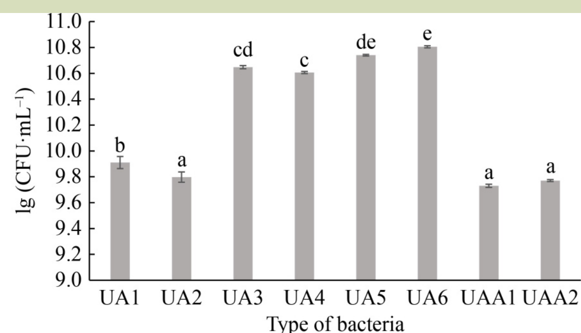

Fig. 1 Growth of bacterial isolates in 5% molasses medium supplemented with 2% KNO_3 at 5 days after incubation. Mean with the same letters are not significantly different at $p < 0.05$ Tukey's multiple comparisons, $n = 3$. Bar indicates the standard error of the treatment's mean.

3.2 Estimation of PGPR Traits

The three types of pure isolated PGPR produced a considerable amount of ethylene after 1 h of incubation which ranged between 17.0 and 18.6 $nmol \cdot mL^{-1} \cdot h^{-1}$ C_2H_4 (Table 3). UA1 and UAA2 resulted almost the same performance in BNF while UA6 resulted the highest BNF value (18.6 $nmol \cdot mL^{-1} \cdot h^{-1}$ C_2H_4) among the three strains. The mixed strains of UA1, UA6, and UAA2 resulted the highest BNF value (21.6 $nmol \cdot mL^{-1} \cdot h^{-1}$ C_2H_4) but the result was not significant ($p < 0.05$) compared to the use of single bacteria inoculum (UA6).

The results from the phosphate solubilization test showed that

Table 3 Estimation of PGPR characteristics isolated from BRIS soil

PGPR traits	Unit	Control	UA1	UA6	UAA2	Mixed strain (UA1 + UA6 + UAA2)
BNF	nmol·mL^{-1}·h^{-1}	0.0±0.00^{a}	17.1±1.20^{b}	18.6±0.48^{bc}	17.0±0.65^{b}	21.6±0.70^{c}
P-solubilization	pH (day 6)	5.9±0.00^{e}	5.8±0.00^{c}	5.9±0.02^{d}	5.8±0.01^{c}	5.9±0.00^{e}
	pH (day 12)	6.0±0.00^{e}	5.4±0.01^{a}	5.6±0.01^{b}	5.0±0.01	5.5±0.00^{b}
	µg·mL^{-1} (day 6)	0.0±0.00^{a}	45.8±2.08^{cd}	29.1±2.65^{b}	43.4±2.18^{abc}	41.1±4.54^{abc}
	µg·mL^{-1} (day 12)	0.0±0.00^{a}	54.4±4.23^{d}	32.0±2.38^{bc}	49.1±2.97^{d}	43.9±3.72^{cd}
K-solubilization	mg·L^{-1}	0.0±0.00^{a}	17.5±0.49^{d}	11.6±0.52^{b}	12.3±0.66^{b}	15.2±0.17^{c}
IRCs (IAA) production	µg·mL^{-1}	0.0±0.00^{a}	17.3±0.33^{e}	13.5±0.24^{c}	9.9±0.48^{b}	15.8±0.25^{d}
Siderophores production	%	0.0±0.00^{a}	14.7±2.32^{b}	31.7±2.32^{b}	20.3±1.34^{c}	43.0±2.66^{d}

Note: The results were expressed as the value of mean ± standard error. Means with different letters show a significant difference for each characteristic at $p < 0.05$ Tukey's multiple comparison, $n = 3$.

bacterial inoculation and prolonged incubation period had decreased the pH of PVK broth medium and increased the value of soluble P. While the pH of the uninoculated PVK broth medium remained constant at 6.0. Total soluble P was calculated from the standard curve of KH_2PO_4 (Fig. S2). The amount of soluble P was directly related to the drop in media pH. It was observed that higher P-solubilization caused a higher pH drop. UA1 reduced the media pH the most from 5.8 on day 6 to 5.4 on day 12 thus resulting in a significantly ($p < 0.05$) the highest soluble P in the media (54.4 µg·mL^{-1}) compared to other single or mixed strains (Table 3). Meanwhile, UA6 showed the lowest P-solubilization (32.0 µg·mL^{-1}) at day 12 with the pH drop from 5.9 to 5.6. UAA2 and mixed strains cultures also showed the same trend with the drop of pH and increment of soluble P from day 6 to day 12.

