



# AGROTECH

ISSN: 2773-4870 eISSN: 2821-3106

DOI: <https://doi.org/10.53797/agrotech.v3i2.9.2024>

## Evaluation of Soil Beneficial Bacteria as Plant Growth Enhancer and Biocontrol Agents

Haron, Farah Farhanah<sup>1\*</sup>, Ismail, Nur Isz Aishah<sup>2</sup>, Sabli, Khairun Nasrin<sup>3</sup> & Zainuddin, Fatin Nadira<sup>3</sup>

<sup>1</sup>Biological Control Programme, Agrobiodiversity and Environment Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400 Serdang, Selangor, MALAYSIA

<sup>2</sup>Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, 43400 Serdang, Seri Kembangan, Selangor, MALAYSIA

<sup>3</sup>Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, MALAYSIA

\*Corresponding author: [farahfarhanah@mardi.gov.my](mailto:farahfarhanah@mardi.gov.my)

Received 07 November 2024; Accepted 27 December 2024; Available online 29 December 2024

**Abstract:** The use of soil beneficial bacteria, particularly plant growth-promoting rhizobacteria (PGPR), provides an eco-friendly alternative to synthetic agrochemicals in sustainable agriculture. This study aimed to isolate and characterize soil bacterial isolates from the rhizosphere of cabbage cultivation areas in Cameron Highlands, Malaysia, and evaluate their potential as PGPR and biocontrol agents. A total of 42 bacterial isolates were obtained, of which 15 were selected for detailed screening. Morphological and molecular characterization using 16S rDNA sequencing identified species from the genera *Bacillus*, *Pseudomonas*, and *Serratia*. These isolates exhibited key plant growth-promoting traits, including nitrogen fixation, phosphate solubilization, and potassium solubilization. Notably, isolates S9 and S30 demonstrated both plant growth-promoting and antibacterial activities against *Xanthomonas campestris*, a major pathogen of cabbage. Meanwhile, isolates S16 and S36, although lacking antibacterial activity, showed strong growth-promoting capabilities. These findings highlight the potential of native soil beneficial bacteria as biofertilizers and biocontrol agents, contributing to sustainable agricultural practices by reducing reliance on chemical fertilizers and pesticides. Future research should focus on field trials to validate their efficacy under diverse environmental conditions.

**Keywords:** Rhizobacteria, Plant growth promotion, Biological control, Sustainable agriculture, Cameron Highlands

### 1. Introduction

Soil beneficial bacteria, particularly rhizobacteria, have become a focal point in sustainable agriculture due to their ability to enhance plant growth and control soil-borne pathogens. Rhizobacteria, a group of bacteria residing in the rhizosphere (the soil zone influenced by root secretions), are recognized for their various plant growth-promoting (PGP) properties. Through complex interactions with plants, rhizobacteria can promote growth by fixing atmospheric nitrogen, solubilizing phosphate and potassium, producing phytohormones, and inducing systemic resistance in plants (Kour et al., 2020). Their application as biological control agents and biofertilizers offers a promising alternative to synthetic chemicals, aiding in the reduction of chemical inputs in agriculture and supporting ecosystem health (Santos & Lin, 2019; Timmusk et al., 2017).

The effectiveness of rhizobacteria as biocontrol agents is mediated through several mechanisms, including the production of antimicrobial compounds, competition for nutrients and space, and direct parasitism of plant pathogens. Rhizobacteria produce antibiotics, siderophores, and lytic enzymes that inhibit pathogen growth in the rhizosphere, enhancing plant resilience (Hussain et al., 2020). Additionally, rhizobacteria can induce systemic resistance in plants, triggering defense responses that protect against a wide range of pathogens, including bacteria, fungi, and nematodes (de Vries et al., 2018). These multifaceted interactions make rhizobacteria an effective component of integrated pest management (IPM), helping to reduce reliance on chemical pesticides and mitigate environmental risks associated with their use.

\*Corresponding author: [farahfarhanah@mardi.gov.my](mailto:farahfarhanah@mardi.gov.my)

<https://www.arsvot.org/> All right reserved.

Cameron Highlands, Malaysia, presents a unique environment for studying rhizobacterial diversity due to its intensive agricultural practices and distinctive microclimate. This high-altitude region, known for its cool and humid conditions, is a significant center for horticultural production in Malaysia, particularly for crops such as tea, vegetables, and flowers (Shaharuddin et al., 2021). The organic-rich soil and extensive use of fertilizers and pesticides make it an ideal setting to explore rhizobacteria that could reduce chemical inputs while maintaining productivity (Mohd Najib et al., 2020). Recent studies indicate that soils in high-altitude, intensively cultivated areas harbor unique microbial communities that play a crucial role in plant growth and health (Naylor et al., 2021).

