HAYATI
Journal of Biosciences

Vol. 32 No. 1, January 2025 212-222
DOI:10.4308/hjb.32.1.212-222
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

Diversity of Rhizospheric Bacterial Community from Kaolin Mining Site and Their Potential as Plant Growth Promoting Bacteria

Khafifah Armanisa, Iman Rusmana*, Rika Indri Astuti

Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, IPB Dramaga Campus, Bogor 16680, Indonesia

ARTICLE INFO

Article history:
Received October 7, 2024
Received in revised form November 11, 2024
Accepted November 18, 2024

KEYWORDS:
Metagenome,
NGS,
PGPR,
Microbe-plant interaction,
Reclamation

ABSTRACT

Tailing from mining activities affects soil fertility resulting in poor soil conditions that are challenging for plants to grow. Plants can interact with rhizosphere bacteria to enhance their growth in harsh environments. Rhizospheric bacteria possess numerous mechanisms that promote plant growth and induced resistance to various abiotic stress. This study aims to determine the diversity of rhizobacteria and their potential as plant growth-promoting rhizobacteria (PGPR) agents. Bacterial communities from rhizosphere soil samples from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia were analyzed using Next Generation Sequencing based on the V3-V4 region of the 16S rRNA gene, while culturable bacteria were isolated from samples and screened for PGP activity. The results showed that the rhizosphere bacterial community was mostly dominated by *Pseudomonadota, Acidobacteria*, and *Verrumicrobiota*. There were 15 bacteria isolated from the sample and RKB-5 bacterial isolate had the potential to be PGP agent. The RKB-5 bacterial isolate was identified as Burkholderia cenocepacia based on its 16S rRNA sequence. The bacterial isolate produced IAA, utilized ACC, dissolved phosphate up to 209,5 mg/L, and formed a high potassium solubilizer index value of 5.00. Therefore, the *B. cenocepacia* RKB-5 has potential application as the PGPR to support plants growth by obtaining nutrients in ex-mining lands with poor soil conditions.

1. Introduction

The tin and kaolin mining industry in Bangka Belitung, Indonesia plays an important social and economic role in the community. Mine production is considered large up to 376,687,532 tons. This mining started in 1711 and some of them are abandoned now, leaving unused land 45,675 Ha in Belitung (Sukarman and Gani 2017). Although this mining area positively affects the community economically, it also leaves behind pollutants that contaminate the surrounding environment.

Kaolin is one of the mining products in Belitung which commonly used in making cement and ceramic (Subari *et al.* 2021). Unfortunately, this mining product contains heavy metal such as Pb, Sn, and As which are recalcitrant (Pasi *et al.* 2020) and contaminate both the inside and outside of the mining area with tailings (Sukarman and Gani 2017), resulting in poor soil condition. The soil structure in ex-mining areas is characterized by sandy composition, low water retention capacity, as well as low organic matter content (Dariah *et al.* 2010). These features create an harsh environment that is challenging for many plant species to thrive in the area. In order to transform ex-mining land suitable for plant growth, microorganisms could potentially serve as effective agents. The microbial community associated with plant root is essential for the survival of the entire plant-microbe system in extreme environment. Native plants that grow in harsh environment may harbour

* Corresponding Author
E-mail Address: irusmana@apps.ipb.ac.id

microbe that capable mitigating the stress (Backer *et al.* 2018).

Plants produce exudates that induce bacterial colonization on their surrounding roots (Backer *et al.* 2018) known as the rhizosphere. Rhizospheric bacteria are essential for plant-soil ecosystem functions, including nutrient cycling, energy transfer, and metal resistance (Duan *et al.* 2021). Certain rhizospheric bacteria provide macronutrients, such as nitrogen, phosphate, potassium, and produce phytohormones that facilitate plant growth (Kong and Glick 2017). These bacteria are classified as plant growth-promoting rhizobacteria (PGPR). Some PGPR can increase plant biomass production (Duan *et al.* 2021) and improve water and nutrient uptake (Backer *et al.* 2018). Consequently, PGPR may support plants in growing and obtaining nutrients in mine land with poor soil conditions, which can later be used to reclaim ex-mining area.

However, the bacterial diversity and community structure of plant rhizosphere originating in kaolin mining areas in Belitung Regency, Indonesia remain undocumented. Therefore, metagenomic analysis based on 16S rRNA using the Next Generation Sequencing-Illumina platform is conducted to determine the diversity of bacterial communities from the rhizosphere of plants from kaolin mining areas and determine their potential as plant growth-promoting agents.

2. Materials and Methods

2.1. Site Description and Sample Collection

The Kaolin mine is located in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia. The area of Belitung Island is 2.293,61 km^2 with a height below 50 m above sea level. The Belitung region has a tropical climate with rainfall of 402.5 mm, air temperature ranging from 22.0-33.0°C with an average of 26.3°C, and air humidity of 89%. Samples were taken around the Kaolin Lake area which is dominated by several types of plants which are nephentes (sample 1), grass (sample 2), moss (sample 3), grass (sample 4), and fern (sample 5). Samples were collected from five points at a depth of 5-15 cm (Figure 1). The samples were then brought to the laboratory and stored at 4°C.

2.2. Soil Physicochemical Characterization

Soil samples were subjected for physicochemical analysis including pH measurement (potentiometric titration), C-organic content (spectrophotometry), N-total (Kjeldahl method), P_2O_5 potential (spectrophotometry), K_2O potential (Atomic Absorption Spectrophotometer), Cation exchange capacity (titrimetry). The analysis was done at The ICBB (PT. Biodiversitas Bioteknologi Indonesia).

Figure 1. Location of rhizosphere sampling in the kaolin mining area in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia. The five sampling sites are marked with blue points

2.3. DNA Extraction from Rhizosphere Soil for Metagenomic Analysis

Rhizosphere soil samples were weighed up to 0.5 g and DNA extraction was carried out following the Powersoil Mobio kit protocol. The extracted DNA was analyzed for its quality using MaestroNano Pro Spectrophotometer (Maestrogen) and 1% agarose gel electrophoresis. The extracted genome was then amplified using specific primers (forward primer 5'TCGTCGGCAGCGCAGAT GTGTATAAGAGACAGCCTACGGGNGGCWGC AG3') and reverse primer 5'GTCTCGTG GGTCGGAGATGTTATAAGAGACAGGACTACHVG GGT ATCTAATCC3') on the variable region V3 and V4 (~460 bp) of the 16S rRNA gene. Library preparation was performed using the Nextera XT DNA Library Preparation Kit and followed by sequencing using the Miseq Illumina platform by using the service from PT Indolab Utama.