The amount of soluble K by the bacterial isolates activities was calculated from the standard curve of KCl (Fig. S3). The results showed that the amount of soluble K at 6 days of inoculation showed that strain UA1 produced significantly ($p < 0.05$) the highest concentration of soluble K (17.5 µg·mL^{-1}) in the media (Table 3). Mixed culture of UA1, UA6, and UAA2 showed the second highest soluble K (15.2 µg·mL^{-1}) followed by UAA2 (12.3 µg·mL^{-1}) and UA6 produced the lowest soluble K (11.6 µg·mL^{-1}) compared to others.

Meanwhile, the concentration of IRC pools containing IAA was estimated from the standard curve of pure IAA stock (Fig. S4). At 5 days of incubation, all the isolated PGPR strains were able to produce a significant different amount of IRCs ranging between 9.9 to 17.3 µg·mL^{-1} (Table 3). UA1 has once again shown the highest value (17.3 µg·mL^{-1}) of IRCs production compared to other bacterial strains. The second highest value of IRCs production was recorded by mixed culture (15.8 µg·mL^{-1}), followed by UA6 (13.5 µg·mL^{-1}) and the lowest was UAA2 (9.9 µg·mL^{-1}).

In the estimation test of siderophores production, mixed culture produced the highest siderophores unit (43.0%) in the Aleksandrov broth medium compared to the single strain culture (Table 3). In comparison to the three selected bacterial strains, UA6 produced the significantly ($p < 0.05$) highest siderophore unit (31.7%), followed by UAA2 (20.3%) UA1 showed the lowest siderophore unit (14.7%).

3.3 Effects of the isolated PGPR on the growth and yield of corn (*Zea mays* L.)

The results showed that inoculation with the selected BRIS soil PGPR does have a significant effect on corn phenology, growth, and yield (Table 4). Bacterial inoculation has significantly accelerated the days to emergence and tasseling from 11 to 8 and 57 to 50 days, respectively. It also elongates the plant life span as the maturity day was extended from 107 to 122 days. Meanwhile, bacterial inoculation does not have any effects on the plant height. However, the number of leaves significantly increased from 13 to 15 leaves per plant. Bacterial treatment also had a significant effect on the plant's yield as the cob length, grain yield, total biomass and harvest index were increased (Table 4). It was found that inoculation with mixed strains of UA1, UA6 and UAA2 had the most significant effects on corn. Among the three isolated PGPRs, the strain UA6 has shown the best effects on corn followed by UA1 and UAA6.

3.4 Molecular identification of BRIS soil PGPR

Molecular identification by partial sequencing of the 16S rRNA

Table 4 Mean values of corn phenology, growth and yield parameters as affected by different treatments of BRIS soil PGPR

Treatment	Emergence (day)	Tasseling (day)	Maturity (day)	Plant height (m)	Leaf no. of plant	Cob length (cm)	Grain yield ($t \cdot ha^{-1}$)	Total biomass ($t \cdot ha^{-1}$)	Harvest index
T_1	11.00^{b}	57.10^{d}	107.67^{a}	2.21^{a}	13.08^{a}	20.34^{a}	2.49^{a}	4.36^{a}	57.11^{a}
T_2	8.50^{a}	53.00^{bc}	115.08^{b}	2.20^{a}	14.33^{b}	22.18^{b}	2.97^{bc}	4.74^{ab}	62.64^{ab}
T_3	8.00^{a}	51.75^{ab}	121.50^{c}	2.28^{a}	14.83^{b}	23.69^{c}	3.09^{bc}	4.88^{b}	63.57^{b}
T_4	8.40^{a}	53.67^{c}	116.75^{b}	2.19^{a}	14.42^{b}	21.79^{b}	2.86^{b}	4.83^{ab}	59.25^{ab}
T_5	7.90^{a}	50.91^{a}	122.83^{c}	2.21^{a}	15.33^{b}	24.49^{d}	3.17^{c}	5.02^{b}	63.77^{b}

Note: T1 = control, T2 = UA1, T3 = UA6, T4 = UAA2, and T5 = mixed strain of UA1, UA6, and UAA2. Means within a column followed by the same letter(s) are not significantly different at $p < 0.05$ (Tukey's).

gene resulted in approximately 1500 bp 16S rRNA fragments were successfully amplified using the 27F and 1492R primers. BLAST comparison searches against the NCBI nucleotide database from the NCBI GenBank and the phylogenetic tree constructed showed the isolated bacterial similarity to known plant-associated bacteria (Table 5). The result showed that UA1 is similar to *Paraburkholderia unamae*, UA6 is similar to *Bacillus amyloliquefaciens*, and UAA2 is similar to *Enterobacter asburiae* (Fig. 2).

