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Enterobacter tabaci and *Bacillus cereus* as biocontrol agents against pathogenic *Ralstonia solanacearum* of eggplant

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Abstract. *Malek AA, Ali NS, Kadir J, Vadamalai G, Saud HM. 2023.* Enterobacter tabaci *and* Bacillus cereus *as biocontrol agents against pathogenic* Ralstonia solanacearum *of eggplant. Asian J Trop Biotechnol 20: 24-30.* Over the world, *Ralstonia solanacearum* caused bacterial wilt disease is responsible for more than 90% of agricultural losses. Therefore, finding a potential biological control agent is extremely important, especially against *R. solanacearum.* The present study aimed to isolate, evaluate and identify potential endophytic bacteria to restrain *R. solanacearum* via in vitro antagonistic assay. A total of 116 endophytic bacteria were isolated, and 55 exhibited abilities to restrain *R. solanacearum* in vitro. However, only two isolates, UPMBC1 and UPMBE2, significantly inhibited R. solanacearum growth by 14.6 mm and 12.6 mm, respectively. Based on the cultural and morphological characteristics, UPMBC1 and UPMBE2 isolates exhibited similar characteristics as the other members of Enterobacteriaceae and Bacillaceae, respectively. Molecular results revealed that UPMBC1 was identified as *Enterobacter tabaci* and UPMBE2 as *Bacillus cereus*. Numerous efficient biological control agents were identified in the present investigation, with *E. tabaci* showing the greatest potential against *R. solanacearum.*

Keywords: Antagonistic assay, *Bacillus cereus*, bacterial wilt disease, *Enterobacter tabaci*, potent endophytic bacteria, *Ralstonia solanacearum*

INTRODUCTION

The eggplant originated from Indo-Burma, based on a Sanskrit document dated as early as 300 BCE (Daunay and Janick 2007). Later, it was brought to China, Japan, East Africa, and the Mediterranean Basin before spreading across the globe as time passed. Eggplant production in Malaysia showed a progressive trend, increasing from 39,103 metric tons (mt) in 2019 to 42,809 mt in 2021 (*Jabatan Pertanian* 2021). The statistical data also reported that Malaysia had achieved over 100% self-sufficiency in eggplant production.

Ralstonia solanacearum (Smith) can infect more than 200 different crop species worldwide that cover 50 families (Achari and Ramesh 2014; Hemelda et al. 2019). Its infection occurs in tropical, sub-tropical, and temperate climates worldwide, with profound severity noted in tropical climate countries (Mondal et al. 2014). The pathogen is capable of destroying crops and causing significant yield losses of up to 90% (Artal et al. 2012). The prominent effects of Bacterial Wilt (BW) disease can be observed in tomatoes, bananas, eggplant, and potatoes, with severity ranging from 80% to 100%. In Ethiopia, 55% of wilt incidence was recorded on tomatoes, which greatly reduced its production and affected the production of other solanaceous crops (Yadessa et al. 2010). In India, BW disease was a major constraint on tomatoes and eggplant in major-growing areas (Mondal et al. 2014). In southwestern Nigeria, 80% of the tomato yield was highly reduced due to BW infection (Popoola et al. 2014). Similar incidents were reported in banana cultivation in Malaysia, affecting 90% of the yields in 2014 (Zulperi and Sijam 2014). The first infection with BW disease was reported in Guyana in 1840 by Thurston and Galindo (1989). Later, Burrill discovered the causal agent when it infected the potato in Japan in 1890 (Tahat and Sijam 2010). Many ongoing studies have focused on finding the most effective control of *R. solanacearum* concerning the environment and human safety, as a pathogen is not easy to eradicate. The non-sporulate bacterium can survive for years in dry soil or the absence of the host (Stander et al. 2013). Infection would occur even if the pathogen population were low (Nair 2013). Moreover, *R. solanacearum* can persist for months or years in the host even after the host has died, providing inoculum for the next infection (Scherf et al. 2010). The variability of the strain and widely distributed hosts even make it difficult to resist the BW infection (Blomme et al. 2017).

