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Research Article
Plant Growth Promoting and Antagonistic Potential of Endophytic Bacteria Isolated from Melon in Indonesia

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Abstract

Background and Objective: Bacterial endophytes, bacteria that reside within plant tissues without causing disease, can act as plant beneficial microbes. This study was carried out to determine the melon endophytic bacteria for their potential in plant growth enhancement and disease management strategies. **Materials and Methods:** The bacterial endophytes were explored from healthy melon plants in Yogyakarta and Central Java, Indonesia. The isolated bacteria were screened for their ability to produce extracellular enzymes (proteases and endoglucanase) and Indole-3-Acetic Acid phytohormone (IAA), to solubilize inorganic phosphate and to perform antagonistic effect against *Acidovorax citrulli* and *Fusarium oxysporum* f.sp. *melonis*. Bacteria that have the potential to be plant growth promoters and antagonists were molecularly identified by partial 16S rDNA sequence analysis. **Results:** Seven endophytic bacterial isolates were obtained, which were isolated from the roots, stems, petioles and leaves of healthy melon plant tissues. All the bacterial isolates were able to produce extracellular protease enzymes, to synthesis IAA and to inhibit the growth of *F. oxysporum* f.sp. *melonis*. Meanwhile, some of them could produce endoglucanase enzymes, solubilize inorganic phosphate and inhibit the growth of *A. citrulli*. Two representative isolates that showed the best results (KP B51 and Klt D01) were identified as genera *Bacillus* and *Burkholderia*, respectively. **Conclusion:** Molecular identification based on 16S rRNA gene sequences analysis suggested that the potential endophytic bacteria were close related to genera *Bacillus* and *Burkholderia*. The explored beneficial bacteria have possibility as alternatives for sustainable eco-friendly plant disease management in melon.

Key words: Endophytic bacteria, melon, *Acidovorax citrulli*, *Fusarium oxysporum* f.sp. *melonis*, 16S rRNA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acidovorax citrulli[1,2] and *Fusarium oxysporum* f.sp. *melonis*[3,4] are important pathogens in melon. *A. citrulli* is a bacterial pathogen that causes fruit blotch diseases in melon[5,6]. Worldwide, this pathogen has emerged rapidly as an economically important seed-borne pathogen of cucurbits[7]. The symptoms of this disease on the leaves are water-soaked lesions on cotyledons, angular spots following light brown to dark brown necrotic lesions on true leaves limited by the veins and irregular, brown to reddish-brown colored necrotic lesions. The symptoms on the fruits are water-soaked, sunken browning in fruit flesh followed by cracks and softening and rotting on melons[8]. Meanwhile, *F. oxysporum* f.sp. *melonis* causes fusarium wilt, one of the most destructive diseases that threaten melon production and causes large yield losses in various parts of the world. The persistence of *F. oxysporum* f.sp. *melonis* in the soil indefinitely is due to the production of chlamydospores and to the colonization of plant residues including roots of non-susceptible crops cultivated in rotation[9]. This pathogen causes yellowing symptoms of the leaves, which is then followed by wilting. In some cases, wilting can occur suddenly without being preceded by the yellowing of the leaves[10].

Control of *A. citrulli* and *F. oxysporum* f.sp. *melonis* is difficult because they have a wide range of hosts[11] in the family of Cucurbitaceae and so far, there are no commercial melon cultivars with resistance to both pathogens[7,12]. In general, the practice of disease controls still relies on chemical compounds that can cause negative impacts such as environmental pollution and poisoning if used continuously[13]. Hence, biological control is considered to reduce the negative impacts of using chemicals. Biological control is performed by utilizing antagonistic microbes that can inhibit or control the development and growth of plant pathogens[14]. One of the microorganisms that are used for biological control is a group of endophytic bacteria. Bacterial endophytes are ubiquitous microorganisms that live in plants without endangering their host plants[15]. Endophytic bacteria are reported to play a role as biocontrol agents and can increase plant growth, thus referred to as Plant Growth Promoting Bacteria[16]. Endophytic bacteria that have been isolated from plants and reported as biocontrol agents, as well as plant growth-promoting agents, include *Bacillus altitudinis*[17], *B. amyloliquefaciens*[18], *B. subtilis*[19], *B. cabrialesii* sp. nov.[20], *Pseudomonas fluorescens*[21], *Burkholderia phytofirmans*[22] and *Pseudomonas putida*[23]. In this research, endophytic bacteria that have a beneficial effect and antagonistic potential in melon plantation were investigated.