4 Discussion

The selected sampling locations represent the area of BRIS soil in Tembila, Besut Terengganu with different criteria. Point 1 is the young coastal forest that showed the highest beneficial bacterial count that can both solubilize phosphate and fix N_2, compared to Point 2 (agricultural land) and Point 3 (housing land). This situation showed that the disturbance of nature and forests can alter soil properties and characteristics. It also caused the bacterial community to shift between natural and disturbed forest soils[20]. Moreover, high land use intensity results in a high-stress environment that could decrease bacterial composition due to competitive exclusion and dominancy of certain competitive species[21]. The soil type will also determine the total bacterial communities at a place. Bacterial counts in different soils ranged from 10^6 to 10^8 $CFU \cdot g^{-1}$ dry soil would be considered healthy soil[22]. The average BRIS soil total bacterial count in this study was low compared to other places with different types of soil such as peat soil and sandy loam soil. This could be related to the soil criteria which is unfertile with too sandy and high temperature and drainage.

Acacia is a plant that adapted to nutrient-poor soils is resistant to drought and grows abundantly in this BRIS soil area. Observation showed that the *Acacia* tree grows near each other and mostly form a cluster. This growth behavior of *Acacia* had managed to decrease temperature and increased humidity at its clump which might be favorable for bacterial growth compared to other places. Interaction between plant species, rhizosphere localization and soil type could influence microbial populations and their action[23]. The size and structure of microbial populations were also affected by plant species[24]. *Acacia* is a leguminous plant that is capable of forming symbiosis interactions with N_2-fixing bacteria or *Rhizobium* species. Besides, the PGPR that is associated with plant rhizosphere could also attach and colonize the *Acacia* roots. These associations are very useful to plants as the bacteria will carry out the process of N_2-fixation or facilitate the uptake of certain nutrients from the soil. At the same time, the plant-particular compounds could be synthesized and the plant diseases could be prevented or suppressed.

Colony morphology and biochemical tests were performed to characterize, categorize, and identify the bacteria. Catalase is the enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. It is very important to protect

Table 5 Molecular bacterial identification using 16S rRNA gene sequences

Isolates	16S rRNA fragment length (bp)	Closest relatives in NCBI	NCBI accession number	Similarity (%)
UA 1	1337	*Paraburkholderia unamae* strain CATux-40	HQ023248.1	99
UA 6	1351	*Bacillus amyloliquefaciens* strain RD7-7	CP016913.1	100
UAA 2	1406	*Enterobacter asburiae* strain ENIPBJ-CG1	CP014993.1	99

Fig. 2 Phylogenetic tree of (a) UA1, (b) UA6, and (c) UAA2 based on the 16S rRNA sequences of the selected strains and related bacteria.

the cell from the harmful byproduct of many normal metabolic processes. Nitrate reductase is the enzyme that reduces nitrate (NO_3^-) to nitrite (NO_2^-). The NO_2^- will be then degraded to various nitrogen products such as nitrogen oxide, nitrous oxide and ammonia which can be absorbed by plants. This reaction is crucial for the production of protein in most crop plants, as nitrate is the predominant source of nitrogen in fertilized soils[23]. Urease hydrolyzes urea to carbon dioxide and ammonia which is an important source of nitrogen for plants. This enzyme is important as the soil urease will hydrolyse urea from fertilizer to ammonia and carbon dioxide to be assimilated by plants. The indole test is to determine the ability of the organism to convert tryptophan by tryptophanase into indole. Indole influences root and plant growth and acts as a plant defense control system against insects, nematodes and herbivore attacks[25]. The selected aerobic bacteria in this study (UA1 and UA6) showed positive results for all the tested biochemical characterization while the facultative anaerobic bacteria (UAA2) also showed the same results except for the indole and urease test.