Since there is no promising control for BW disease, farmers rely on the resistant cultivar to resist the infection (Artal et al. 2012). However, the resistant cultivar is also restricted to certain *R. solanacearum* strains. Alternatively, researchers switched to the biological control approach to control BW and found it effective in controlling this soil-borne disease (Lwin and Ranamukhaarachchi 2006). Endophytic Bacteria (EB) were known for their symbiotic relationship with the host plant and were widely used to suppress *R. solanacearum*. Generally, EB colonizes internal plant tissues and exists in the root and xylem of plants. Extensive colonization by EB acts as a barrier to prevent the wilt pathogen from establishing in the vascular tissues (Bell et al. 1995). Some genera proved effective in suppressing the growth of *R. solanacearum*, such as *Streptomyces* sp. *Bacillus* sp., *Pseudomonas* sp., and *Enterobacter* sp. (Liu et al. 2009; Xue et al. 2009). EB's benefit the host plant by contributing to plant growth by fixing the nitrogen, solubilizing phosphate, and producing a siderophore for the iron uptake (Egamberdieva et al. 2017). In addition, EB acted as a root colonizer, capable of expelling the wilt pathogen during nutrient source competition (Toyota et al. 2000). EB's secretion of volatile compounds and metabolites could help the plant defense system (Tahir et al. 2017). In a nutshell, EB has the potential to act as a phytostimulant, biological control agent, biofertilizer, and soil remediation agent. However, it was hard to find novel and potent EB against *R. solanacearum* as this pathogen was genetically diverse, had a broad geographical distribution, and had long persistence in soil. Therefore, the objectives of this study were (i) to isolate and evaluate the virulence of potential EB against *R. solanacearum* and (ii) to characterize and identify the potent EBs against *R. solanacearum* via cultural, morphological, and molecular techniques.

MATERIALS AND METHODS

Isolation of pathogen and pathogenicity test

R. solanacearum was isolated from wilted eggplant on a small private farm in Kuala Selangor, Malaysia. The infected stem was recognized by the wilting symptom and thick slime running out when placed in water. Wilted stems were surface sterilized by dipping them in 20% sodium hypochlorite (NaOCl) for 30 seconds and then washing them three times with sterile distilled water. Next, 1 cm of each end of the wilted stem was discarded, while the remaining length was cross-sectioned and dipped in sterile distilled water to collect the bacterial slime (ooze). One loop of the infected distilled water was streaked on Kelman Tetrazolium Chloride (TZC) agar to confirm the pathogen and incubated for 48 hours at 25°C. The most virulent strain was chosen for the pathogenicity test. *R. solanacearum* was inoculated in Casein-Protease-Glucose (CPG) broth and shaken for 48 hours at 25°C at 140 rpm. The concentration was determined via spectrophotometer at 600 nm absorbance using Multiscan™ GO equipment (Thermo Scientific, Germany) and adjusted to 10^{10} CFU/mL. Ten replicates of 30-day-old commercial eggplant seedlings, Antaboga hybrid (F1), were soil-drenched with 30 mL of *R. solanacearum* inoculated CPG broth. The soil of the control pots was drenched with 30 mL of sterile distilled water. An adequate amount of water was applied to the seedlings twice daily. The seedlings were monitored for wilting symptoms for three weeks, and Disease Severity (DS) was calculated according to the formula of Winstead and Kelman (1952).

$$\text{Disease severity (DS) (\%)} = [\sum (ni \times v_i) \div (V \times N)] \times 100$$

Where:

ni : the number of plants with the respective disease rating v_i : disease rating

V : highest disease rating

N : number of plants observed.

The disease rating was measured on the following scale: 1: no symptoms; 2: one leaf wilted; 3: two-three leaves wilted; 4: more than 4 leaves wilted; and 5: the whole plant wilted.

Isolation of endophytic bacteria

Therefore, the healthy stems of eggplant, chili, and tomato (8-12 weeks old) were collected from seven states of Peninsular Malaysia: Johor, Selangor, Penang, Kedah, Perak, Pahang, and Negeri Sembilan. 11 cm of the main stem for each sample was excised, and the bark was discarded. The later, excised stem was dipped in 20% NaOCl for one minute and washed using sterile distilled water thrice. The washed water was streaked onto the Nutrient Agar (NA) as a control to ensure sterilization. One cm of each end of the stem was discarded, and the remaining stem was cut into 3 cm lengths horizontally to expose the interior part using a sterile blade. The pieces were transferred into a 200 mL 1×PBS (Phosphate Buffered Saline) buffer and shaken at 140 rpm for 48 hours at 25°C. After 48 hours, one loopful of EB-containing buffer was streaked on NA and incubated for four days at 25°C. After four days, different colonies of EB were selected based on their shape, color, colony, and texture. Subsequently, EB was purified onto fresh NA until a single colony was obtained. In addition, five replications were performed for each different crop.