2.4. Isolation and Characterization of Rhizospheric Bacteria

The rhizosphere soil sample was weighed as much as 10 grams and mixed with 90 ml of 0.9% w/v NaCl. The suspension was then homogenized with an orbital shaker for 90 minutes at 300 rpm, then centrifuged for 10 minutes at 150 g to concentrate the soil particles in the pellet (Barillot *et al.* 2013). Furthermore, the supernatant was serially diluted and 10^{-3}, 10^{-4}, and 10^{-5} dilutions were taken up to 100 µL and inoculated on NA (Nutrient Agar) media and incubated for 48 hours at room temperature. The isolates that grew were then purified and characterized based on colony morphology and Gram staining.

2.5. Pathogenicity Assay of Rhizospheric Bacterial Isolates

The pathogenicity of bacterial isolates towards animal erythrocytes was evaluated through haemolysis test by culturing them on blood agar for 48 h at room temperature. Positive results were indicated by clear zone formation around the colony. Whereas, to determine the pathogenicity of bacteria towards plants, hypersensitivity test was performed by culturing bacterial isolates in TSB (Tryptone Soya Broth) medium (tryptone 17 g/L, soya peptone 3 g/L, NaCl 5 g/L, dextrose 2.5 g/L, K_2HP_o4 2.5 g/L, pH 7.3) and incubated for 24-48 h. A total of 1 ml of the culture was injected into the abaxial part of the tobacco leaf using a sterile syringe. Positive results were indicated by necrosis on the leaf area.

2.6. Screening for Isolates with PGPR Potential

2.6.1. Indole Acetic Acid (IAA) Production

Bacterial isolates were cultured in TSB (Tryptone Soya Broth) media (tryptone 17 g/L, soya peptone 3 g/L, NaCl 5 g/L, dextrose 2.5 g/L, K_2HPO_4 2.5 g/L, pH 7.3) added with 1% tryptophan 0.5 mM and incubated for 48 h. The culture was then centrifuged at 10,000 g for 10 minutes (Rana *et al.* 2011), and the supernatant was rejected with Salkowski reagent ($FeCl_3$•$6H_2O$ 0.05% 7.5 ml, H_2SO_4 150 ml, aquadest 250 ml) in a ratio 1:4 (Wahyudi *et al.* 2019). The solution was then incubated for 30 minutes under dark conditions, followed by the absorbance measurement at 530 nm. The standard curve was made using IAA at concentrations 0, 20, 40, 60, 80, dan 100 mg/L.

2.6.2. ACC Utilization

The ability of bacteria to utilize ACC was tested qualitatively. Bacteria isolates were first inoculated on DF (Dworkin Foster) media (KH_2PO_4 4 g, Na_2HPO_4 6 g, $MgSO_4$•$7H_2O$ 0.2 g, $FeSO_4$•$7H_2O$ 1 mg, 10 µg MoO_3, glucose 2 g, gluconic acid 2 g, citric acid 2 g, agar 12 g and 1,000 ml aquadest; filter sterilized 0.3033 g ACC/ liter and 2 g ammonium sulphate/liter) and incubated for 72 h (Husen 2012).

2.6.3. Phosphate Solubilization

Bacterial isolates were inoculated on Pikovskaya agar medium (Glucose 10 g/L, $Ca_3(PO)_4$ 5 g/L, $(NH_4)_2SO_4$ 0.5 g/L, NaCl 0.2 g/L, $MgSO_4$•$7H_2O$ 0.1 g/L, KCl 0.2 g/L, yeast extract 0.5 g/L, $MnSO_4$•H_2O 0.0025 g/L, $FeSO_4$•H_2O 0.0025 g/L, Agar 20 g/L, pH 7.0) and incubated for 72 h. The clear zone formed was measured and the phosphate solubilization index was calculated using the following (Mursyida *et al.* 2015):

$$\text{Phosphate solubilization index} = \frac{\text{Clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

Isolates that were able to form the clear zone were subjected to quantitative tests. Bacterial isolates were cultured 1% on liquid Pikovskaya media and incubated for 72 h. The culture was centrifuged at 10,000 g for 5 minutes. The 600 µL of supernatant were taken and reacted with 1.4 ml of reagent (10% ascorbic acid and 0.42% ammonium molybdate in 1 N H_2SO_4), and heated at 45°C for 20 minutes. The phosphate solubilization activity was measured using a spectrophotometer at 827 nm. The standard curve was prepared using KH_2PO_4 at concentrations 0, 20, 40, 60, 80, dan 100 mg/L.

2.6.4. Potassium Solubilization

Isolate was inoculated on Aleksandrov agar medium ($MgSO_4$ 0.5 g/L, $CaCO_3$ 0.1 g/L, $AlKO_6Si_2$ 2 g/L, Glucose 5 g/L, $FeCl_3$ 0.005 g/L, $Ca_3(PO_4)_2$ 2 g/L, agar 20 g/L, pH 7.2) and incubated for 15 days at room temperature. The ability of the isolate to dissolve K is indicated by the clear zone formation. The potassium solubilization index was calculated using the following equation (Anwar *et al.* 2022):

$$\text{Phosphate solubilization index} = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

2.7. Molecular Identification of Bacterial Isolate Based on 16S rRNA Gene

Genomic DNA of potential bacteria was extracted using the Zymo Research Quick DNATM Fungal/ Bacterial Miniprep Kit, following the manufacturer's protocol. DNA quality was measured using MaestroNano Pro Spectrophotometer (Maestrogen). 16S rRNA gene was amplified using universal primers 63F (5'CAGGCCTAACACATGCAAGTC3') and 1387R (5'GGGCGGCGTGTACAAGGCC3') that targeted approximately 1300 bp of DNA fragment (Venkataramanamma *et al.* 2022). The PCR mix contained: 25 µL Gotaq Green Mastermix (Promega), 5 µL 63F primer, 5 µL 1387R primer, 2 µL DNA template, and diluted with 13 µL Nuclease Free Water. The PCR conditions were performed in 35 cycles with the following profile: pre-denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 30 second, annealing at 55°C for 45 second, elongation at 72°C for 1 minutes 45 second, post-elongation at 72°C for 10 minutes. The PCR product was visualized in 1% agarose gel and sent for sequencing to PT. Genetika Science. Both forward and reverse sequences were aligned using Seqtrace 0.9.0 and a consensus sequence was obtained. The isolates were identified using the BLAST-N program in the GeneBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using MEGA 11.0.13, by the Minimum Evolution method with 500x bootstrap replications.