The long-term environmental impacts of intensive agricultural inputs, especially fertilizers and pesticides, have raised concerns regarding soil health and biodiversity in Cameron Highlands (Shaharuddin et al., 2021). This has sparked interest in exploring biological solutions, such as utilizing native rhizobacteria, to promote sustainable farming practices. Research suggests that rhizobacteria adapted to local conditions may be more effective in enhancing plant growth and protecting crops, thus representing valuable assets for sustainable agriculture (Gumiere et al., 2020). Given the environmental concerns associated with chemical fertilizers and pesticides, this research addresses the pressing need for sustainable agricultural solutions by exploring the potential of native rhizobacteria as eco-friendly alternatives to conventional agrochemicals, thereby supporting soil health and promoting long-term agricultural sustainability. This study aims to evaluate the potential of beneficial bacteria isolated from the rhizosphere soil in cabbage cultivation areas of Cameron Highlands as plant growth enhancers and biocontrol agents.

## 2. Material and methods

### 2.1 Isolation of Soil Bacteria

Soil sampling was conducted at five cabbage cultivation areas in Cameron Highlands: (i) Bertam (4.40023° N, 101.45997° E), (ii) Kampung Taman Sedia (4.47990° N, 101.37942° E), (iii) Brinchang (4.49063° N, 101.40688° E), (iv) Habu (4.45346° N, 101.39133° E), and (v) Tanah Rata (4.45270° N, 101.39145° E). Soil samples were collected specifically targeting the rhizosphere, a region of soil directly influenced by root secretions and associated microorganisms. The collected soil was air-dried and passed through a 2 mm sieve to remove debris and ensure uniform particle size, facilitating subsequent microbial isolation steps. The soil sample was mixed with a sterile saline solution at a ratio of 1 gram of sieved soil to 9 mL of liquid, creating a 1:10 ( $10^{-1}$ ) dilution to form a soil suspension. This suspension was then serially diluted, typically from  $10^{-1}$  to  $10^{-6}$ , to decrease bacterial concentration and facilitate isolation. Aliquots from each dilution were plated onto Nutrient Agar (NA) to isolate various bacterial species, and the plates were incubated at room temperature for 24 to 48 hours. Colonies with distinct morphological characteristics were selected and transferred to fresh agar plates to obtain pure cultures, with further sub-culturing carried out until single, pure colonies were achieved.

### 2.2 Morphological Characterization of Soil Bacteria

The pure cultures of bacterial isolates were further characterized based on their phenotypic morphology, including cell shape, arrangement, and colony characteristics (Sulistiyan et al., 2024). Morphologically, bacteria were classified as cocci (round), rods, or spirilla (spiral-shaped), with arrangements such as single cells, pairs (diplo-), chains (strepto-), or clusters (staphylo-) depending on their cell organization. The colony morphology of the pure cultures was assessed on NA, considering factors such as size (pinpoint, small, moderate, large), shape (circular, irregular, rhizoid), margin (entire, filamentous, lobate, serrate, undulate), and elevation (flat, raised, convex, umbonate). Additionally, optical property like transparency (transparent, translucent, opaque) was evaluated, along with pigmentation, which ranged from white and cream to yellow, red, or other colours.

Following morphological characterization, the isolates were further classified by Gram staining to differentiate them into Gram-positive and Gram-negative groups based on cell wall structure (Dimri et al., 2020). The Gram staining procedure involved preparing a bacterial smear on a sterile glass slide, heat-fixing it, applying crystal violet dye, and adding iodine solution to form a crystal violet-iodine complex. The slide was then decolorized with alcohol or acetone, enabling differentiation. Gram-positive bacteria retained the crystal violet stain due to their thicker peptidoglycan layer, appearing purple under the microscope, while Gram-negative bacteria were counterstained with safranin, appearing pink or red.

### 2.3 Molecular Identification of Soil Beneficial Bacteria

A total of 42 bacterial isolates were screened and sequenced for identification. From these, 15 isolates that are most relevant to agriculture were selected for further presentation based on their identification as beneficial soil bacteria, particularly those with plant growth-promoting and biocontrol properties. Isolates identified as plant pathogens, human pathogens, or opportunistic pathogens were excluded to maintain the focus of the study on agricultural applications. This approach ensures that the results logically reflect the soil environment in cabbage cultivation areas.

Genomic DNA was extracted using the Geneaid Genomic DNA Mini Kit (GBB100) according to the manufacturer's guidelines. The 16S rRNA gene was amplified via polymerase chain reaction (PCR) using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The PCR procedure involved an initial denaturation at 94°C for 2 minutes, followed by 25 cycles consisting of denaturation at 98°C for 10 seconds, annealing at 53°C for 30 seconds, and extension at 68°C for 1 minute. The amplified DNA fragments were separated on a 1.5% agarose gel, stained with SYBR Safe, and visualized under UV light. The PCR products were then purified using a standard clean-up method and sequenced using the Sanger method. The resulting sequences were analyzed against the NCBI GenBank database using the BLAST algorithm to determine their closest phylogenetic relatives.