In vitro antagonistic assessment

Purified EB (individually) and *R. solanacearum* were cultured in 5 mL of Nutrient Broth (NB) and CPG broth, respectively, and shaken at 140 rpm for 48 hours at 25°C. Next, 250 mL of NA was prepared, and 150 µL of *R. solanacearum* broth was added to every 100 mL of NA, swirled, and poured onto a Petri plate. Three wells were made for each plate using a sterile 1 cm cork borer (in diameter). Next, 25 µL of EB suspension was pipetted into each well, and the plates were sealed. In the control plates, distilled water was used. All plates were incubated for four days at 25°C, and the growth radius of EB (inhibition radius) was measured from the edge of the well in mm (direction: top, below, left, and right). Ten replications were performed for each EB. Isolates with the highest radius inhibition (>10 mm) were selected for further experiments.

Cultural and morphological characterization

The colony was characterized by evaluating the diameter, margin, pigmentation, opacity, texture, and form. In addition, Gram-staining was performed to observe the types of cell walls and cell shapes described by Tankeshwar (2015). The Gram-staining slide was observed on a light microscope Olympus CX31 RBSF (Olympus Corporation, Japan) under 40X, 100X, 400X, and 1000X magnification (using oil immersion). The images were magnified using the Dino-Lite USB microscope camera AM 4515ZTL (AnMo Electronics Corporation, Taiwan) and captured using the Dino Capture 2.0 software (AnMo Electronics Corporation, Taiwan).

Molecular identification

Molecular identification was performed using the Polymerase Chain Reaction (PCR) method (Walch et al. 2016). Only EB with more than 10 mm of mean inhibition zone measurement in in vitro inhibition bioassay was subjected to molecular identification. Next, 50 µL of master mix for each EB was prepared. The master mix contained 1.25 µL of Taq polymerase, 1.0 µL of dNTPs, 2.5 µL each of 1429R (5' GGTTACCTTGTTACGACTT 3'), and 27F primers (5' AGAGTTTGATCCTGGCTCAG 3'), 5.0 µL of PCR buffer and 37.75 µL of sterile distilled water. A sterile 10 µL tip was used to dip slightly on the EB colony and mixed with the 50 µL master mix mixture. As for the control, no EB was added. Polymerase Chain Reaction tubes (0.5 mL) containing the prepared master mix were transferred into the PCR machine (c1000 Bio-Rad Thermal Cycler, United States) for amplification.

Next, 1% agarose gel was prepared using 1 × Tris-acetate-EDTA (TAE) buffer, microwaved, and poured into a casting tray. The gel was left to solidify for 20 minutes and subsequently transferred into an electrophoresis gel documentation system (Thermo Fisher Scientific, United States) containing 1 × TAE buffer (Appendix A). Next, 1 µL of 100 bp Thermo Fisher Scientific ladder was mixed with 1 µL loading dye and pipetted into the first well as a marker. Next, the amplified PCR products were pipetted into the wells of solidified agarose gel. Finally, 1 µL of PCR product was mixed with 1 µL of loading dye (Thermo Fisher Scientific) to give color. Electrophoresis was run for 45 minutes at 85 volts until the dye reached approximately 80% of the gel. The gel obtained from the electrophoresis was placed in the Bio-rad Gel Doc XR Imaging System (Bio-rad, United States) and viewed under Ultraviolet (UV) light for the band. Subsequently, the PCR products of each EB were sent to NHK Bioscience Solution Sdn. Bhd. for sequencing.

Analysis of Variance (ANOVA)

Statistical analysis was done using Tukey's Honest Significant Difference (HSD) with probability ($P<0.05$) and performed via the Statistical Analysis System (SAS) software version 9.4 (SAS Institute, United States).

