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ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA FROM *Mangifera indica* (MANGO) AS A POTENTIAL BIOCONTROL AGENT AGAINST THE EMERGING BACTERIA CAUSING BACTERIAL BLIGHT

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ABSTRACT

Mango (*Mangifera indica*), the national fruit of the Philippines and an economically important crop, faces various challenges, including diseases such as bacterial blight, which has impacted its production in recent years. This study isolated and identified bacterial endophytes from mango plants and evaluated their antagonistic activity against the emerging bacteria causing bacterial blight pathogen *Enterobacter asburiae*. A total of thirty-three bacterial isolates were obtained and characterized morphologically and biochemically. Antagonistic assays demonstrated that four isolates inhibited the growth of *E. asburiae*. 16S rRNA sequencing identified these isolates as members of the genera *Bacillus*, *Alcaligenes*, *Proteus*, *Paenibacillus*. Among them, isolate CTL S2-R1 (*Alcaligenes* sp.) exhibited the most significant inhibitory effect, with a mean zone of inhibition of 16.67 ± 1.53 mm. Further investigations are recommended to characterize and identify the antibacterial compounds produced by these endophytes.

Keywords: Bacterial endophytes, bacterial blight, biocontrol, *Enterobacter asburiae*, mango.

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INTRODUCTION

Endophytes are known as microorganisms that can often be bacteria or fungi, which reside and thrive within plant cells and in all species of plants. They have been known to promote plant growth, help reduce and cope with the stress tolerance of plants, improve the plant's immunity, and aid in suppressing harmful pathogens (Omomowo et al., 2019). This interaction between the endophytes and plants has created lots of possibilities for the endophytes to become a potential biocontrol agent against the threat of pests and pathogens. Biocontrol agents, or biological control agents, are microorganisms or organisms utilized to control and resist the growth of plant pathogens that cause plant disease. Plant diseases caused by many plant pathogens could have severe effects on the plant they affect, and for this reason, studies regarding the potential application of different endophytes from different plant species as biocontrol agents became important (Pandit et al., 2022).

Mangifera indica, known as the mango, is the Philippine national fruit, loved by the Filipino people, eaten fresh, processed as flavorings for ice creams, pastries, and other delicacies. According to the Philippine Mango Industry Roadmap (2017−2022) developed by the Department of Agriculture, mango is one of the most important fruit crops in the Philippines, next to banana and pineapple. However, the production volume and mango yield have significantly deteriorated over the last several years. In the study of Sossah et al. (2024), mango trees have been suffering from an emerging plant disease called bacterial black spot caused by the bacterium *Xanthomonas citri pv. mangiferaeindica* that affects mango production in the Americas, Africa, Asia, and Oceania. The infected plants of this disease exhibit multiple tiny water-soaked black lesions on leaves, stems, and fruits.

Bacterial black spot is one of the types of bacterial blight disease. Leaf spots and blights in many vegetable crops are commonly caused by species of genera *Pseudomonas* and *Xanthomonas* (Madeiras, 2020). However, several first reports of *Enterobacter asburiae*, a Gram-negative bacterium, causing bacterial blight to different plant crops have emerged. *E. asburiae* has been identified as causing bacterial leaf blight on sorghum and tuber rot on ginger and radish (Chen et al., 2023; Zhang et al., 2020; Wang et al., 2023). Management and prevention of plant diseases and pests include the utilization of pesticides.

Pesticides have advantages in crop production and disease management, but the use of pesticides presents serious environmental risks and impacts to public health (Ahmad et al., 2024). One of the sustainable alternatives to the use of pesticides is the utilization of endophytic bacteria that have biocontrol properties. However, comprehensive studies regarding the isolation and identification of endophytic bacteria from mango as effective biological control agents against the bacterial blight can still be considered insufficient.

Diversity exists in the number and morphological characteristics of isolating endophytes from varying plant tissues in different varieties of mango trees (Dashyal et al., 2019). This could suggest that isolation and identification of bacterial endophytes in mango from other locations can display different antimicrobial activity against the harmful pathogens causing bacterial blight and increase the possibility of using endophytic bacteria as biocontrol agents against plant diseases. Thus, the study aims to isolate endophytic bacteria from selected parts of the mango tree (leaves, stems, and roots) and determine the potential use of the isolated endophytic bacteria as a biocontrol agent against the bacterial blight.