MATERIALS AND METHODS

Study area: Healthy plant samples were collected from three locations in Sleman and Kulon Progo District (Yogyakarta) and Klaten District (Central Java), Indonesia. Samples were put on paper and into a plastic bag before processed in the laboratory for bacterial isolation[24]. Isolation and characterization of endophytic bacteria were carried out at the Laboratory of Plant Disease, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta (Indonesia). The study was conducted during the year 2018-2019.

The isolation of endophytic bacteria: The medium used to isolate bacteria from plant tissue is Nutrient Agar (NA) medium and Tap Water Yeast Extract (TWYE) medium (Yeast extract 0.25 g, K_2HPO_4 0.5 g, agar 18 g, tap water 1000 mL), plus 50 µg mL^{-1} $K_2Cr_2O_7$. Melon plant tissues (roots, stems, petioles and leaves) were washed, then the tissue samples were cut into small pieces. The tissue surface was sterilized by soaking in 70% ethanol for 1 min and sodium hypochlorite solution for 5 min, respectively. The tissue was washed three times with distilled water and dried on sterile filter paper. The tissues then were dipped in 70% ethanol for 1 min and passed over the flame for surface sterilization and then placed on TWYE medium and incubated at 26°C for 10-20 days[25]. Sterilization is successful if no bacteria grow on the media until the third day after incubation[26].

Sterile plant tissues were immersed in sterile water in a test tube then shaken out for 20 min to release endophytic bacteria inside the plant tissues. A one bacterial loop was etched on the NA medium and incubated for 3-5 days. Further isolation was carried out by moving each different type of colony that was then inoculated into the Yeast Peptone Agar (YPA) medium (Yeast extract 5 g, peptone 10 g, agar 20 g). Incubation was carried out at 25°C for 1-2 days[27].

***In vitro* antagonistic assay against *Acidovorax citrulli* and *Fusarium oxysporum* f.sp. *melonis*:** Antagonistic activity of the endophytic bacteria was analyzed by *in vitro* challenging against pathogenic pathogen (*A. citrulli*) by using the double-layer method. A 5 mL of YPA medium was prepared in a petri dish and allowed to condense. The 5 mL of 0.6% Water Agar (WA) at 50°C that had been added with 100 µL of *A. citrulli* suspension was added to solid YPA medium. A pure culture of endophytic bacterial isolates was then grown on WA medium and incubated for 48-78 hrs and the inhibition zone was formed[28].

Antagonistic activity assay of the endophytic bacteria against the pathogenic fungi (*F. oxysporum* f.sp *melonis*) was carried out by the dual culture method. In the dual culture method, pure cultures of *F. oxysporum* f.sp *melonis* isolates and endophytic bacteria were grown on Pepten Dextrose Agar (PDA) in pairs and the fungus was cultured in the middle of the petri dish. The culture was incubated for seven days and the inhibition zone formed was measured[29].

Production of protease enzyme: The production of protease enzyme was analyzed using Skim Milk Agar (SMA) medium (0.7 g K_2HPO_4, 0.3 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}_7H_2O$, 0.01 g $FeSO_4{\cdot}_7H_2O$, 0.001 g $ZnSO_4$, 2.5 g skim milk and 1000 mL of distilled water). The medium was prepared in a petri dish until solidified. Bacteria were grown at two points on the medium and incubated for 24 hrs. Positive results are indicated by the presence of clear zones on the surface of the SMA medium[30,31].