3. Results

3.1. Soil Physicochemical Characteristics

The soil in kaolin mining is considered poor fertility and is acidic. The nitrogen, organic carbon, potassium, and phosphorous contents were low in all samples, except sample 5 which contain high phosphorous. The soil ability to retain nutrient was low as indicated by the low cation exchange capacity in all samples. Based on the measured parameters, sample 2 found to be the least fertile, on the other hand sample 3 found to be the most fertile compared to other samples (Table 1).

3.2. Rhizospheric Bacterial Community Based on Metagenomic Analysis

Alpha diversity of rhizosphere sample was estimated using Shannon, Gini-Simpon, and Chao 1. Chao 1 value is 628 and based on Shannon index and Gini-Simpson (Figure 2), the diversity of rhizosphere bacteria was considered high as well as diverse and even.

Based on the 97% similarity of the 16S rRNA gene sequences, the bacteria were grouped into 628 OTU (Operational Taxonomic Unit) which are classified into 16 phyla (Figure 3), 38 classes, 69 orders, 109 families, and 178 genera. The dominant phylum was Pseudomonadota, also known as Proteobacteria, with an abundance 28.17%, followed by Acidobacteria 19.77%, Verrumicrobiota at 17.13%, Vulcanimicrobiota 8.34%, Actinomycetota at 4.54%, and Bacteroidetes at 4.09%. Among the 16 phyla, Nitrosphera belonged to the Archaea group with an abundance of 0.01%. Additionally, at the genus level, *Chthoniobacter* had the largest population size, followed by *Vulcanimicrobium, Pseudaciobacterium,* and *Acidibacter* (Figure 4).

Table 1. Physicochemical characteristics of soils from kaolin mining site in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

Sample	pH	Parameter						
		Organic C		Total N		P_2O_5 potential	K_2O potential	Cation exchange capacity
		mg/100 g	%	mg/100 g	%	mg/100 g	mg/100 g	Cmol(+)/kg
1	3.9	1.67	0.002	0.12	0.0001	3.38	5.34	4.31
2	4.7	0.23	0.0002	0.03	0.00003	1.93	10.62	6.32
3	5.1	4.25	0.004	0.09	0.0001	3.94	13.91	8.40
4	5.7	1.52	0.001	0.10	0.0001	3.85	7.26	7.57
5	5.4	0.43	0.0004	0.04	0.00004	12.41	7.06	8.17

Figure 2. Alpha diversity analysis of rhizospheric bacterial community in the rhizosphere soil samples of kaolin mine area in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

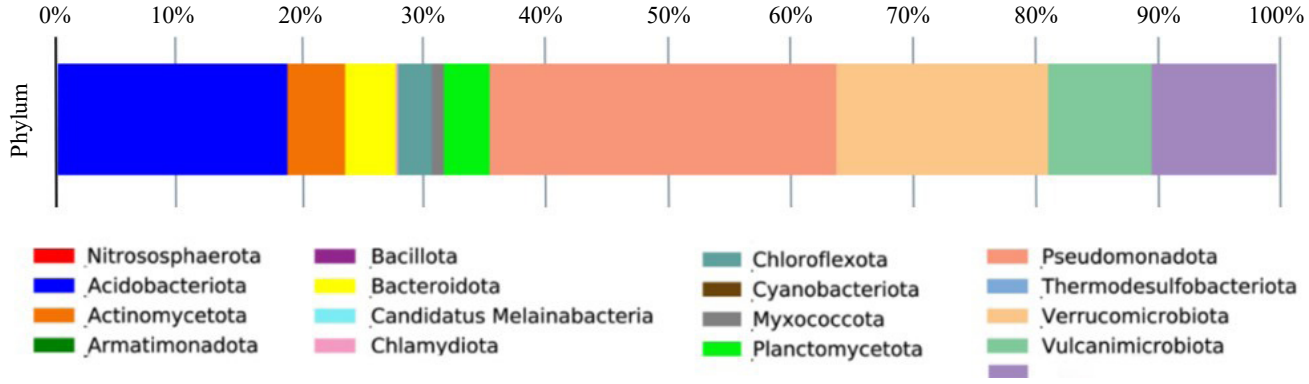

Figure 3. Relative abundance of dominant bacterial community phyla found in the rhizosphere soil samples of kaolin mine area in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