MATERIALS AND METHODS

Sample collection

Mango (*M. indica*) plant samples were collected from different locations in San Jose, Camarines Sur. Leaves, stems, and roots were randomly collected. Samples were transported in a cooler and immediately processed in the laboratory. Surface sterilization was done by washing the samples with distilled water to remove attached materials. Samples were

subsequently immersed in 70% ethanol, washed with 1.0% hypochlorite solution, rinsed with 70% ethanol, and finally washed with sterile distilled water (Sharma et al., 2022; Khanam et al., 2017).

Isolation of endophytic bacteria

Surface-sterilized samples were cut into small sections about 2−3 cm long and placed onto petri plates containing Nutrient Agar (5 g peptone, 3 g beef extract, 5 g NaCl, and 15 g agar for 1 liter of distilled water) medium supplemented with nystatin, at a concentration of 30 μg/mL, for bacterial growth (Basumatary et al., 2021). Three replicated plates with 10 segments were prepared from each sample. Plates were incubated at 28 ^{o}C for 72 hours (Sangwan et al., 2021). Following incubation, morphologically distinct bacterial colonies were isolated and purified on NA (Anjum & Chandra, 2015).

Morphological and biochemical characterization of endophytic bacteria

The isolated endophytic bacteria were characterized morphologically and biochemically. Morphological features like shape, size, and arrangement were determined. Gram staining was utilized to identify the morphology of the isolates. After the morphological characterization, isolates were subjected to perform the biochemical tests, such as starch hydrolysis (amylase) and catalase test to characterize and assess their metabolic capabilities based on the chart of Bergey's manual of determinative bacteriology for systematic identification (Kiros et al., 2023). The starch hydrolysis or amylase test was performed by streaking the bacterial culture onto a starch agar plate, and incubated at 28 ^{o}C until growth was observed. After incubation, the plate was flooded with an iodine solution. The iodine reacted with the starch, turning the medium blue-black, indicating that no starch-splitting enzymes were present. If the starch had been hydrolyzed, a clear zone of hydrolysis was observed, showing a positive result. For the catalase test, this involved the addition of 3% of hydrogen peroxide (H_2O_2) to the glass slides containing a bacterial colony from Nutrient Agar (NA) plates. A bubble formation indicated a positive result (Cappuccino & Welsh, 2017).

Isolation and characterization of bacteria causing bacterial blight

Mango leaves samples that exhibited black spots surrounded by a yellow halo were collected and surface sterilized, as mentioned in the above section. After sterilization, cut leaves were macerated using a sterile pestle and mortar. The extract was transferred onto the Yeast Peptone Glucose Agar (YPGA) plates and spread evenly across the agar surface using a sterile L-shaped glass rod. YPGA is a growth medium composed of 18 g/L of Agar, 7 g/L of glucose, 7 g/L of yeast, and 7 g/L of peptone for 1 liter of distilled water, subsequently sterilized through autoclaving at 121 ^{o}C under a pressure of 1 bar for 15 minutes. Plates were incubated at 28 ^{o}C for 24 hours (Jean-Martial et al., 2021). Following incubation, morphologically distinct bacterial colonies were isolated and purified on YPGA and were characterized morphologically and biochemically.

Molecular identification

The isolates of both endophytic bacteria and bacterial blight-causing pathogens were identified through sequencing and analysis. A pure culture of each bacterial isolate was prepared, and cells were collected and sent to Macrogen Inc., South Korea, for 16S rRNA sequencing. Partial 16S rRNA sequences were imported, checked for sequencing quality, and trimmed using the Staden Package software (Staden, 1996), after which the consensus sequences were compiled and matched to the reference strains found in the National Center of Biotechnology Information (NCBI) GenBank database through BLASTn (Basic Local Alignment Search Tool) (NCBI, 2013). Rooted phylogenetic tree construction used MEGA5 (Tamura et al., 2007).