RESULTS AND DISCUSSION

Isolation of pathogen and pathogenicity test

The isolation result showed that three different *R. solanacearum* strains were isolated based on cultural appearances. Among all three strains, only one strain produced a fluidal pinkish colony with a whitish margin, indicating a virulent strain (Figure 1A). The other isolated strains produced a deep red colony with a non-fluidal and non-whitish margin, indicating a non-virulent strain. In the pathogenicity test, eggplant showed low wilting in the first and second weeks with 20% Disease Severity (DS). However, in the third week, DS was accelerated to 80%. The symptoms were wilted green leaves remained attached to the stem. The comparison between infected and control eggplant seedlings in the pathogenicity test is shown in Figure 1B.

In vitro antagonistic assessment

Furthermore, a total of 116 isolates were obtained from healthy chili, eggplant, and tomato plants. The highest (39) isolates of EB were obtained from chili, while eggplant and tomato yielded the lowest EB with 11 isolates. Out of 116 isolates, 55 showed inhibition against *R. solanacearum* growth in in vitro antagonistic assessment. However, only 27 isolates were subjected to ANOVA analysis based on their consistency in inhibiting the growth of *R. solanacearum.* Based on the analysis, the highest (14.64 mm) mean radius of inhibition was observed by UPMBC1, followed by UPMBE2, with a mean radius of 12.55 mm. The UPMBC1 was isolated from chili in Selangor, while UPMBE2 was isolated from eggplant in Selangor. Among the 27 isolates, the highest, i.e. (7) isolates of potential EB, was recorded from Selangor. Nevertheless, chili and eggplant had the highest number of eight isolates. The growth inhibition of EB against *R. solanacearum* via in vitro antagonistic assessment is shown in Figure 2, and the inhibition radius is tabulated in Table 1.

Cultural and morphological characterizations

Enterobacter tabaci was cocci-shaped, and its cell wall was stained with pink safranin, which indicates a Gram-negative bacterium. On the Petri plate, the colony exhibited a round margin, smooth and translucent appearance, and yellowish coloration with a butyrous texture. The colony size was 2 mm in diameter; the *Bacillus cereus* was rod-shaped, and the cell wall was stained with purple-violet crystal color, indicating a Gram-positive bacterium. On the plate, *B. cereus* had a round margin, a rough, opaque appearance, and a white coloration with a dry texture. The colony size was 4 mm in diameter. The characteristics of both colonies under the microscope and on a plate are shown in Figure 4.

Molecular identification

The result revealed that UPMBC1 and UPMBE2 displayed a bright single band on gel electrophoresis. The nucleotide size for both EB was about 1500 bp (Figure 3). Sequences obtained from the sequencing company were compared with the sequence database in GenBank via the National Center for Biotechnology Information (NCBI). From the database, UPMBC1 was identified as *Enterobacter tabaci* (MH794127.1) and UPMBE2 as *Bacillus cereus,* with 99% (NR 146667.2) and 98% (KC 248214.1) nucleotide similarity, respectively. The actual nucleotide size of *E. tabaci* and *B. cereus* was 1405 bp and 1126 bp, respectively.

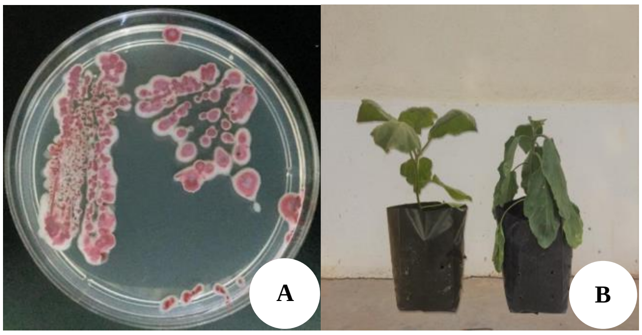

Figure 1. A. Isolated *Ralstonia solanacearum* strains on Kelman's Tetrazolium Chloride (TZC) media. B. The treated eggplant seedling showed wilting symptoms where all the leaves drooped on the third week (*right*) and the control eggplant seedling showed no symptoms of wilting (*left*)