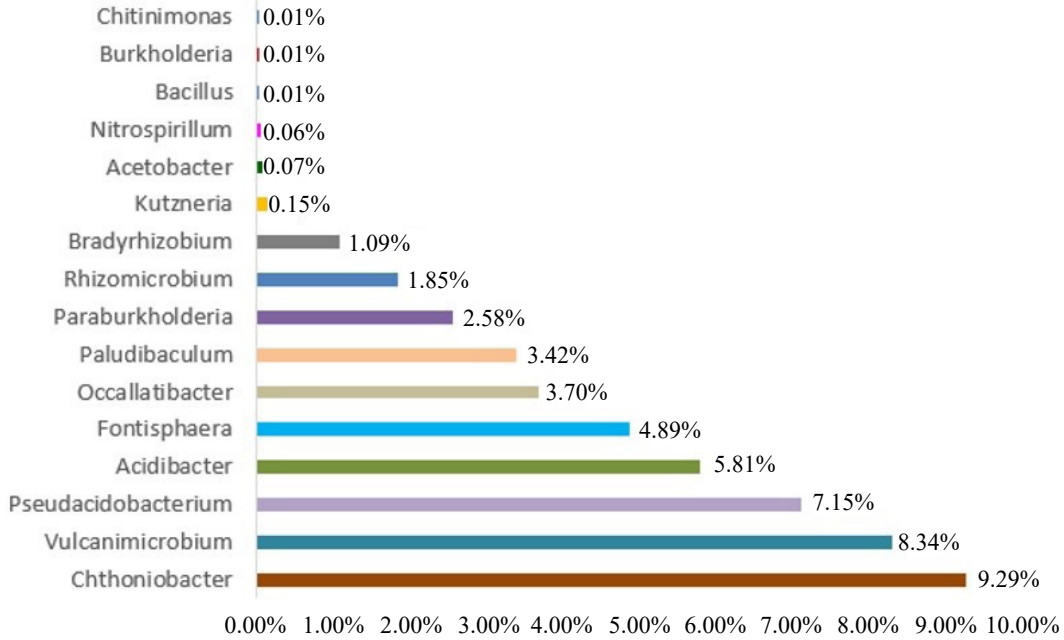

Figure 4. Relative abundance of several rhizospheric bacterial community at the genus level found in the rhizosphere soil samples of kaolin mine area in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

3.3. Culturable Rhizospheric Bacteria

The highest population of bacterial cells was found in rhizosphere sample taken from location 3 (rhizosphere 3), while the lowest population was found in rhizosphere sample taken from location 1(rhizosphere 1) (Table 2). There were 15 bacteria with different characteristics were isolated from the five samples. Based on cell morphologies and Gram staining, 93% of the isolated were bacilli and 66.7% were Gram-positive. All bacterial isolates were selected to avoid potential pathogenicity in animals and plants. Based on the selection results of the pathogenicity assay, only six isolates showed negative results in both haemolysis and hypersensitivity test: RKB-1, RKB-5, RKB-10, RKB-13, RKB-19, and RKB22 bacterial isolates. These six bacterial isolates were screened for PGPR activity.

3.4. PGPR Activity of Rhizospheric Bacteria

3.4.1. IAA Production

Based on the results of the IAA production test, the six isolates were able to produce different amounts of IAA after incubation for 48 hours in the range of 1.7-7.89 mg/L. The highest IAA was produced by RKB-5 bacterial isolate (7.89 mg/L) (Figure 5).

Table 2. The number of culturable bacteria in the rhizosphere soil samples of kaolin mine area in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia. The culture used Nutrient Agar (NA) medium incubated for 48 hours at a room temperature (28-30°C)

Soil sample code	Number of culturable bacteria (CFU/g)	Number of isolated bacteria
Nephentes's Rhizosphere 1	2.9×10^3	2
Grass's Rhizosphere 2	2.0×10^4	5
Moss's Rhizosphere 3	4.5×10^5	4
Grass's Rhizosphere 4	4.1×10^3	2
Fern's Rhizosphere 5	4.7×10^4	2
Total		15

3.4.2. ACC Utilization

Qualitative results showed that out of six isolates, only four isolates (RKB-19, RKB-13, RKB-1, and RKB-5 bacterial isolates) were able to grow on DF medium + ACC (aminocyclopropane-1-carboxylic acid), indicating their ability to utilize ACC as a nitrogen source (Figure 6). The RKB-5 bacterial isolate grew best and formed thick colony on DF medium supplemented with ammonium sulphate (Figure 6C). This result suggested that despite its ability to utilize ACC, the bacteria could also use another nitrogen source, such as ammonium sulphate, which has a simpler molecule than that of ACC.

3.4.3. Phosphate and Potassium Solubilization Activity

In the phosphate solubilization assay, only 2 bacteria isolates had the P-solubilization activity. RKB-1 and RKB-5 bacterial isolates were able to dissolve tricalcium phosphate in Pikovskaya agar medium, creating a clear zone around the colony within 72 h incubation (Figure 7). The qualitative assay showed that RKB-5 isolate has a higher solubility index than that of RKB-1 isolate (Table 3). However, the different result showed in the quantitative assay, it was found that RKB1isolate solubilizes twice tricalcium phosphate in Pikovskaya liquid medium up to 401 mg/L (Table 3). This shows that a larger clear zone does not always indicate a higher solubilization activity.

Out of six bacterial isolates, RKB-5 bacterial isolates is the only one that was able to form a clear

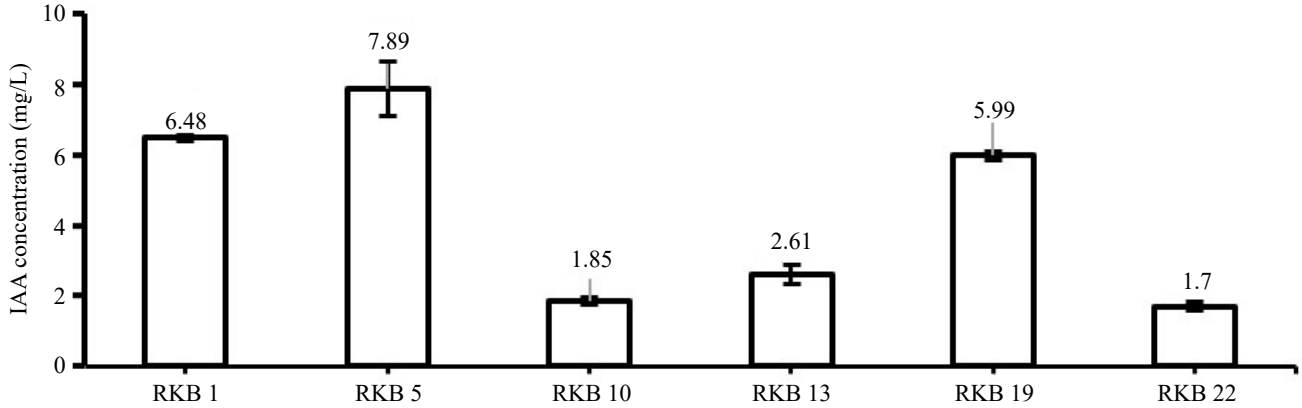

Figure 5. Production of IAA by the rhizospheric bacterial isolates from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia. The IAA concentration was measured from 48-hours bacterial cultures in TSB medium supplemented with 1% 0.5 mM tryptophan. Three replicates assay was conducted