Antagonistic activity

The agar well diffusion assay was used to test the ability of bacterial endophytes to

inhibit pathogenic microorganisms identified. Test organisms were swabbed into Mueller-Hinton Agar, MHA (Sigma-Aldrich), and 7 mm wells were bored on the agar. Bacterial endophytes were also grown in Nutrient Broth (NB) and incubated for 24 hours at 37 ^{o}C. After incubation, cultures were centrifuged at 12,000 rpm for 3 minutes, and the supernatants were collected. Then, 100 µL of supernatant was added to the respective wells. Plates were incubated for 24 hours at 37 ^{o}C. Chloramphenicol was the positive control, and sterile distilled water was used as the negative control. Antimicrobial activity was detected by measuring the zone of inhibition that appeared after the incubation period (Balouiri et al., 2015).

Statistical analysis

All data obtained were subjected to one-way analysis of variance (ANOVA), and the mean differences were compared by a least significant deviations (LSD) test. P values $\leq$ 0.05 were considered significantly different. Statistical analysis of the data was done using SPSS Statistics version 20.

RESULTS AND DISCUSSION

Isolation of bacterial endophytes

A total of 20 different samples were collected from a mature and healthy mango tree, from various locations within the barangays of Camagong, Telegrafo, and Catalotoan in San Jose, Camarines Sur. The multiple samples aimed at ensuring the comprehensive potential variability of the bacterial endophytic communities across different tissues and geographical areas. This method increases the probability of identifying a broader range of beneficial endophytes potentially adapted within the tree (Dashyal et al., 2019). Thirty-three bacterial endophytes were obtained from the peripheral regions of the cut samples. Out of which seven isolates were from the leaves, 11 were from the stems, and 15 isolates were from the root tissues (Table 1).

The results showed that more endophytic bacteria were isolated from the root tissues than from the leaves and stems. These findings strengthen the existing literature and studies suggesting that the roots are known for possessing a more diverse and abundant microbial community, such as bacteria. Few studies have notably assessed that the composition of root microbiota differs within and between the plant species under the natural conditions (Aleklett et al., 2014). Plant roots are the interface between the plants and soil, which provides a diverse and nutrient-rich environment to support the bacterial microorganisms that influence the plant's growth and tolerance to biotic and abiotic stress (Philippot et al., 2013).

The stems, although less directly influenced by the soil conditions than roots, according to the result of isolation, demonstrated more bacterial endophyte isolates than the leaves. According to Lin et al. (2022), root tissues possess the most diverse endophytes for endophytic bacteria, followed by stems and old leaves, while new leaves have the lowest diversity. Most of the dominant endophytes showed an obvious range and tissue preferences. The vascular system, particularly the xylem in the stem, allows for the adaptation of certain bacteria to help their survival and growth (De La Fuente et al., 2022).

The low number of endophytic bacterial isolates on leaves may result from extreme environmental conditions, such as low moisture, nutrient deficiency, and high temperature, which challenge the bacteria to survive and limit their populations on the leaf surface. The number of phyllobacteria and their species composition vary with the host and the geographical area (Countinho & Bophela, 2021).

Characterization of bacterial endophytes

Colony and cell morphology were used to characterize the bacterial isolates morphologically (Table 1).

The biochemical characterization shows the diverse enzymatic profile among the endophytic bacterial isolates (Table 2). These extracellular enzymes or hydrolases target

various macromolecules such as carbohydrates, lignin, organic phosphate, proteins, and sugars. Hydrolases help plants to establish systemic resistance against pathogenic invasion (Shivalingaiah et al., 2024). The release of extracellular enzymes helps overcome the competition with other microbial agents.

Table 1. Morphological characterization of endophytic bacterial isolates

Isolate code	Cell morphology	Colony morphology				
	Shape	Surface	Color	Margin	Elevation	Opacity
Leaves						
TLG S2-L1	Rod/Circular	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
TLG S2-L3	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CMG S2-L1	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S2-L1	Rod	Dry, Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S2-L2	Rod	Smooth	White	Entire	Umbonate	Opaque (not clear)
CTL S2-L3	Rod	Dry	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S3-L3	Rod	Smooth, wrinkled	White	Undulate	Umbonate	Opaque (not clear)
Stems						
CMG S1-S1	Rod	Dry, Wrinkled	White	Undulate	Umbonate	Opaque (not clear)
CMG S1-S2	Circular	Rough	Creamy-white	Filiform	Raised	Opaque (not clear)
CMG S2-S1	Rod	Rough	Creamy-white	Undulate	Umbonate	Opaque (not clear)
CMG S2-S3	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S1-S1	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S1-S2	Rod	Rough	Creamy-white	Undulate	Umbonate	Opaque (not clear)
CTL S1-S3	Rod	Rough	Creamy-white	Undulate	Umbonate	Opaque (not clear)
CTL S2-S1	Rod	Smooth, Glistening	Creamy-white	Entire	Convex	Opaque (not clear)
CTL S2-S2	Rod	Smooth, Glistening	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S3-S2	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)