N and P are two important essential nutrients for all stages of plant growth and development. The key to maximum crop growth and yield is by providing the crops with a sufficient amount of these elements. It is crucial in biofertilizer formulation to use the bacteria inoculant that has both abilities to fix N_2 and solubilize P. It is an advantage if the bacteria inoculant has other PGP characteristics such as the ability to solubilize K, and produce plant hormone, enzyme or siderophore. K solubilizing bacteria (KSB) may enhance the soluble K availability in the soil, thereby increasing plant uptake and potentially increasing plant growth and yield. Meanwhile, IRCs in particular the IAA is another fundamental plant growth substance that belongs to a class of phytohormones known as auxins. While siderophore is the ferric ion chelating agent produced by bacteria may enhance soil fertility and plant growth and act as a biocontrol agent against bacterial and fungal pathogens on the plant's root. Most plants are able to use bacterial iron siderophore complexes as a source of iron from the soil with competitive root colonization by phytopathogens[26].

Estimation of the PGP characteristics of the isolated BRIS soil PGPR either in single or mixed form shows considerable significant results. It is often said that a mixed culture is more effective than a single culture because of its diverse function

and synergistic effects. However, it was strongly dependent on the inoculant strain formulations and parameters evaluated[27]. Competition for nutrients and growth space between bacterial strains could be the biggest limitation in mixed culture[28]. This makes the more dominant bacterial strain dominate the culture and suppress the weak one. That explained how some of the PGP characteristics showed by single strain culture are higher than mixed strain culture in this study. Nevertheless, the difference is not significant and some of the mixed culture results are still higher than the use of certain single strains.

The amount of N_2 fixed by bacteria and transferred to their host plant varies greatly between species. ARA is among the established quantitative methods used worldwide for BNF. ARA is considered positive if the activity is more than 6 nmol·CFU^{-1}·h^{-1} C_2H_4[27] thus indicating the significance of BNF rate by the selected BRIS soil bacterial strains in this study. The ARA value obtained was in agreement with the nitrogenase activity of diazotrophs from the rhizosphere of pine (*Pinus sylvestris* L.) and oak (*Quercus robur* L) which were within the range from 4 to 20 nmol·CFU^{-1}·h^{-1} C_2H_4[28]. However, the ethylene produced by the selected bacteria was slightly lower compared to the indigenous species such as UPMB 10, UPMB 12, and UPMB 14 obtained from the rhizosphere of oil palm at Selangor, Malaysia which ranged from 18.66 to 19.90 nmol·CFU^{-1}·h^{-1} C_2H_4[29]. Nevertheless, the ARA values were higher compared to the values recorded by the diazotroph isolated from rice in Tanjong Karang, Malaysia only produced 6.1×10^{-8} to 1.2×10^{-3} nmol·CFU^{-1}·h^{-1} C_2H_4[30].

The phosphate solubilization index is generally reliable for the preliminary characterization of phosphate-solubilizing microorganisms. The vanadomolybdophosphoric acid method is used to get a more accurate result in quantification of phosphate solubilization rate by the bacterial isolates[13]. The amount of soluble P in this study was found to be negatively correlated to the pH of the PVK medium. It is because the pH decrease is due to the production of organic acid and acid phosphatase by the microbes as the by-products of P-solubilization in the medium[31]. Inoculation of PSB on seed or soil was known to improve solubilization of fixed soil P and applied phosphates resulting in higher crop yields[32]. Biofertilizer with PSB inoculant not only improves the crop's growth and yield but can also reduces the use of chemical fertilizers thus minimising the excessive input of chemical fertilizers to the environment[33]. Hence, the ability to solubilize the insoluble form of phosphate to an accessible form is another important PGPR criteria to be considered.

Plant and soil inoculation with KSB has been reported to have beneficial effects on the growth of various crops such as eggplant, rice, pepper, sorghum, and maize. A wide range of bacteria such as *Paenibacillus mucilaginosus*, *Bacillus megaterium*, *Bacillus circulans*, *Pseudomonas*, *Burkholderia*, and *Paenibacillus* have been reported to release K in accessible form from K-bearing minerals in soils[34–36]. The K-solubilization rates by the bacterial isolates in this study ranged from 11.55 to 17.45 μg·mL^{-1} which were lower compared to a few *Bacillus* species such as *Bacillus cereus*, *mycoides*, and *firmus* that was at 44.80–72.80 μg·mL^{-1} soluble K[37]. However, the rate of K-solubilization by the isolated bacteria in this study was higher than *Paenibacillus mucilaginous* ability to solubilize K at the rate of 4.29 μg·mL^{-1}[38].