## 2.4 Screening of Potential Soil Beneficial Bacteria

### 2.4.1 In Vitro Plant Growth Promoting Activities

#### 2.4.1.1 Nitrogen Fixation Assay

The nitrogen-fixing potential of each bacterial isolate was evaluated using Jensen's nitrogen-free medium, a selective medium without nitrogen to assess the isolates' ability to fix atmospheric nitrogen. Each isolate was inoculated into the medium and incubated at room temperature, with four replicates for each isolate to ensure accuracy and reproducibility. Observations were made daily over a period of seven days to monitor bacterial growth. Successful growth in this nitrogen-free medium suggested that the isolate could fix atmospheric nitrogen, as nitrogen fixation would provide the essential nitrogen source needed for cell proliferation.

#### 2.4.1.2 Phosphate Solubilization Assay

For assessing phosphate solubilization, each isolate was streaked onto Pikovskaya Agar, which contains insoluble phosphate compounds that can be converted to soluble forms by phosphate-solubilizing bacteria. The isolates were streaked on the agar plates in four replicates and incubated at room temperature. Plates were observed periodically for the development of a clear halo around the colonies, which indicated phosphate solubilization. The clear zone results from the conversion of insoluble phosphate into soluble forms by the enzymatic activity of the bacteria, making phosphate accessible for plant uptake.

#### 2.4.1.3 Potassium Solubilization Assay

A similar approach was used to evaluate the potassium solubilization capacity of each isolate, employing Aleksandrow Agar, a medium specifically formulated for potassium solubilization studies. Each isolate was streaked onto the Aleksandrow Agar plates in four replicates and incubated at room temperature. After 72 hours, the plates were checked for the presence of clear zones around the bacterial colonies, signifying successful potassium solubilization. The clear zone forms due to the bacterial conversion of insoluble potassium compounds into soluble forms that can be utilized by plants.

### 2.4.2 Antibacterial activity as Biocontrol Agents

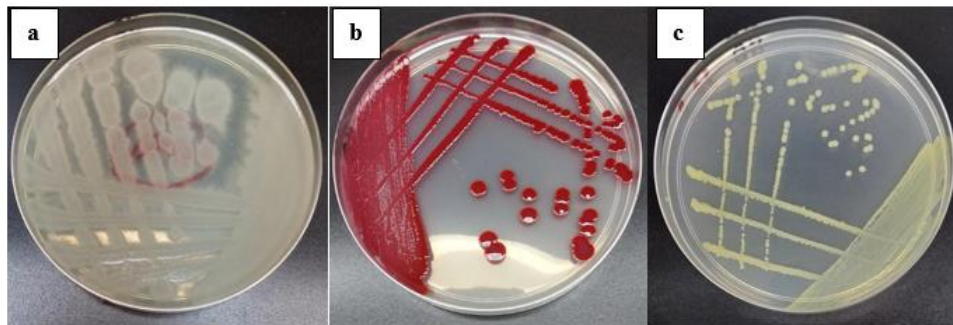
The antibacterial activity of bacterial isolates was evaluated against *Xanthomonas campestris*, a major pathogen of cabbage, using the disc diffusion assay. Rhizobacterial isolates were cultured in Nutrient Broth and incubated at room temperature for 24 hours, while *X. campestris* was cultured on Nutrient Agar (NA) plates at room temperature for 48 hours. The NA plate surface was uniformly swabbed with a suspension of *X. campestris*. Sterile filter paper discs (6 mm in diameter) were impregnated with 20 µL of the rhizobacterial culture (OD<sub>600</sub> = 0.5) and placed on the inoculated NA plate with *X. campestris*. Discs soaked in sterile Nutrient Broth served as the control. The plates were incubated at room temperature for 24 hours, and the clear zone around each disc indicated positive effect.

## 3. Results and Discussion

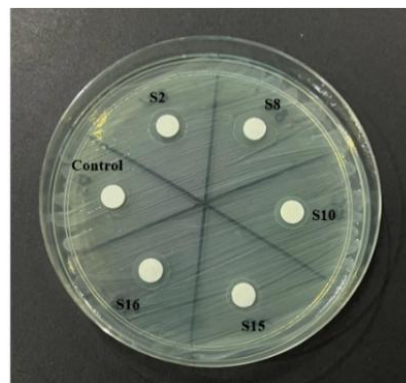
Based on the isolation of bacteria from soil samples collected in cabbage cultivation areas around Cameron Highlands, 42 bacterial isolates were obtained. Gram staining and microscopic observation revealed that 22 of these isolates were Gram-positive, comprising rod and cocci forms. The majority formed circular, raised colonies with entire margins, appearing opaque and ranging in color from white to yellow (Table 1, Fig.1). The results also revealed numerous rod-shaped, yellow-pigmented colonies among the isolates. This finding aligns with the characteristics of *Pseudomonas* species, which are typically Gram-negative, rod-shaped bacteria that display yellow to green pigmentation due to pyoverdine production under specific growth conditions (Kirisits et al., 2005).