Isolate code	Cell morphology	Colony morphology				
	Shape	Surface	Color	Margin	Elevation	Opacity
CTL S3-S3	Rod	Rough	Creamy-white	Entire	Raised	Opaque (not clear)
Roots						
CMG S1-R2	Rod	Rough	Creamy-white	Undulate	Flat	Translucent (clear)
CMG S1-R3	Rod	Rough	Creamy-white	Undulate	Umbonate	Opaque (not clear)
CMG S3-R3	Rod	Smooth, Glistening	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S1-R1	Rod	Rough	Creamy-white	Undulate	Umbonate	Opaque (not clear)
CTL S1-R2	Rod	Dry, Wrinkled	Creamy-white	Lobate	Umbonate	Opaque (not clear)
CTL S1-R3	Rod	Rough	Creamy-white	Filiform	Raised	Opaque (not clear)
CTL S2-R1	Rod	Rough	Creamy-white	Filiform	Umbilicate	Opaque (not clear)
CTL S2-R2	Rod	Rough	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S2-R3	Rod	Rough	Creamy-white	Undulate	Umbilicate	Opaque (not clear)
CTL S3-R1	Rod	Smooth	Creamy-white	Undulate	Raised	Opaque (not clear)
CTL S3-R2	Rod	Rough	Creamy-white	Undulate	Raised	Translucent (clear)
TLG S1-R1	Rod	Rough	Creamy-white	Entire	Raised	Opaque (not clear)
TLG S1-R2	Enlarged, Rod	Dry	Creamy-white	Undulate	Flat	Opaque (not clear)
TLG S1-R3	Rod	Smooth, Glistening	Creamy-white	Filiform	Pulvinate	Opaque (not clear)
TLG S2-R1	Rod	Dry, Rough	Creamy-white	Filiform	Flat	Opaque (not clear)

Legend: Camagong (CMG); Catalotoan (CTL); Telegrafo (TLG).

According to Bodalo et al. (2023), endophytic bacteria secrete enzymes to defend against pathogens by protecting the plant. The biocontrol activity of any endophytes can be evaluated by measuring the enzymes secreted by the biocontrol agents. The involvement of these enzymes in the degradation of pathogenic compounds in suppressing the growth of pathogens can assess the potential of biocontrol agents (Kashyap et al., 2023).

Table 2. Biochemical characterization of endophytic bacterial isolates

Test Isolates	Isolate Code	Starch/ Amylase Test	Catalase Test	Test Isolates	Isolate Code	Starch/ Amylase Test	Catalase Test
Leaves	TLG S2-L1	–	+	Roots	CMGS1-R2	–	+
	TLG S2-L3	–	+		CMGS1-R3	–	+
	CMG S2-L1	–	+		CMGS3-R3	+	+
	CTL S2-L1	–	+		CTL S1-R1	–	+
	CTL S2-L2	+	+		CTL S1-R2	+	+
	CTL S2-L3	+	+		CTL S1-R3	+	+
Stems	CMG S1-S1	+	+		CTL S2-R1	+	+
	CMG S1-S2	–	+		CTL S2-R2	–	+
	CMG S2-S1	–	+		CTL S2-R3	+	+
	CMG S2-S3	+	+		CTL S3-R1	–	+
	CTL S1-S1	–	+		CTL S3-R2	+	+
	CTL S1-S2	–	+		TLG S1-R1	–	+
	CTL S1-S3	–	+		TLG S1-R2	–	+
	CTL S2-S1	–	+		TLG S1-R3	+	+
	CTL S2-S2	–	+		TLG S2-R1	–	+
	CTL S2-S3	–	+				
	CTL S3-S3	–	+				