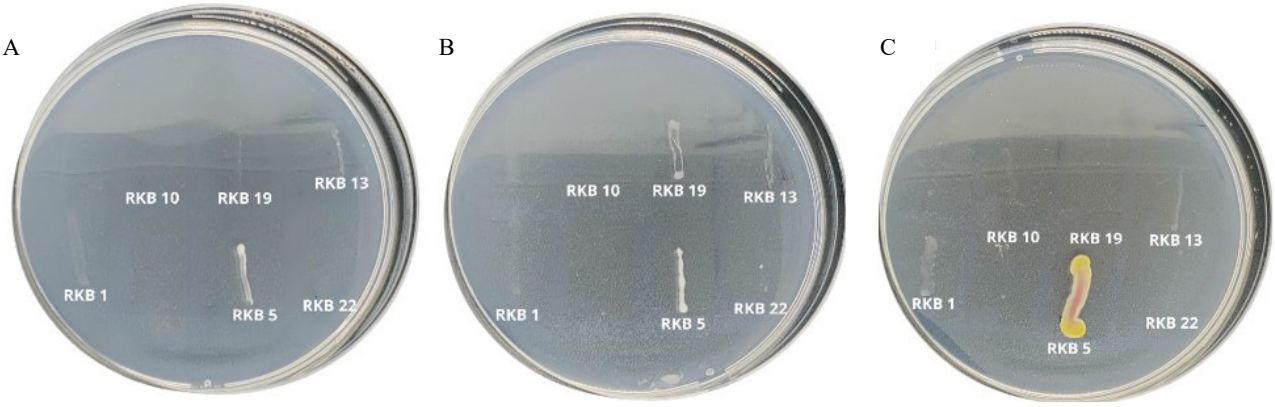

Figure 6. The colony growth of the six culturable rhizospheric bacterial isolates from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia in DF medium up to 48 h of incubation to examine ACC utilization. (A) DF medium, (B) DF medium + ACC, (C) DF medium + $(NH_4)_2SO_4$

Figure 7. Clear zone around the bacterial colony that solubilize tricalcium phosphate in Pikovskaya medium. The bacterial isolates were isolated from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

Table 3. Bacterial activity in solubilizing phosphate (qualitative and quantitative) and potassium of the bacterial isolates from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia

Bacterial Isolate Code	P solubilization index	P-solubilization in liquid media (mg/L)	K-solubilization index
RKB-1	0.20	401±58.32	-
RKB-5	0.22	209.5±8.60	5.23±1.86
RKB-10	-		-
RKB-13	-		-
RKB-19	-		-
RKB-22	-		-

zone on Aleksandrov media (Figure 8). This indicates that the isolate can dissolve K contained in the media in the form of potassium aluminium silicate. The K solubilizing index formed after 24 h of incubation and kept increasing until 15 days of observation, reaching 5.23 mm (Table 4).

3.5. Molecular Identification of Bacterial Isolate Based on 16S rRNA Gene

RKB-5 bacterial isolate was the only isolate that showed positive results in all PGPR activity assays. Based on 16S rRNA gene sequences, RKB-5 bacterial isolate is closely related to Burkholderia cenocepacia BKP SB54 with a similarity value of 99,76% (Table 4) and the phylogenetic tree results also showed that the two isolates were on the same branch (Figure 9).

4. Discussion

Mining activities leave more than 90% of the sand tailings (Putra *et al.* 2017), resulting in infertile soil conditions unsuitable for certain plant growth. Based on physicochemical characteristics, the soil in kaolin mines is found to be acidic. Soil pH is one of the factors that determines soil quality, as it affects nutrient availability, nutrient exchange, and soil microorganism activity (Neina

2019). Low soil pH can reduce microbial activity in soil and impede nutrient exchange in soil; in this case, low soil pH might be the reason for the low cation exchange capacity (CEC), which could impact nutrient exchange between plant and the surrounding soil matrix. This finding aligns with Sukarman and Gani (2017), who reported that the CEC value in ex-mining land was very low at 2.48-6.95 Cmol(+)/kg, and soils from mining areas are

Table 4. The 16S rRNA gene sequences analysis of RKB-5 bacterial isolate using BLAST-N program of NCBI

Accession number	Reference bacterial name	Base pair length	% Identity
MW383910.1	*Burkholderia cenocepacia* BKP SB54	1527	99.76
MW383912.1	*Burkholderia cenocepacia* BKP SB56	1527	99.76

Figure 8. Potassium solubilizing activity of culturable rhizospheric bacterial isolates from kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia. K-solubilization is confirmed by a clear zone around the bacterial colony