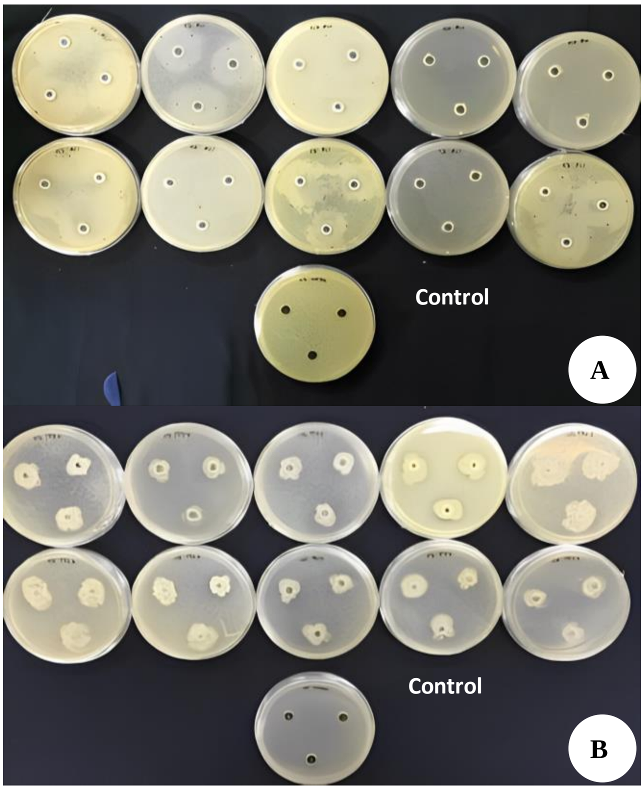

Figure 2. In vitro antagonistic assessment of (A) UPMBC1 and (B) UPMBE2

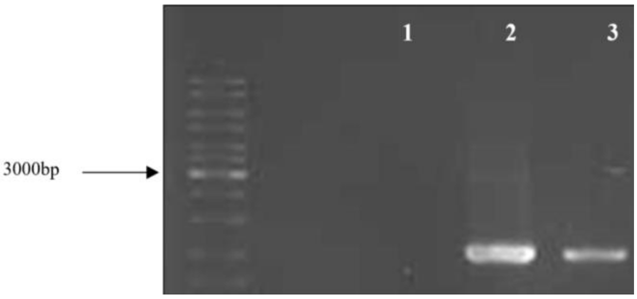

Figure 3. A bright band for *Enterobacter tabaci* (Lane 2) and *Bacillus cereus* (Lane 3) in gel electrophoresis. No band yielded by the control (Lane 1).

Table 1. The Tukey's mean separation analysis of 27 endophytic bacteria against *Ralstonia solanacearum* in the in vitro antagonistic assay

Endophytic bacteria	Radius (mm)	Host plants	States
UPMBC1	14.64^{a}	Chili	Selangor
UPMBE2	12.55^{ab}	Eggplant	Selangor
UPMJT2	9.72^{abc}	Tomato	Johor
UPMBRE4	9.19^{bc}	Eggplant	Selangor
UPMBRE5	8.47^{bcd}	Eggplant	Selangor
UPMAWE2	7.97^{bcde}	Eggplant	Perak
UPMAC3	6.84^{cdef}	Chili	Perak
UPMAC1	6.61^{cdef}	Chili	Perak
UPMJT4	6.51^{cdef}	Tomato	Johor
UPMBC2	6.49^{cdef}	Chili	Selangor
UPMAWE3	5.72^{cdef}	Eggplant	Perak
UPMAC2	5.50^{cdef}	Chili	Perak
UPMNWE3	5.26^{cdef}	Eggplant	Negeri Sembilan
UPMJT3	4.98^{cdef}	Tomato	Johor
UPMPLE2	4.54^{cdef}	Eggplant	Penang
UPMPLE4	4.39^{cdef}	Eggplant	Penang
UPMCLE7	3.57^{def}	Eggplant	Pahang
UPMBRE6	3.53^{def}	Eggplant	Selangor
UPMPC3	3.45^{def}	Chili	Penang
UPMCLE5	3.40^{def}	Eggplant	Pahang
UPMCLE6	3.27^{def}	Eggplant	Pahang
UPMCC1	2.86^{ef}	Chili	Pahang
UPMPLE1	2.72^{ef}	Eggplant	Penang
UPMNWE2	2.49^{f}	Eggplant	Negeri Sembilan
UPMCLE5	2.44^{f}	Eggplant	Pahang
UPMBLE6	2.09^{f}	Eggplant	Selangor
UPMJC4	1.56^{f}	Chili	Johor