Gram staining is a crucial step in selecting beneficial bacteria for plant growth and biocontrol, as it differentiates Gram-positive from Gram-negative bacteria, each with distinct ecological roles and plant interactions. Many Gram-positive bacteria, particularly *Bacillus* species, produce spores that enable them to thrive in soil and promote plant growth through bioactive compounds (Swain et al., 2021). Gram-negative bacteria, such as *Pseudomonas* species, are effective biocontrol agents due to their production of antimicrobial compounds and competitive colonization in the rhizosphere, which suppresses soil pathogens and supports plant health (Rangarajan et al., 2022).

Morphological traits such as cell shape, colony appearance, pigmentation, and motility are valuable indicators for selecting effective bacterial strains. For example, *Bacillus* species, known for their rod shape and spore-forming ability, exhibit resilience in soil environments. Similarly, *Pseudomonas* pigmentation is linked to antimicrobial activity. Additionally, red-pigmented *Serratia* species, such as isolate S1, produce the characteristic pigment prodigiosin, although certain strains may appear white or colorless. These morphological characteristics, combined with Gram staining, provide critical insights for selecting bacterial strains suitable for various agricultural applications (Mahlen, 2011).



**Fig. 1:** Colonies of isolated soil bacteria on NA medium showing different sizes and characteristics: (a) Isolate S8, irregularly shaped, filamentous, large, and opaque white; (b) Isolate S1, circular, smooth-edged (entire), moderate-sized, and opaque red; and (c) Isolate S6, circular, smooth-edged (entire), small, and opaque light yellow.



**Fig. 2:** Antibacterial activity of selected soil bacterial isolates against *Xanthomonas campestris*, demonstrated by the formation of clear zones on disc diffusion plates after 24 hours of incubation