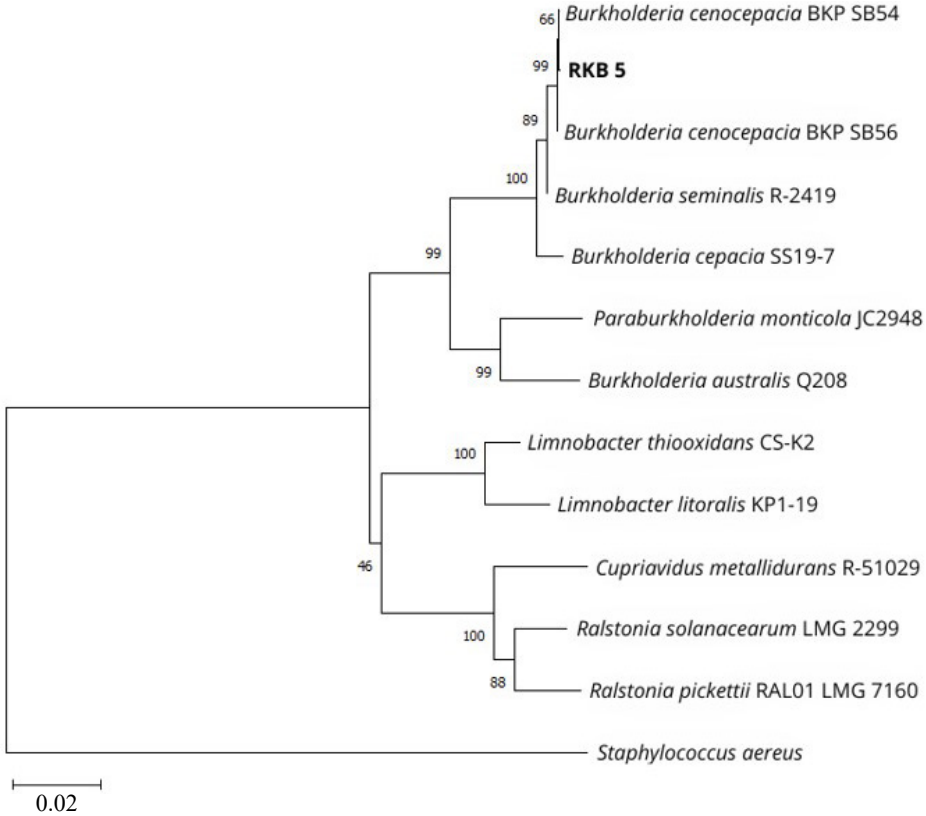

Figure 9. Phylogenetic tree based on 16S rRNA gene sequences of RKB 5 bacterial isolate. The tree was constructed using the minimum evolution method with a bootstrap value 500x. A scale 0.02 indicates the evolutionary distance in branch length

known to have low pH in the range of 4-5 (Nurtjahya *et al.* 2009; Sukarman *et al.* 2020). Furthermore, the results also indicate that the soil from the kaolin mines lacks essential nutrients. The organic carbon and total nitrogen contents are under 0.1%, which is considered extremely low. Similarly, the phosphorus and potassium contents are also considered to be low. This poor soil condition could affect biological properties, one of which is the microbial community.

The number of culturable rhizosphere bacteria from the five soil samples is still relatively low range from 10^3-10^5, according to McNear (2013) generally the number of bacteria in the rhizosphere is between 10^{10}-10^{12}. The low number of viable bacterial cells can be influenced by poor soil conditions, resulting in low nutrient availability and water retention (Sutono *et al.* 2019). On the other hand, it can also influence by its direct interaction with plants. Since the root produces exudate, bacteria colonize the rhizosphere making them a hotspot for nutrition. The presence of exudate may affect the viability of bacterial cells so that bacteria require specific nutrients that make them difficult to isolate. Therefore, a metagenome analysis approach is needed to determine the diversity of rhizosphere bacteria.

The bacterial diversity in rhizospheric area is considered high, wherein this region is directly influenced by root exudates that contain a myriad of nutrients bacteria, enabling them to establish microhabitats and form diverse communities. The phylum that makes up the composition of rhizosphere bacteria on kaolin mine generally plays a role in biogeochemical cycles, remediation, and promotes plant growth. The two most dominant phyla identified in this study (Pseudomonadota and Acidobacteria) are consistent with the finding of Gou *et al.* (2018) and Xie *et al.* (2022), which indicate that the diversity of rhizosphere bacterial communities in mine areas contaminated with heavy metal is dominated by Pseudomonadota, Acidobacteria, Actinobacteria, and Bacteroidetes. Pseudomonadota have been reported to have a high tolerance to extreme environments contaminated with heavy metals (Gou *et al.* 2018) and Acidobacteria are commonly found in environmental conditions that are low in nutrients, such as in contaminated soil, low organic carbon content, and acidic pH (Kielak *et al.* 2016).

Most genera found at genus level play role in nutrient cycling and as decomposer such as *Chthoniobacter*. About 9.55% bacterial genera commonly known as PGPR were also found, including *Burkholderia, Paraburkholderia* (Madhaiyan *et al.* 2021), and *Bradyrhizobium* (Zhong *et al.* 2024) as well as S*treptomyces, Bacillus, Paenibacillus, Acetobacter*, which known to play roles in nitrogen fixation, phosphate solubilization, and phytohormone production (Govindasamy *et al.* 2010; Vu *et al.* 2019; Nonthakaew *et al.* 2022).

Based on the results of metagenomic analysis, Chthoniobacter was identified as the predominant genus found in the rhizosphere; however, this genus has not been detected in laboratory-scale isolations. In contrast, the genus *Burkholderia* was detected, despite its low abundance of 0.01%. This discrepancy can be attributed to the fact that bacterial identification primarily focuses on isolates with potential as plant growth-promoting bacteria, particularly those exhibiting the ability to produce IAA, utilise ACC, solubilize phosphate, and solubilize potassium. Out of 25 isolates, only RKB-5 bacterial isolated from grass's rhizosphere (sample 2) demonstrated all these abilities and was subsequently identified as *Burkholderia*.

The RKB-5 bacterial isolate which is isolated from a grass rhizosphere is found to be able to produce IAA up to 7.89 mg/L. This result similar with others rhizobacteria from wheat plants can produce IAA of 12–46 µg/ml (Rana *et al.* 2011) as well as rhizobacteria *Agrobacterium* sp. and *Rhizobium* produce IAA 74.3 µg/ml and 90.21 µg/ml respectively (Lebrazi *et al.* 2020). Although the production of IAA by RKB-5 bacterial isolate was still relatively low compared to some studies. Wahyudi *et al.* (2011) reported that even low IAA production up to 3.25 mg/L, the bacterial isolates still can increase primary root growth in soybean plants. The bacteria ability to produce IAA might facilitate plant root elongation and development, thereby supporting water and nutrient uptake of plants.

The utilization of ACC (aminocyclopropane-1-carboxylic acid) by RKB-5 bacterial isolate possibly demonstrated its ability to produce ACC deaminase. ACC is known to be an ethylene precursor whose production is affected by environmental conditions such as stress (Chandwani & Amaresan 2022). In response to stress conditions, plants produce a myriad of ACC resulting high ethylene levels, which leads to plant growth and plant death (Gupta and Pandey 2019). Therefore, in the root system, rhizobacteria that able to utilize and degrade the ACC could enhance plant adaptability to unfavourable environments.