Note: The mean separations marked with similar letters are not significantly different according to Tukey's Honest Significant Difference (HSD) test (P<0.05)

Discussion

In this study, *R. solanacearum* colonies were isolated from the infected eggplant. Fresh colonies were required because this pathogen could easily lose viability when continuously subcultured on laboratory media (Champoiseau et al. 2009). Moreover, it may also be viable but not culturable if kept at 4°C for an extended period (Milling et al. 2009). From the experiment, three colonies were yielded; however, only one virulent colony of *R. solanacearum* was successfully isolated. The virulent colony was determined by its cultural appearance, and further pathogenicity test was conducted. *R. solanacearum* on culture media showed white color with a pinkish center and watery appearance, whereas in the pathogenicity test, it successfully induced 80% wilting in the tested eggplant. In a previous study, *R. solanacearum* enhanced its biosynthesis of virulence factors by utilizing the host plant metabolites (Shen et al. 2020). The L-glutamic acid acquired from the host plant was proven to be associated with increased Exopolysaccharide (EPS) production, swimming motility, cellulase activity, and biofilm formation of *R. solanacearum*. Besides, the acid compound also helps *R. solanacearum* colonize the root and stem cells, thus accelerating DS. Another study also found that *R. solanacearum* assimilated nitrate to promote its EPS virulence, enhanced root attachment, and initiated infections (Dalsing and Allen 2014).

The distribution of EB among states varies since each state experiences different rainfall distributions, soil fertility, physiology, chemical, pH, and temperature. Based on the in vitro antagonistic assessment, only 27 isolates could inhibit the growth of *R. solanacearum* effectively and consistently (inhibited the growth of *R. solanacearum* in all replicates). Only two EB were subjected to identification as both were the most efficient at outgrowing the *R. solanacearum* in in vitro antagonistic assessment. 16s rDNA was performed to identify the EB because the colony phenotype cannot be trusted 100% (Sousa et al. 2013). That is because the environment always influences the phenotypic appearance of bacterial characteristics (Young 2007). Cultural and morphological characterizations were carried out to support molecular identification. Therefore, as a Gram-negative bacterium, *E. tabaci's* cell-stained pink safranin color parallels with other Enterobacteriaceae, indicating that the cell wall has a thin peptidoglycan layer.

Even with a thin cell wall layer, the bacterium can withstand the ultimate pH and temperature (Beveridge 1999). Furthermore, it has been reported to withstand three atmospheric pressure (atm) and turgor pressure (Koch 1998). Nevertheless, in the present study, the cocci shape of *E. tabaci* was in contrast to the rod shape of Enterobacteriaceae. This can be explained by the abiotic and biotic stresses from the environment, for instance, salinity, predation, and nutrient competition imposed upon the bacterium (Philippe et al. 2009). Furthermore, numerous studies used *Escherichia coli* as a model in shape-changing studies (Kerksiek 2009; Philippe et al. 2009; Pilizota and Shaevitz 2014). Therefore, the bacterial shape changes from rod to cocci when there are differences in surrounding osmotic pressure. Thus, enduring a harsh environment triggers *E. coli* to reduce its size from rod to cocci. Therefore, the binding protein synthesizing the peptidoglycan is distracted when facing a difficult condition. This distraction disturbed the formation of cell walls and led to a smaller bacterium (Philippe et al. 2009). Therefore, it can be deduced that the odd shape of *E. tabaci* in this study was due to evolution and adaptation from the wild environment the bacterium faces.