The colorimetric detection using Salkowski reagent was commonly used for the estimation of IRCs production. IAA is the indole-based compound and the most common auxin found in nature. It is an important compound in the plant life cycle as it promotes the production of other hormones and control the growth of stems, roots, and fruits. Over 80% of rhizosphere bacteria may be capable of synthesizing IAA[39]. IAA production is believed to be one of the bacterial colonization strategies on plants other than phytostimulation of the basal plant defense mechanisms[40]. It was reported by many researchers that IAA production by PGPR has significantly promoted growth and rooting in many crops such as rice, spring wheat, maize, kiwifruit, and oil palm. The IRCs (in particular the IAA) production by the selected PGPR isolates in this study is in agreement with the bacterial strains of *Bacillus* sp., *Burkholderia* sp., and *Enterobacter* sp. in the presence of L-tryptophan as the precursor[41]. IAA promotes the proliferation and elongation of root hair development thus increasing the root's surface area for greater water and nutrient absorption[42]. Optimum root growth increases root vigour thus protecting the plant, especially from pest and disease infections that originate from soil[42]. There are many factors that influent the production of IAA by bacterial isolates. Bacterial species and their growth stage, environmental conditions and medium conditions such as the availability of substrate could influence the production and concentration of IAA[36].

Inoculation to the root rhizosphere needs the introduced PGPR to compete with other soil microorganisms. Thus, the ability to produce siderophore gives an advantage for successful plant colonization by the introduced PGPR. Siderophore-producing PGPR has also been used as a biocontrol agent against several plant diseases. This technique has gained more attention as they are safer and at the same time provide iron nutrition to

the crops[43]. Siderophores enhance plant growth by increasing the availability of iron near the roots for plant uptake[44]. PGPR ability to produce siderophores will reduce the availability of iron to other soil microbes. This activity will inhibit the growth and survival capability of other microorganisms such as phytopathogenic soil microbes.

Plant requires only a trace amount of iron for optimal growth. Although Fe is one of the most abundant metals in the soil, its availability to plant roots is very limited depending on the soil redox potential and pH[45]. Under the situation of iron-limiting conditions, microorganisms will produce the small organic molecules of siderophores. This compound will enhance the iron uptake to the microorganisms and plants by chelating ferric ions and making them more available for absorption. Thus, siderophores play an important role in enhancing plant growth and iron competition also protecting the host plant from phyto-pathogens.

All the isolated bacterial strains in this study either in single or mixed form have shown a considerable amount of siderophores production. Most of the siderophore-producing bacteria fall under the *Bacillus* sp.[46]. Nevertheless, the *Burkholderia* sp. including *Burkholderia unamae* also have the ability to produce siderophores[46]. The result of this present study is also in agreement with the higher production of siderophores unit by *Bacillu*s sp. compared to *Enterobacte*r sp. using glucose and fructose as carbon sources[13].

Complex nutrients are preferred in bacterial fermentation media as they often support higher yields[7]. Chemically defined media are widely used for laboratory needs but could be costly for commercial purposes. Of all the medium compositions, carbon and nitrogen sources are the most important components in the medium for bacterial growth. Molasses is among the major carbon and nitrogen sources of fermentation media other than soybean meal, corn liquor and yeast extract[7]. Cane molasses used as a carbon source contains 60% of sucrose in addition to growth-promoting components[47]. Cane molasses between 3.5% and 17.5% (w/v) total sugar were as good as sucrose and peptone in the enzyme and fructooligosaccharides production and has been successfully used in the formulation of media for the cultivation of *Aspergillus japonicas* FCL-119T and *Aspergillus niger* ATCC 20611[47].

Meanwhile, application of KNO_3 usually will increase the soil or fertilizer salinity which could affect the bacterial cell. Therefore, in the crop and fertilizer industry, it is an advantageous if the isolated bacteria can grow in an alternative organic medium and retain a high concentration of KNO_3 because the use of high KNO_3 in growth medium and fertilizer formulation will increase salinity which might affect plant growth and soil microbial functions. High salinity reduces microbial biomass because of the osmotic stress, resulting in the drying and lysis of cells. Even though plants and microbes could adapt to low osmotic potential by accumulating osmolytes, this process requires a large amount of energy, resulting in reduced growth and activity[48]. The isolated PGPRs in this study have shown the ability to grow in the molasses medium with KNO_3 meaning that these types of PGPRs might have potential in biofertilizer production.

Three selected strains (UA1, UA6, and UAA2) were identified as the strain and member of genera which were previously reported by many researchers to have all the plant growth characteristics that were discussed in this study and beneficial to various crops. The selected strains have shown a significant effect in increasing corn phenology, growth and yield. Bacterial inoculation has generated a greater increase in grain yield (27%) and total biomass (15%). This result is in agreement with the study of other research that used the *Bacillus* and *Enterobacter* strains in enhancing crop growth and yield[49]. The growth and yield enhancement might be related to the strains PGP characteristics. It might be also associated with the fact that the use the isolated PGPR which is the indigenous microflora might be better adapted and more effective than exogenous bacteria[50].