The ability of RKB-5 bacterial isolate to form clear zone in Pikovskaya medium supplemented with calcium phosphate indicates that the isolate has ability to solubilize phosphate as well as calcium. The RKB-5 bacterial isolate activity in solubilizing phosphate is considered high (up to 209.5 mg/L) compared to Actinomycetes ARJ which

dissolve phosphate in the range of 55.84-144.55 mg/L (Amri *et al.* 2022). One of bacterial mechanisms to dissolve phosphate is by secreting organic acids such as gluconic acid, citric acid, succinate, fumaric acid, and lactic acid. These organic acids convert tricalcium phosphate into di- or mono-phosphate (Awais *et al.* 2019). In this study, it was found that the pH of Pikovskaya media inoculated with RKB-5 bacterial isolate was decreased from 7 to 5, indicating that the rhizobacterium produces organic acids. The RKB-5 bacterial isolate also exhibits a high potassium solubilizing activity and capable of solubilizing potassium aluminium silicate on Aleksandrov media. As the availability of phosphate and potassium contents in kaolin mining land is generally low, the presence of this rhizosphere bacteria could help plants to absorb phosphate and potassium from the soil and utilize it to help plant growth and development.

RKB-5 bacterial isolate was identified as *Burkholderia cenocepacia*, this genus makes up 0.01% of rhizosphere bacterial communities in the kaolin mining soil. *B. cenocepacia* is commonly known as PGPR, some studies shows that *B. cenocepacia* SRD isolated from rice produces IAA, dissolves phosphate, and also potassium (Sherpa *et al.* 2021) and *B. cenocepacia* CR318 from corn plant roots has high activity in dissolving phosphate in the form of tricalcium phosphate (You *et al.* 2020). Therefore, *B. cenocepacia* RKB-5 isolated from rhizosphere soils of kaolin mining sites in Perawas, Tanjung Pandan district, Belitung Regency, Bangka Belitung Island, Indonesia has potential application as a PGPR agent for reclamation of the ex-mining areas due this bacterial can support plants growth by obtaining nutrients in a mine land with poor soil conditions.

Acknowledgements

This study was funded and supported by the Productive Innovative Research Program (RISPRO) of the Ministry of Finance, Indonesia with the theme of UK-Indonesia Consortium for Interdisciplinary Sciences (UKICIS) (Contract No: 8986/IT3.L1/PT.01.03/P/B/2022).

References

Amri, M.F., Husen, E., Tjahjoleksono, A., Wahyudi, A.T., 2022. Alkaline phosphate activity of plant growth-promoting Actinomycetes and their genetic diversity based on the phoD Gene. *Hayati J Biosci*. 29, 360-369. DOI:10.4308/hjb.29.3.360-369

Anwar, A.R., Ala, A., Kuswinanti, T., Syamun'un, E., 2022. The ability of potassium-solubilizing fungi isolated from leucite potassium rock deposits. *Biodiversitas*. 23, 6580-6585. DOI:10.13057/biodiv/d231257

Awais, M., Tariq, M., Ali, Q., Khan, A., Ali, A., Nasir, I.A., Husnain, T., 2019. Isolation, characterization and association among phosphate solubilizing bacteria from sugarcane rhizosphere. *Cytol Genet*. 53, 86-95. DOI:10.3103/S0095452719010031

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action, and roadmap to commercialization of biostimulan for sustainable agriculture. *Front Plant Sci*. 9, 1-17. DOI:10.3389/fpls.2018.01473

Barillot, C.D.C., Sarde, C.O., Bert, V., Tarnaud, E., Cochet, N., 2013. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria to a herbaceous root system. *Ann Microbiol.* 63, 471-476.

Chandwani, S., Amaresan, N., 2022. Role of ACC deaminase producing bacteria for abiotic stress management and sustainable agriculture production. *Environ Sci Pollut Res*. 29, 22843–22859. DOI:10.1007/s11356-022-18745-7

Dariah, A., Abdurachman, A., Subardja, D., 2010. Reklamasi lahan eks penambangan untuk perluasan areal pertanian. *J. Sumberd Lahan*. 4, 1-12.

Duan, R., Lin, Y., Zhang, J., Huang, M., Du, Y., Yang, L., Bai, J., Xiang, G., Wang, Z., Zhang, Y., 2021. Changes in diversity and composition of rhizosphere bacterial community during natural restoration stages in antimony mine. *PeerJ.* 9, :e12302. DOI:10.7717/peerj.12302

Govindasamy, V., Senthikumar, M., Magheshwaran, V., Kumar, U., Bose, P., Sharma, V., Annapura, K., 2010. *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture. In: Maheswari D. (Eds). *Plant Growth and Health Promoting Bacteria. Microbiology Monographs, Vol 18.* Berlin: Springer. DOI:10.1007/978-3-642-13612-2_15

Guo, D., Fan, Z., Lu, S., Ma, Y., Nie, X., Tong, F., Peng, X., 2018. Changes in rhizosphere bacterial communities during remediation of heavy metal-accumulating plants around the Xikuangshan mine in Southern China. *Sci Rep.* 9, 1-11. DOI: 10.1038/s41598-018-38360-2

Gupta, S., Pandey, S., 2019. ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in french bean (*Phaseolus vulgaris*) plants. *Front Microbiol.* 10, 1-17. DOI:10.3389/fmicb.2019.01506

Husen, E., 2012. Studi *Pseudomonas* penghasil ACC Deaminase dalam Meningkatkan Pertumbuhan dan Ketahanan Tanaman Kedelai terhadap Penyakit [Disertasi]. Bogor, Indonesia: IPB University.