**Table 1: Morphological characteristics and Gram staining of soil bacterial isolates**

| Bacterial isolate | Cell description |           |           | Colony description |             |             |           | Colony colour   | Gram staining |
|-------------------|------------------|-----------|-----------|--------------------|-------------|-------------|-----------|-----------------|---------------|
|                   | Shape            | Structure | Form      | Size               | Optical     | Margin      | Elevation |                 |               |
| S1                | Rod              | Single    | Circular  | Moderate           | Opaque      | Entire      | Convex    | Red             | Negative      |
| S2                | Rod              | Strepto   | Rhizoid   | Large              | Opaque      | Filamentous | Flat      | Off white       | Positive      |
| S3                | Rod              | Staphylo  | Circular  | Small              | Opaque      | Lobate      | Convex    | White           | Positive      |
| S4                | Rod              | Single    | Irregular | Large              | Translucent | Serrate     | Umbonate  | Beige           | Negative      |
| S5                | Cocci            | Diplo     | Circular  | Moderate           | Opaque      | Entire      | Convex    | Yolk yellow     | Negative      |
| S6                | Cocci            | Diplo     | Circular  | Small              | Translucent | Entire      | Convex    | Beige           | Positive      |
| S7                | Cocci            | Staphylo  | Circular  | Small              | Translucent | Entire      | Convex    | Yellowish white | Negative      |
| S8                | Rod              | Strepto   | Irregular | Large              | Opaque      | Filamentous | Raised    | Off white       | Positive      |
| S9                | Rod              | Diplo     | Circular  | Small              | Translucent | Entire      | Umbonate  | Yellowish white | Negative      |
| S10               | Rod              | Strepto   | Irregular | Large              | Opaque      | Serrate     | Umbonate  | Copper          | Positive      |
| S11               | Cocci            | Staphylo  | Circular  | Small              | Opaque      | Entire      | Convex    | Off white       | Negative      |
| S12               | Cocci            | Diplo     | Irregular | Moderate           | Translucent | Undulate    | Raised    | Yellowish white | Positive      |
| S13               | Cocci            | Strepto   | Circular  | Large              | Translucent | Entire      | Raised    | Yellowish white | Positive      |
| S14               | Rod              | Strepto   | Irregular | Large              | Translucent | Lobate      | Raised    | Beige           | Negative      |
| S15               | Rod              | Strepto   | Irregular | Large              | Opaque      | Lobate      | Raised    | Off white       | Positive      |
| S16               | Rod              | Staphylo  | Irregular | Large              | Translucent | Undulate    | Raised    | Off white       | Positive      |
| S17               | Rod              | Diplo     | Irregular | Moderate           | Translucent | Entire      | Raised    | Yellowish white | Positive      |
| S18               | Cocci            | Staphylo  | Irregular | Moderate           | Opaque      | Entire      | Flat      | Yellowish white | Positive      |
| S19               | Rod              | Staphylo  | Circular  | Large              | Opaque      | Lobate      | Raised    | Beige           | Positive      |
| S20               | Cocci            | Staphylo  | Circular  | Small              | Translucent | Entire      | Umbonate  | Yellow          | Negative      |
| S21               | Cocci            | Staphylo  | Irregular | Large              | Transparent | Filamentous | Raised    | Yellowish white | Negative      |
| S22               | Rod              | Staphylo  | Irregular | Small              | Opaque      | Serrate     | Convex    | Off white       | Positive      |
| S23               | Cocci            | Staphylo  | Irregular | Moderate           | Translucent | Lobate      | Raised    | Beige           | Positive      |
| S24               | Rod              | Strepto   | Irregular | Small              | Opaque      | Lobate      | Flat      | Off white       | Positive      |
| S25               | Cocci            | Staphylo  | Circular  | Small              | Opaque      | Entire      | Convex    | Yellowish white | Positive      |
| S26               | Cocci            | Strepto   | Circular  | Large              | Opaque      | Undulate    | Flat      | Yellowish white | Positive      |
| S27               | Rod              | Strepto   | Circular  | Small              | Translucent | Serrate     | Raised    | Yellowish white | Negative      |
| S28               | Rod              | Strepto   | Irregular | Large              | Opaque      | Lobate      | Flat      | Yellowish white | Positive      |
| S29               | Cocci            | Staphylo  | Circular  | Moderate           | Translucent | Entire      | Convex    | Yellow          | Negative      |
| S30               | Rod              | Diplo     | Circular  | Small              | Translucent | Entire      | Raised    | Yellow          | Negative      |
| S31               | Cocci            | Diplo     | Circular  | Small              | Transparent | Entire      | Convex    | Yellowish white | Negative      |
| S32               | Cocci            | Staphylo  | Irregular | Small              | Translucent | Entire      | Convex    | Yolk yellow     | Negative      |
| S33               | Rod              | Staphylo  | Circular  | Moderate           | Translucent | Undulate    | Convex    | Yellowish white | Negative      |
| S34               | Cocci            | Staphylo  | Circular  | Moderate           | Opaque      | Serrate     | Convex    | Yellowish white | Negative      |
| S35               | Cocci            | Staphylo  | Irregular | Moderate           | Translucent | Undulate    | Convex    | Off white       | Negative      |
| S36               | Rod              | Diplo     | Circular  | Moderate           | Transparent | Entire      | Raised    | Off white       | Negative      |
| S37               | Cocci            | Staphylo  | Irregular | Moderate           | Opaque      | Undulate    | Raised    | Off white       | Positive      |
| S38               | Cocci            | Strepto   | Irregular | Large              | Translucent | Entire      | Flat      | Off white       | Positive      |
| S39               | Cocci            | Staphylo  | Circular  | Moderate           | Translucent | Entire      | Convex    | Yellowish white | Negative      |
| S40               | Cocci            | Staphylo  | Circular  | Pinpoint           | Opaque      | Filamentous | Convex    | Off white       | Positive      |
| S41               | Cocci            | Staphylo  | Circular  | Pinpoint           | Opaque      | Entire      | Convex    | Yolk yellow     | Positive      |
| S42               | Cocci            | Staphylo  | Irregular | Moderate           | Translucent | Lobate      | Raised    | Yellow          | Positive      |

A total of 15 selected bacterial isolates were identified through 16S rDNA gene sequencing, revealing diverse species with potential plant growth-promoting and biocontrol properties (Table 2). Among the isolates, several belonged to the genera *Bacillus* and *Pseudomonas*, which are well-known for their beneficial effects on plant health. Specifically, *Bacillus pseudomycolides*, *Bacillus thuringiensis*, *Bacillus megaterium*, and *Bacillus vietnamensis* were identified with 100% similarity. Isolates *Pseudomonas atagonensis*, *Pseudomonas resinovorans*, and *Pseudomonas germanica* displayed high similarity ( $\geq 98\%$ ), demonstrating their genetic closeness to known beneficial strains. These results indicate a wide distribution of beneficial bacterial genera in the soil samples collected from cabbage cultivation areas in Cameron Highlands.

The identified bacterial isolates belong to genera widely recognized for their plant growth-promoting and biocontrol capabilities. *Bacillus* species, such as *B. megaterium* and *B. thuringiensis*, are known for their ability to produce enzymes, phytohormones, and antimicrobial compounds, which enhance nutrient availability and suppress plant pathogens (Kumar et al., 2021). Their spore-forming capability also ensures resilience under harsh environmental conditions, making them reliable candidates for field applications. *Pseudomonas* species, including *P. atagonensis* and *P. germanica*, are effective biocontrol agents due to their production of siderophores, hydrogen cyanide, and antifungal compounds, which inhibit soil-borne pathogens and promote root health (Rangarajan et al., 2022). Additionally, the

identification of *Serratia surfactantfaciens* highlights its potential role in nutrient solubilization and disease suppression, although further studies are required to confirm its specific functions.