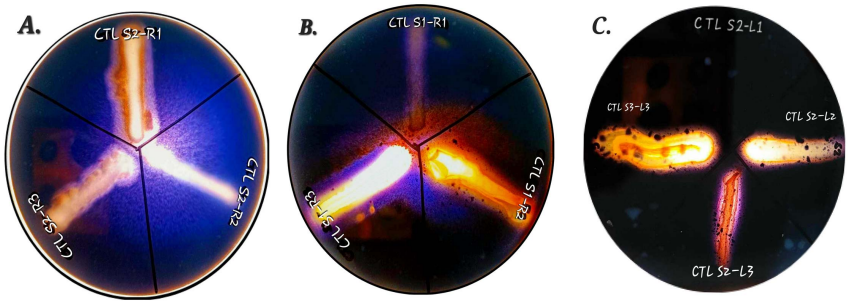

Figure 1. Amylase test result. Showcasing plates inoculated with the bacterial isolates (Kiros et al., 2023)

Isolation and characterization of bacteria causing bacterial blight

Pathogens primarily cause bacterial blight on mango, an emerging disease affecting mango-producing areas, including Asia. This disease exhibits various symptoms, such as dark lesions on leaves and fruits. Early symptoms on leaves include water-soaked patches with angular black dots and, at times, have chlorotic spots, which impact the yield and fruit quality (Sossah et al., 2024). The bacterial blight-causing pathogen was isolated, and the pure culture bacterial colonies on the Yeast Peptone Glucose Agar (YPGA) plates (Fig. 2) were cream-white colored, smooth and circular.

The bacterial isolates were morphologically and biochemically characterized. Table 3 showed that all the isolates were Gram-negative and rod-shaped. The biochemical tests revealed that all isolates were positive for amylase, catalase, and potassium hydroxide (KOH) tests. A positive amylase test indicates the bacterial strain's ability to degrade starch. A positive catalase test revealed that the bacteria can produce the enzyme catalase,

which helps the bacteria to survive in an oxygen-rich environment. Furthermore, a positive KOH test indicates that the bacteria are Gram-negative (Aryal, 2022), confirming the Gram stain result.

Figure 2. Pure culture of bacteria causing bacterial blight isolates (Xcc - 1) on YPGA plate

Using 16S rRNA, the isolate Xcc. was identified as *E. asburiae. E. asburiae* is an opportunistic pathogen (Mardaneh & Soltan-Dallal, 2016). It caused several types of infections, such as pneumonia, septicemia, and urinary tract infection (Marchetti et al., 2024). However, *E. asburiae* has been found as an emerging pathogen causing bacterial blight in various types of crops, causing bacterial leaf blight on sorghum, and tuber rot on ginger and radish (Chen et al., 2023; Zhang et al., 2020; Wang et al., 2023). The study isolated *E. asburiae* from leaves of mango exhibiting bacterial blight symptoms and found that it can cause bacterial blight disease in mango. To our knowledge, this is the first detection of *E. asburiae* causing bacterial blight in mango.

Table 3. Morphological and biochemical characterization of isolates (bacterial causing bacterial blight)

Classifications	Feature	Xcc-1	Xcc-2	Xcc-3
Cell Morphology	Shape/Form	Rod-shaped	Rod-shaped	Rod-shaped
Biochemical Characterization	Amylase Test (+/-)	+	+	+
	Catalase Test (+/-)	+	+	+
	KOH Test (+/-)	+	+	+