The strain UA1 has been identified as *Paraburkholderia unamae*, the important plant-associated N_2-fixation endophyte rhizobacteria. *Paraburkholderia* genera have been reported to exhibit PGP activities such as phosphate solubilization, IAA biosynthesis and other activities of biocontrol and bioremediation[46,51]. Meanwhile, UA6 has been identified as *Bacillus amyloliquefaciens* which is a common non-pathogenic soil bacterium. It has been reported to be used commercially as a biofertilizer and biocontrol agent in agriculture. This species triggers pathways of induced systemic resistance, which protects plants against attacks from pathogenic microbes, viruses, and nematodes[52]. It is also capable of producing endospores that make it resistant to certain unfavorable conditions allowing it to survive for extended periods of time[53]. These criteria make *Bacillus amyloliquefaciens* a great potential candidate as bioinoculant in biofertilizer production. Meanwhile, UAA2 was identified as *Enterobacter asburiae* which is usually reported as an opportunistic pathogen isolated from a variety of clinical and environmental specimens. However, this species was also reported to be isolated as an endophyte PGP bacteria in many plants and crops.

Enterobacter asburiae was reported to have the ability to solubilize various inorganic phosphate and ammonia, producing plant growth hormones such as IAA, plant polymer hydrolyzing enzymes (pectinase and cellulase) and has the potential for bioremediation of pesticide-contaminated agricultural fields[54–56].

5 Conclusions

A total of 24 pure bacterial strains that have abilities to fix N_2 and solubilize P were isolated from the rhizosphere of the *Acacia* tree in the young coastal forest at BRIS soil area. Only eight bacterial strains were selected for further studies based on their colony morphology and biochemical characteristics. Finally, three superior strains with different colony morphology and showed the best PGP characteristics were selected and identified as *Paraburkholderia unamae* (UA1), *Bacillus amyloliquefaciens* (UA6), and *Enterobacter asburiae* (UAA2)

Among the three isolates, UA1 produced the highest soluble P (54.4 $\mu g \cdot mL^{-1}$), soluble K (17.5 $mg \cdot L^{-1}$), and IAA (17.3 $\mu g \cdot mL^{-1}$). UA6 showed the highest BNF traits (18.6 $nmol \cdot mL^{-1} \cdot h^{-1}$) and siderophores production (31.7%). Meanwhile, UAA2 showed the weakest performance of PGP traits. However, the use of mixed strains showed a better performance in N_2 fixing characteristics (21.6 $nmol \cdot mL^{-1}$ h^{-1}) and siderophores production (43.0%). The three isolates also showed significant growth in 5% molasses medium supplemented with 2% KNO_3 with UA6 showing the highest growth (lg10.81 $CFU \cdot mL^{-1}$), followed by UA1 (lg9.91 $CFU \cdot mL^{-1}$) and UAA2 (lg9.77 $CFU \cdot mL^{-1}$). Nevertheless, the isolated PGPR have significantly enhance corn phenology, growth, and yield either in single or in mixed strains inoculations.

These types of PGPR have been previously reported by many studies, isolated from various places and beneficial to various plants and crops. The PGPR from this study might be resistant and hardy since they were isolated from the problematic BRIS soil. The inoculation of single or multiple strains of PGPR which have multiple beneficial characteristics will increase crop growth and yield while minimizing the chemical fertilizer inputs. These kinds of microbes should be exploited either in single or mixed form as they might have the potential to be used as an inoculant in biofertilizer production and may be applied worldwide, especially in the sandy soil region.

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2024559 contains supplementary materials (Figs. S1–S4).

Acknowledgements

This research was supported by Ministry of Higher Education Malaysia for the Knowledge Transfer Program—KTP Community grant [KTP/Bil 003/16 (KTP-R5)] and Center for Research Excellence and Incubation Management of UniSZA for the Pre-Commercialization grant (UniSZA/16/DPP/RR217).

Compliance with ethics guidelines

Zakiah Mustapha, Radziah Othman, Nik Nurnaeimah Nik Muhammad Nasir, Dhiya Dalila Zawawi, Mohd Khairi Che Lah, and Hafizan Juahir declare that they have no conflict of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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