The high similarity percentages (98–100%) to reference strains from GenBank validate the accuracy of the 16S rDNA sequencing approach. These findings align with previous studies that emphasize the importance of *Bacillus* and *Pseudomonas* genera in sustainable agriculture (Jha & Saraf, 2019; Radhakrishnan et al., 2020). The multifunctional roles of these isolates could reduce the need for chemical fertilizers and pesticides, promoting environmentally friendly farming practices.

**Table 2: Identification of selected soil beneficial bacteria based on 16S rDNA gene sequencing**

| Bacterial isolate | Identified species                | Similarity (%) | Reference Sequence ID |
|-------------------|-----------------------------------|----------------|-----------------------|
| S2                | <i>Bacillus pseudomycolides</i>   | 100            | NR_113991.1           |
| S3                | <i>Serratia surfactantfaciens</i> | 99.9           | NR_169468.1           |
| S8                | <i>Bacillus thuringiensis</i>     | 100            | NR_114581.1           |
| S9                | <i>Pseudomonas atagonensis</i>    | 99.8           | NR_180743.1           |
| S10               | <i>Bacillus vietnamensis</i>      | 100            | NR_024808.1           |
| S14               | <i>Pseudomonas resinovorans</i>   | 98             | NR_112062.1           |
| S15               | <i>Bacillus aryabhattai</i>       | 100            | NR_115953.1           |
| S16               | <i>Bacillus megaterium</i>        | 99.8           | NR_117473.1           |
| S17               | <i>Bacillus salipaludis</i>       | 100            | NR_180481.1           |
| S22               | <i>Bacillus aerius</i>            | 100            | NR_118439.1           |
| S24               | <i>Bacillus altitudinis</i>       | 100            | NR_042337.1           |
| S28               | <i>Bacillus cytotoxicus</i>       | 98.8           | NR_074914.1           |
| S30               | <i>Pseudomonas germanica</i>      | 99.6           | NR_181838.1           |
| S33               | <i>Pseudomonas alcaligenes</i>    | 99.5           | NR_113646.1           |
| S36               | <i>Pseudomonas glycinae</i>       | 99.6           | NR_179889.1           |

The selected bacterial isolates were evaluated for their plant growth-promoting and antibacterial properties to determine their potential as effective plant growth enhancers and biocontrol agents (Table 2). All tested activities were observed in isolates S9 and S30, while S16 and S36 exhibited all plant growth-promoting activities. In the antibacterial assays, 9 out of 15 tested soil bacterial isolates showed clear zones, indicating positive effects. Isolates S2, S3, S8, S9, S22, S24, S28, S30, and S33 showed positive antibacterial activity, demonstrating their ability to inhibit the growth of *X. campestris*. These soil bacteria show great potential as effective plant growth-promoting rhizobacteria (PGPR) and biocontrol agents. The dual activity observed in isolates S9 and S30 underscores their potential as multifunctional PGPR and biocontrol agents. Similar findings have been reported in *Bacillus* and *Pseudomonas* species, which are known for their robust plant growth-promoting traits and ability to suppress plant pathogens (Ahmad et al., 2021; Ali et al., 2022). The antibacterial activity, demonstrated by clear zones in 9 isolates, further supports their role in reducing pathogen pressure, a crucial feature for sustainable agricultural practices. Isolates S16 and S36, while lacking antibacterial activity, exhibited strong growth-promoting traits, suggesting their utility in non-pathogen-stressed environments.

Nitrogen, phosphorus, and potassium are essential macronutrients for plant growth, but their availability in the soil is often limited. Nitrogen is a critical component of amino acids, proteins, and chlorophyll; however, plants cannot directly utilize atmospheric nitrogen ( $N_2$ ). Nitrogen-fixing rhizobacteria play a crucial role in converting atmospheric nitrogen into ammonia ( $NH_3$ ) or ammonium ( $NH_4^+$ ), which are accessible forms for plant uptake. This process reduces the reliance on chemical nitrogen fertilizers, promoting more sustainable and cost-effective farming practices (Jha & Saraf, 2019). Similarly, phosphorus is indispensable for energy transfer, photosynthesis, and root development, yet it often exists in insoluble forms in soil. Phosphate-solubilizing bacteria, such as *Pseudomonas* and *Bacillus*, enhance phosphorus availability by releasing organic acids and enzymes that convert insoluble phosphate into soluble forms, thereby improving root development and overall plant growth (Kumar et al., 2021). Potassium, another vital macronutrient, plays a key role in water regulation, enzyme activation, photosynthesis, and disease resistance. Potassium-

solubilizing bacteria contribute to making insoluble potassium available for plant uptake, ensuring optimal potassium levels and enhancing plant resilience (Kour et al., 2020).