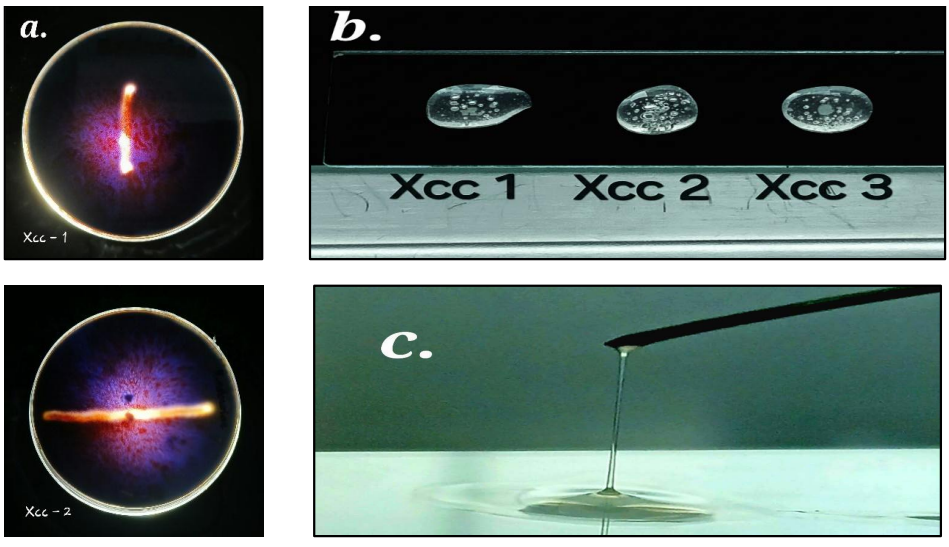

Figure 3. Biochemical test results. (a) The amylase test of isolate Xcc-1 showed a positive result (Kiros et al., 2023). (b) As indicated by bubble formation, the catalase test showed positive results for all three bacterial isolates (Xcc-1, Xcc-2, and Xcc-3) (Cappuccino et al., 2014). (c) KOH test of isolated Xcc-3 showing a positive result string formation (Aryal, 2022)

Table 4. Molecular identification of bacterial pathogen

Isolate	Genbank Accession Number	Identity (%)	Consensus Sequence Length	Genus
Xcc.	JQ682630	99%	1469	*Enterobacter asburiae*

The evolutionary history was inferred using the Neighbor-Joining method (Saitou, 1987). The optimal tree with the sum of branch length = 0.18098040 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004) and are in the units of the number of base substitutions per site. The analysis involved five nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 648 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Kumar, 2024).

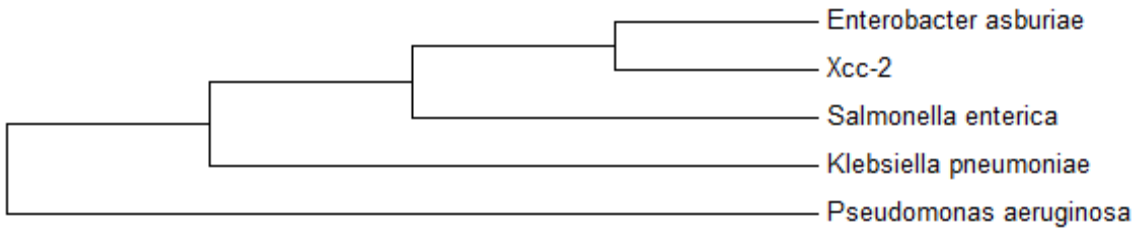

Figure 4. Rooted phylogenetic tree of a bacterial pathogen isolated, inferred from the nearest neighbor of bacterial isolates, based on the Neighbor-Joining method (Tamura et al., 2004) using MEGA5 (Kumar et al., 2024)

Antagonistic activity of endophytic bacteria against *Enterobacter asburiae*

About thirty-three bacterial endophytes were isolated from various parts of the mango tree, including seven isolates from leaves, 11 isolates from stems, and 15 isolates from roots (Table 1). The antibacterial activities of the bacterial isolates were assayed against *E. asburiae.*

According to the obtained result, the inhibitory effect of the isolates was classified as inactive, partially active, or active. The gained results (Table 5) demonstrated a significant antibacterial activity against *E. asburiae* from bacterial isolates CMG S1-S1 (11.33 mm), CTL S1-R1 (10.67 mm), CTL S2-R1 (16.67 mm), and CTL S3-R2 (11 mm) that were isolated from *M. indica.* The growth of *E. asburiae* was suppressed by the endophytic bacterial isolate CTL S2-R1, belonging to the genus *Alcaligenes*, that exhibited the highest growth inhibition against the bacterium causing bacterial blight *E. asburiae*.