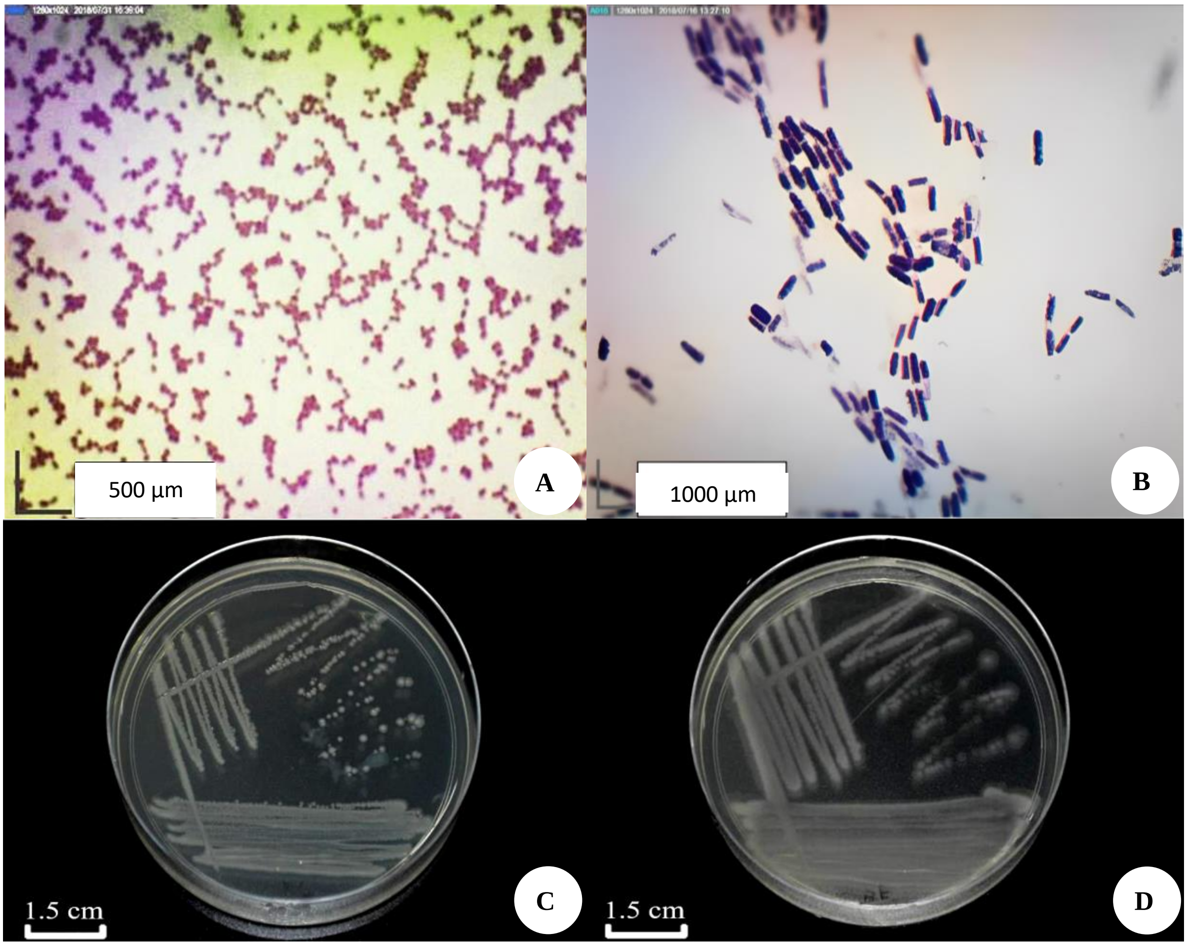

Figure 4. Gram-staining images of endophytic bacteria captured with the Dino-Lite microscope camera (A) *Enterobacter tabaci* with cocci cell shape and stained pink safranin color, (B) *Bacillus cereus* with rod cell shape and stained purple crystal violet color, (C) colony appearance of *Enterobacter tabaci* on nutrient agar and (D) colony of *Bacillus cereus* on nutrient agar