Kielak, A.M., Barreto, C.C., Kowalchuk, G.A., Veen, J.A., Kuramae, E., 2016. The ecology of Acidobacteria: moving beyond genes and genomes. *Front Microbiol*. 7, 744. DOI:10.3389/fmicb.2016.00744

Kong, Z., Glick, B.R., 2017. The role of plant growth-promoting bacteria in metal phytoremediation. *Adv Microb Physiol*. 71, 97-132. DOI:10.1016/bs.ampbs.2017.04.001

Lebrazi, S., Fadil, M., Chraibi, Fikri-Benbrahim, K., 2020. Screening and optimization of indole-3-acetic acid production by *Rhizobium* sp. strain using response surface methodology. *J. Genet. Eng. & Biotechnol.* 18, 1-10. DOI:10.1186/s43141-020-00035-9

Madhaiyan, M., Selvakumar, G., Alex, T.H.H., Cai, L., JI, L., 2021. Plant growth promoting abilities of novel Burkholderia-related genera and their interactions with some economically important tree species. *Front. Sustain. Food Syst.* 5, 1-14. DOI:10.3389/fsufs.2021.618305

Mcnear Jr, D.H., 2013. The Rhizosphere-roots, soil and everything in between. *Nat Edu Knowl*. 4, 1.

Mursyida, E., Mubarik, N.R., Tjahjoleksono, A., 2015. Selection and identification of phosphate-potassium solubilizing bacteria from the area around the limestone mining in Cirebon quarry. *Res J Microbiol.* 10, 270-279. DOI:10.3923/jm.2015.270.279

Neina, D., 2019. The role of soil pH in plant nutrition and soil remediation. *Appl Environ Soil Sci*. 3, 1-9. DOI:10.1155/2019/5794869

Nonthakaew, N., Panbangred, W., Songnuan, W., Intra, B., 2022. Plant growth-prooting properties of Streptomyces spp. Isolated and their impact on mung bean plantlets' rhizosphere microbiome. *Front Microbiol.* 13, 1-17. DOI:10.3389%2Ffmicb.2022.967415

Nurhidayati, Hasfianti, F.E., Wahyudi, K., 2020. Kajian teknoekonomi kaolin Belitung sebagai bahan substitusi impor pada produksi fiber cement board. *JKGI.* 29, 152-164.

Pasi, N.I., Bratadireja, M.A., Chaerunnisa, A.Y., 2020. Physicochemical characteristic of kaolin from Belitung regency. *IJPST.* 7, 38-45.

Putra, H.F., Sulistijorini, Aryanti, N.S., 2017. Landscape function of post tin-mining land after reclamation in Bangka, Indonesia. *IOP Conf Ser Earth Enviro. Sci.* 58, 1-7. DOI:10.1088/1755-1315/58/1/012018S

Rana, A., Saharan, B., Joshi, M., Prasanna, R., Kumar, K., Nain, L., 2011. Identification of multi-trait PGPR isolates and evaluating their potential as inoculants for wheat. *Ann Microbiol.* 61, 893-900.

Sherpa, M.T., Sharma, L, Bag, N., Das, S., 2021. Isolation and characterization, and evaluation of native rhizobacterial consortia developed from the rhizosphere of rice grown in organic state Sikkim,India and their effect on plant growth. *Front Microbiol*. 12, 1-15. DOI:10.3389/fmicb.2021.713660

Subari, Erlangga, B.D., Maryani, E., Arifin, D.N., 2021. Potential utilization of quartz sand and kaolin from tin mine tailings for whiteware. *Min of Mineral Depos.* 15, 1-6. DOI:10.33271/mining15.03.001

Sukarman, Gani, R.A., Asmarhansyah, 2020. Tin mining process and its effects on soils in Bangka Belitung Islands Province, Indonesia. *Sains Tanah: J Soil Sci Agroclimatol.* 17, 180-189. DOI:10.20961/stjssa.v17i2.37606.

Sukarman, Gani, R.A., 2017. Lahan bekas tambang timah di Pulau Bangka dan Belitung, Indonesia dan Kesesuaiannya untuk Komoditas Pertanian. *J Tanah dan Iklim.* 41, 101-112.

Sutono, S., Haryati, U., Agus, F., 2019. Karakteristik tanah dan strategi rehabilitasi lahan bekas tambang timah di Provinsi Kepulauan Bangka Belitung. *J Sumberd Lahan.* 12, 99-116.

Wahyudi, A.T., Priyanto, J.A., Fijrina, H.N., Mariastuti, H.D., Nawangsih, A.A., 2019. *Streptomyces* spp. from rhizosphere soil maize with potential as plant growth promotor. *Biodivers.* 20, 2547-2553. DOI:10.13057/biodiv/d200916

Wahyudi, A.T., Astuti, R.P., Widyawati, A., Meryandini, A., Nawangsih, A.A., 2011. Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria. *J Microbiol Antimicrob.* 3, 34-40.

Venkataramanamma, K., Reddy, B.V.B., Jayalakshmi, R.S., Jayalakshmi, V., Rajendran, L., 2022. Isolation *in vitro* evaluation of *Bacillus* spp. against *Fusarium oxysporum* f.sp. ciceris and their growth promotion activity. *Egypt J Biol Pest Control.* 32, 123. DOI:10.1186/s41938-022-00618-3

Vu, H.T.L., Yukphan, P., Bui, V.T.T., Charoenyingcharoen, P., Malimas, S., Nguyen, L.K., Muramatsu, Y., Tanaka, N., Tanasupawat, S., Le, B.T., Nakagawa, Y., Yamada, Y., 2019. *Acetobacter sacchari* sp.nov., for a plant growth-promoting acetic acid bacterium isolated in Vietnam. *Ann Microbiol*. 69, 1155-1163. DOI:10.1007/s13213-019-01497-0

Xie, X., Gu, S., Hao, L., Zhang, T., Guo, Z., 2022. Rhizosphere microbial communities and geochemical constraining mechanism of Antimony mine waste-adapted plants in South western China. *Microorganism*. 10, 1-18. DOI:10.3390/microorganisms10081507

You, M., Fang, S., Macdonald, J., Xu, J., Yuan, Z.C., 2020. Isolation and characterization of *Burkholderia cenocepacia* CR318, a phosphate solubilizing bacterium promoting corn growth. *Microbiol Res.* 233, 1-8. DOI:10.1016/j.micres.2019.126395

Zhong, C., Hu, G., Hu, C., Xu, C., Zhang, Z., Ning, K., 2024. Comparative genomins analysis reveals genetic characteristics and nitrogen fixation profile of *Bradyrhizobium. J Isci.* 27, 1-11. DOI:10.1016/j.isci.2024.108948