A one-way analysis of variance was used to determine whether the three test isolates were significantly different from each other in terms of statistical analysis. Table 6 showed p-values of less than the level of significance 0.05, which are highly significant, leading to the rejection of the null hypothesis (Ho). According to Thiese et al. (2016), a p-value less than 0.05 (typically < 0.05) level of significance is considered statistically significant, and a p-value higher than 0.05 (> 0.05) is not statistically significant. Specifically, the analysis resulted in an F-value of 5.354 (Leaves), 15.665 (Stems), and 23.449 (Roots) with corresponding p-values of 0.005, 0.000, and 0.000, respectively. It indicates that test isolates (leaves, stems, and roots) are statistically significant in their antagonistic activity against the pathogen. Endophytic bacterial isolates with strong enzymatic

activities often exhibited both antimicrobial and plant growth-promoting properties (Tsipinana et al., 2024). This finding aligned with insights about plant growth-promoting bacteria (PGPB), which usually express hydrolytic enzymes such as amylase, cellulase, protease, and lipase, to help inhibit a pathogenic microbe. In Table 2, the positive isolates on the catalase and amylase test corresponded with a higher inhibition zone in the in-vitro antagonistic assay (Table 5), effectively inhibiting the growth of *E. asburiae*. The findings showed that the isolates have a strong correlation between enzymatic activity and antimicrobial efficacy, which served as a selection for identifying promising isolates as biocontrol agents against emerging bacterial blight. Hence, the test isolates from leaves, stems, and roots significantly differ in their antimicrobial effects.

Table 5. Antagonistic activity of isolated endophytic bacteria against *Enterobacter asburiae*

Isolate code	Average zone of inhibition (mm)	Interpretation
CMG S1-S1	11.33	Partially Active
CTL S1-R1	10.67	Partially Active
CTL S2-R1	16.67	Active
CTL S3-R2	11	Partially Active
● The average positive control (Chloramphenicol) from the following isolates is 10.92 mm, and the average negative control (sterile distilled water) is **0 mm**.		

Table 6. One-way ANOVA

Test Isolates	f	p-value	Interpretation
Leaves	5.354	0.005	Significant
Stems	15.655	0.000	Significant
Roots	23.449	0.000	Significant

Molecular identification of isolates

Using 16S rRNA sequences, the isolates CTL S2-R1, CTL S1-R1, CTL S3-R2, and CMG S1-S1 were consecutively identified as belonging to the genera *Alcaligenes, Paenibacillus*, *Proteus*, and *Bacillus*.

Table 7. Molecular identification of endophytic bacteria

Isolate	Genbank Accession Number	Identity (%)	Consensus Sequence Length	Genus
CMG S1-S1	KX354355	98%	1529	*Bacillus*
CTL S1-R1	AB746175	99%	1502	*Paenibacillus*
CTL S2-R1	JF710954	98%	1501	*Alcaligenes*
CTL S3-R2	CP021852	99%	4209445	*Proteus*

The evolutionary history was inferred using the Neighbor-Joining Method (Saitou, 1987). The optimal tree with the sum of branch length = 0.549 is shown in Figure 5. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004) and are in the units of the number of base substitutions per site. The analytical procedure encompassed 14 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions. The pairwise deletion option was applied to all ambiguous positions for each sequence pair, resulting in a final data set comprising 2,398 positions. Evolutionary analyses were conducted in MEGA12 (Kumar, 2024).