Several studies have been published on the potential Gram-negative bacterium as BCA against *R. solanacearum*, such as *Pseudomonas* sp. Some mechanisms employed by the species are the production of secondary metabolites, enzymes, and antimicrobial compounds to induce the host plant's defense, apart from the ability to expel the pathogen in a nutrient competition physically. *Pseudomonas brassicacearum* was reported to release 2, 4-diacetylphloroglucinol (2,4-DAPG), a siderophore, a protease, and hydrogen cyanide (HCN) to inhibit the growth of *R. solanacearum* (Zhou et al. 2012). A different study documented Pseudomonas strains to stimulate endophytic *Azospira* populations and induced systemic resistance to inhibit *R. solanacearum*. In addition, the plant's defense-related enzymes and defense signaling marker gene expression levels were escalated upon inoculation with *Pseudomonas* strains (Shang et al. 2021). Nonetheless, the induction of systemic resistance was determined to involve the activities of superoxide dismutase, catalase, and polyphenol oxidase (Shang et al. 2021). In another experiment, *Pseudomonas mosselii* triggered tobacco's ethylene- and jasmonate-dependent defense signaling pathways and reduced the DS by 9.1% (Zhuo et al. 2019). Even though Enterobacteriaceae is commonly associated with a pathogen, some of it has the capability of a BCA. Other than *E. tabaci*, *Enterobacter cloacae* were also effective against *R. solanacearum*. The bacterium could suppress and produce some specific antibiotics to induce plant systemic resistance (Mohamed et al. 2020). The *E. cloacae* reduced DS from 93.25% to 10.7% under greenhouse conditions and 26.6% under field conditions, improving crop yield by 20.44%. The BCA-treated potato proved the induction of systemic resistance by the increased: total phenol, salicylic acid contents, and levels of lipoxygenase, peroxidase, and polyphenol oxidase. The *E. cloacae* has also been reported to be effective against RS infection in tomatoes, with 65% biocontrol activity (Xue et al. 2009). Previously, *E. cloacae* were found effective against damping-off disease caused by *Pythium* sp. by producing hydroxamate siderophore aerobactin (Loper et al. 1993). The siderophore production under iron-limited conditions expelled *Phytium* sp. from surviving, thus eliminating the pathogen. Apart from that, *E. cloacae* can also reduce the damping-off incidence by inactivating the stimulatory activities of root exudates. Thus, it could minimize *Phytium ultimum* infection (Kageyama and Nelson 2003). In a different experiment, hyphae of *Pythium aphanidermatum* were deformed and shriveled upon inoculation with *E. cloacae* (Kazerooni et al. 2020).

As for *B. cereus*, the characteristics in this study match those of Bacillaceae. *B. cereus* has a purple-violet stained color, indicating a Gram-positive bacterium. The gram-positive bacterium has a thick peptidoglycan layer, making it more rugged than the Gram-negative. *Bacillus* sp. is one of the famous BCAs and has been widely studied for its potential ability to control phytopathogens, including BW disease. *Bacillus niabensis* and *Bacillus subtilis* produced a halo zone, indicating the production of metabolites against the *R. solanacearum* in vitro (Tuhumury et al. 2021). The antibiosis mechanism is believed to be due to the ability of *B. subtilis* to emit antibiotics, such as Bacillomycin D and iturin. In different studies, *Bacillus velezensis* and *Pseudomonas fluorescens* significantly reduced the population of *R. solanacearum*, thus minimizing the occurrence of BW disease (Elsayed et al. 2020). Both strains enhanced the prokaryotic communities and promoted plant defense against BW disease. *P. fluorescens* was detected attached to the plant's root hairs, lateral roots, epidermal cells, and xylem vessels. Besides *B. subtilis* and *B. velezensis*, *Bacillus thuringiensis* was also used to suppress *R. solanacearum.* Compared to the control, B. thuringiensis cell-free supernatant could suppress BW disease by one-third (Hyakumachi et al. 2013). The Volatile Organic Compounds (VOC) of *Bacillus amyloliquefaciens* significantly suppressed the motility, antioxidant enzymes, EPS production, biofilm production, and root colonization of *R. solanacearum* (Raza et al. 2016). In the experiment, 9 VOCs exhibited 1-11% antibacterial activity against *R. solanacearum,* but the consortium of all VOCs reduced the growth of *R. solanacearum* by 70%. The proteomic study suggested that VOCs of *B. amyloliquefaciens* downregulated *R. solanacearum* protein, which is related to its virulence, antioxidant activity, carbohydrate, protein folding and translation, and amino acid metabolism. Tahir et al. (2017) stated that the VOCs of *B. subtilis* Induced Systemic Resistance (ISR) by significantly increasing Polyphenol Oxidase (PPO) and Phenylalanine (PAL) activities, besides over-expressing the gene related to wilt resistance. In the present study, *E. tabaci* and *B. cereus* significantly inhibited *R. solanacearum* growth in vitro. Further experimentation needs to be conducted to find these strains' mode of action models on *R. solanacearum* growth suppression. The excellent performance of both EB in controlling *R. solanacearum* is an important parameter that may provide new hope in e control of BW disease, particularly *E. tabaci.*

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