Soil bacteria exhibiting these nutrient-solubilizing traits not only improve plant nutrient uptake but also enhance plant tolerance to environmental stressors, such as drought, salinity, and pathogen attacks. This dual role of enhancing plant growth while reducing dependency on chemical fertilizers aligns with sustainable agricultural practices and helps improve soil health (Pandey & Maheshwari, 2021; Radhakrishnan et al., 2020). Moreover, certain isolates in this study demonstrated strong antibacterial activity, reinforcing their potential as biocontrol agents. Soil bacteria that act as biocontrol agents suppress harmful pathogens, including fungi and bacteria, by producing antimicrobial compounds or competing for resources in the rhizosphere. This reduces the need for chemical pesticides, which are costly and environmentally damaging, further supporting sustainable farming practices (Zhang et al., 2019). Thus, isolates like S9 and S30, which exhibited a combination of plant growth-promoting and biocontrol activities, demonstrate great potential for use as multifunctional PGPR, contributing to enhanced crop productivity and sustainability in agriculture.

**Table 3: Screening of potential beneficial soil bacteria for plant growth promoting and antibacterial activities**

| Bacterial isolate | Plant growth promoting activity |                          |                          | Antibacterial activity |
|-------------------|---------------------------------|--------------------------|--------------------------|------------------------|
|                   | Nitrogen fixation               | Phosphate solubilization | Potassium solubilization |                        |
| S2                | +                               | -                        | -                        | +                      |
| S3                | +                               | +                        | -                        | +                      |
| S8                | +                               | -                        | -                        | +                      |
| S9                | +                               | +                        | +                        | +                      |
| S10               | +                               | -                        | -                        | -                      |
| S14               | +                               | +                        | -                        | -                      |
| S15               | +                               | +                        | -                        | -                      |
| S16               | +                               | +                        | +                        | -                      |
| S17               | +                               | +                        | -                        | -                      |
| S22               | -                               | +                        | -                        | +                      |
| S24               | +                               | +                        | -                        | +                      |
| S28               | -                               | +                        | -                        | +                      |
| S30               | +                               | +                        | +                        | +                      |

Notes: The symbol "-" indicates the absence of activity, while "+" denotes the presence of activity.

#### 4. Conclusion

This study demonstrates the potential of soil bacterial isolates from cabbage cultivation areas in Cameron Highlands as effective plant growth-promoting rhizobacteria (PGPR) and biocontrol agents. Among the selected isolates, species from the genera *Bacillus*, *Pseudomonas*, and *Serratia* exhibited key plant growth-promoting traits, including nitrogen fixation, phosphate solubilization, and potassium solubilization. Notably, isolates S9 and S30 displayed both growth-promoting and antibacterial activities, highlighting their multifunctional role in enhancing plant health and controlling pathogens such as *Xanthomonas campestris*. These isolates can reduce reliance on chemical fertilizers and pesticides, promoting sustainable agricultural practices and improving soil health. Isolates S16 and S36, while lacking antibacterial activity, exhibited strong growth-promoting capabilities, making them suitable for use in non-pathogen-stressed environments. These findings underscore the importance of native rhizobacteria in sustainable farming, with future research needed to validate their efficacy in field conditions and explore their potential for commercial biofertilizer and biocontrol applications.

## Acknowledgement

The authors would like to extend their gratitude to all those involved in this study, conducted under MARDI's international special project funded by the UN Environment Programme titled "The Impacts of MyGAP Certification among Farmers in Cameron Highlands: The Economics of Ecosystems and Biodiversity for Agriculture (K-RE347)".

## Conflict of Interest

The authors declare no conflicts of interest.