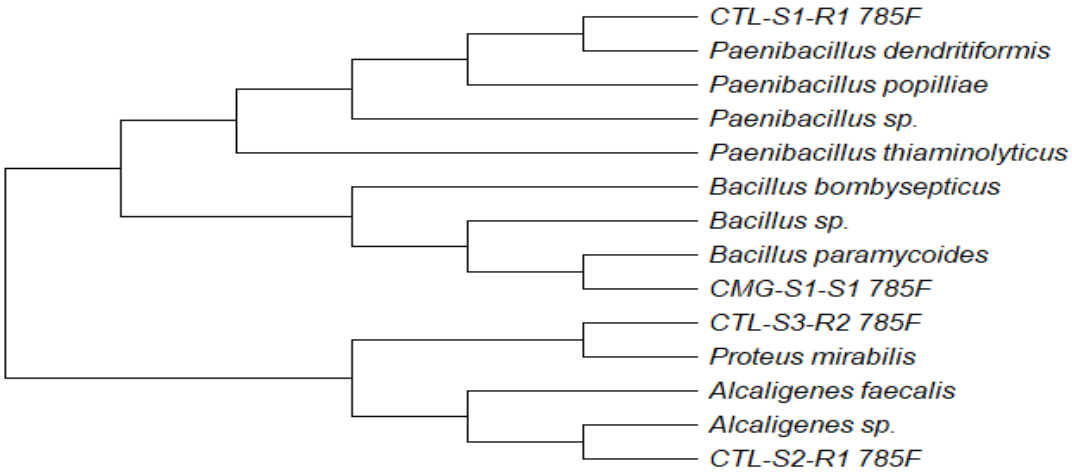

Figure 5. Rooted phylogenetic tree of bacterial endophyte isolated, inferred from the nearest neighbor of bacterial isolates, based on Neighbor-Joining method (Tamura et al., 2004) using MEGA12 (Kumar et al., 2024)

The genus *Bacillus* is everywhere, as it can reside and be found on the surface, in air, water, soil, within the plants and rhizosphere, gastrointestinal tract, and other extreme environments. Some of the *Bacillus* are used in agriculture for various uses such as industrial production, environmental safety, and sufficient biocontrol efficacy (Ali et al., 2024). *Bacillus* spp. can inhibit the growth of different pathogens by producing various biologically active compounds (Liang et al., 2022). According to Kim et al. (2023), *Bacillus* species are promising biocontrol agents against various plant pathogens. These bacteria can suppress pathogen growth through multiple mechanisms, such as antibiosis, nutrient and space competition, and systemic resistance, notably contributing to plant growth.

The genus *Paenibacillus*, a plant growth-promoting bacterium (PGPB), is highly effective as an environmentally friendly alternative in reducing the use of chemicals. *Paenibacillus* spp. have various mechanisms, such as the production of lipopeptides, the induction of systemic resistance (ISR), hydrolytic enzymes, and volatile organic compounds that help combat plant phytopathogens. These properties gave the *Paenibacillus* strains the capability to suppress the growth of fungi, and some strains of *Paenibacillus*, such as *Paenibacillus polymyx*, have shown efficacy in controlling fungal diseases in plants (Dobrzynski et al., 2024).

Numerous strains from the genus *Alcaligenes* have recently been reported to be suppressive bacteria of plant pathogens. Specifically, *Alcaligenes faecalis* strains isolated from the rhizosphere of plants are being utilized broadly in the agriculture and pharmaceutical industries these days. The *A. faecalis* strains have been reported to have antifungal activities and have been used as biocontrol agents in controlling sheath blight and blast disease in rice (Kakar et al., 2017).

Proteus spp. bacteria are known to be human opportunistic pathogens, as they are isolated from urine, wounds, and other clinical sources. However, the ability of *Proteus* spp. to tolerate or use polluting compounds and promote plant growth has made it possible to utilize these microorganisms for bioremediation and environmental protection (Drzewiecka, 2016).

Based on the previous studies, the genera *Bacillus, Paenibacillus, Alcaligenes,* and *Proteus* can suppress and control the growth of different pathogens. As they were reported to have other mechanisms for producing

biologically active compounds and have antifungal activities, they can inhibit the growth of pathogens and fungi. This provided the knowledge and supported the study that the identified isolates, *Bacillus, Paenibacillus, Alcaligenes*, and *Proteus*, can potentially be used as biocontrol agents against plant diseases such as the bacterial blight disease.

CONCLUSION

Thirty-three endophytic bacteria were successfully isolated and characterized from mango trees in San Jose, Camarines Sur. Morphological and biochemical characterization tests revealed a predominance of Gram-negative bacteria. Four isolates were distinguished as potential biocontrol agents against the emerging bacteria causing bacterial blight through the agar well diffusion. They identified CTL S2-R1 as it exhibits the most vigorous antagonistic activity against bacterial blight, with a zone of inhibition of 16.67 ±1.53 (mm). At the same time, the other isolated endophytic bacteria, CTL S1-R1, CTL S3-R2, and CMG S1-S1 were consecutively identified as *Paenibacillus, Proteus*, and *Bacillus,* which showed moderate activity. These findings suggest the potential of these endophytic bacteria, particularly CTL S2-R1, identified as *Alcaligenes*, as biocontrol agents against mango bacterial blight.

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