## References

- Ahmad, M., Nadeem, S. M., Naveed, M., & Zahir, Z. A. (2021). Plant growth-promoting rhizobacteria: Ecological role in sustainable agriculture. *Sustainability*, 13(3), 1145. <https://doi.org/10.3390/su13031145>
- Ali, S., Khalid, N., & Glick, B. R. (2022). Plant growth-promoting bacteria: Mechanisms and applications in agriculture. *Frontiers in Microbiology*, 13, 786125. <https://doi.org/10.3389/fmicb.2022.786125>
- de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., & Bardgett, R. D. (2018). Soil bacterial networks are less stable under drought than fungal networks. *Nature communications*, 9(1), 3033.
- Dimri, A. G., Chaudhary, S., Singh, D., Chauhan, A., & Aggarwal, M. L. (2020). Morphological and biochemical characterization of food borne gram-positive and gram-negative bacteria. *Science Archives*, 1(1), 16-23.
- Gumiere, T., Durrer, A., Bohannan, B. J. M., Andreote, F. D., & Andreote, F. D. (2020). Biotic and abiotic factors influencing the soil microbiome in agriculture. In M. N. Timmusk (Ed.), *Microbiome in Plant Health and Disease: Challenges and Opportunities* (pp. 1–20). Springer. [https://doi.org/10.1007/978-3-030-50395-6\\_1](https://doi.org/10.1007/978-3-030-50395-6_1)
- Hussain, A., et al. (2020). Siderophore-producing rhizobacteria and their role in plant growth promotion and disease resistance. *Microbiological Research*, 239, 126532. <https://doi.org/10.1016/j.micres.2020.126532>
- Jha, P., & Saraf, M. (2019). Evaluation of multispecies plant-growth-promoting consortia for the growth promotion of Glycine max. *Brazilian Journal of Microbiology*, 50(2), 229-236. <https://doi.org/10.1007/s42770-019-00037-4>
- Kirisits, M. J., Prost, L., Starkey, M., & Parsek, M. R. (2005). Characterization of colony morphology variants isolated from *Pseudomonas aeruginosa* biofilms. *Applied and Environmental Microbiology*, 71(8), 4809-4821. <https://doi.org/10.1128/AEM.71.8.4809-4821.2005>
- Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Dhaliwal, H. S., & Saxena, A. K. (2020). Potassium solubilizing microorganisms for sustainable agriculture. *Environmental Sustainability*, 3(2), 211-220. <https://doi.org/10.1007/s42398-020-00102-7>
- Kumar, A., Verma, J. P., & Shukla, L. (2021). Phosphate solubilizing bacteria in sustainable agriculture: A review. *Agronomy*, 11(2), 412. <https://doi.org/10.3390/agronomy11020412>
- Mahlen, S. D. (2011). *Serratia* infections: From military experiments to current practice. *Clinical Microbiology Reviews*, 24(4), 755-791.
- Mohd Najib, N., Shamsuddin, Z. H., & Halimi, M. S. (2020). Organic farming practices and soil microbial diversity in Cameron Highlands. *Applied Soil Ecology*, 156, 103696. <https://doi.org/10.1016/j.apsoil.2020.103696>
- Naylor, D., Fansler, S. J., Brislawn, C. J., Nelson, W. C., Hofmockel, K. S., & Jansson, J. K. (2021). High-throughput sequencing reveals distinctive microbial populations in high-altitude soils. *Frontiers in Microbiology*, 12, 691121. <https://doi.org/10.3389/fmicb.2021.691121>
- Pandey, A., & Maheshwari, D. K. (2021). Plant growth promoting rhizobacteria for sustainable agriculture. *Environmental Sustainability*, 4, 227-240. <https://doi.org/10.1007/s42398-021-00134-4>



- Radhakrishnan, R., Hashem, A., & Abd\_Allah, E. F. (2020). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, 11, 13. <https://doi.org/10.3389/fphys.2020.00013>
- Rangarajan, S., Saleena, L. M., & Nair, S. (2022). Pseudomonas spp. as biofertilizers and biocontrol agents in sustainable agriculture. *Agronomy*, 12(3), 754. <https://doi.org/10.3390/agronomy12030754>
- Santos, V. L., & Lin, B. (2019). Microbial biofertilizers for sustainable agricultural systems. In G. Panpatte & Y. Jhala (Eds.), *Microbial Ecology in Sustainable Agroecosystems* (pp. 187–215). Springer. [https://doi.org/10.1007/978-981-13-8335-9\\_9](https://doi.org/10.1007/978-981-13-8335-9_9)
- Shaharuddin, N. A., Praveena, S. M., & Aris, A. Z. (2021). Assessing the impact of intensive vegetable farming on water quality in Cameron Highlands, Malaysia. *Water, Air, & Soil Pollution*, 232(4), 120. <https://doi.org/10.1007/s11270-021-05068-w>
- Sulistiyani, T. R., Meliah, S., Kusmiati, M., Budiyo, A., & Muslichah, D. A. (2024). Molecular approach and preliminary screening of culturable free-living Rhizobium as plant growth enhancer. *Malaysian Journal of Science*, 21-29.
- Swain, M. R., Naskar, S. K., & Ray, R. C. (2021). Bacillus species in agriculture: A sustainable approach to enhance plant growth. *Frontiers in Sustainable Food Systems*, 5, 669598. <https://doi.org/10.3389/fsufs.2021.669598>
- Timmusk, S., Behers, L., Muthoni, J., Muraya, A., & Aronsson, A. C. (2017). Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Science*, 8, 49. <https://doi.org/10.3389/fpls.2017.00049>
- Zhang, Y., Xu, D., Feng, X., & Wang, L. (2019). Biocontrol potential of beneficial rhizobacteria against plant diseases. *International Journal of Molecular Sciences*, 20(21), 5205. <https://doi.org/10.3390/ijms20215205>