Production of endoglucanase enzyme: The 24 hrs-old bacterial isolates were suspended in sterile water, then 100 µL of bacterial suspension was cultured on 5 mL YPB medium. The culture was shaken for 24 hrs, which was subsequently centrifuged at a speed of 10,000 rpm for 5 min, forming 2 layers that consist of supernatants and deposits. The supernatants were used for the test. Endoglucanase activity was tested on Carboxymethyl Cellulose (CMC) selective medium (4.5 g Na_2HPO_4, 1 g CM cellulose, 2 g NaN_3, 8 g agarose and 1000 mL distilled water). The medium was perforated to make two holes using a cork drill and the bottoms were sealed with 15 µL of 0.6% (w/v) agarose. The supernatant was added to the two holes that already contained 0.6% agarose medium. The culture was incubated for 24-48 hrs at 37°C and added with 0.8% Congo red by pouring it evenly throughout the surface of the medium and allowed to stand for 15 min and the color was washed with 5 M NaCl. Positive results are indicated by a clear zone[32,33].

Production of indole-3-acetic acid: The IAA activity assay was performed with 20 µL of bacterial suspension grown in 5 mL of yeast peptone broth (YPB) medium that had been added with L-tryptophan (500 µg L^{-1}). The culture was incubated at room temperature for 48 hrs. A 1 mL of bacterial culture was centrifuged at a speed of 10,000 rpm for 15 min. A total of 1 mL of the supernatant was then reacted with 4 mL Salkowski reagents (50 mL of 50% $HClO_4$ and 1 mL $FeCl_3.6H_2O$ 0.5 M). The suspension was then incubated for 15 min without light exposure. The brick red color formed indicates that the bacteria produce IAA[34,35].

Ability to solubilize phosphate: Pure culture of the isolate was grown at two points on the Pikovskaya medium (10 g glucose, 2.5 g $Ca_3(PO_4)$, 5 g $MgCl_{2\cdot6}H_2O$, 0.25 g $MgSO_4{\cdot}_7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_3$, 15 g agar and 1000 mL distilled water) and incubated for seven days. Clear zones formed around the colony indicate that bacteria can dissolve phosphate[36].

Molecular identification based on 16S rRNA gene fragments: Bacterial genomic DNA was extracted based on the modified CTAB method[37,38]. As much as 1.5 mL of bacterial culture was centrifuged at 5,000 rpm for 2 min. The DNA pellet was dissolved with 540 µL TE buffer (0.1 M Tris-HCl, 0.1 M ETDA pH 8)+30 µL 10% Sodium Dodecyl Sulfate (SDS) and incubated at 37°C for 60 min. The resultant pellets were added with 100 µL NaCl 5 M+80 µL cetrimonium bromide (CTAB)/ NaCl, incubated at 65°C for 10 min, added with 750 µL of isoamyl alcohol/chloroform (24:1) and centrifuged at 12,000 rpm for 5 min. The top layer was moved into a 1.5 mL tube and added with 600 µL phenol/chloroform/isoamyl alcohol (25:24:1) then centrifuged at 12,000 rpm for 5 min. The top layer was then moved to a new 1.5 mL tube. As much as 0.6 times the volume of isopropanol was added and centrifuged at a speed of 12,000 rpm for 5 min. The pellets were washed with 70% ethanol, dried and dissolved with 20 µL TE buffer.

DNA obtained was then amplified by PCR using universal 16S rRNA primers with 63f forward primers (5'-AACGCGAAGAACCTTAC-3') and 1378r reverse primer (5'-CGGTGTGTACAAGGCCCGGGAACG-3')[39]. The PCR reaction was used with a total volume of 25 µL using the GoTaq Green Kit (Promega) with a composition of 2.5 mL DNA template, 1.5 µL forward and reverse primers, 12.5 µL GoTaq Green master mix and 7 µL nuclease-free water. Furthermore, amplification was performed on a PCR machine (Biorad T100, Germany) with an initial denaturation program at 94°C for 2 min, followed by 34 denaturation cycles at 94°C for 15 sec, annealing at 55°C for 30 sec, extension at 68°C for 30 sec and a final extension at 72°C for 5 min. The DNA amplicons were then visualized by electrophoresis on 0.8% agarose gel, which was electrophoresed at 70 volts for 50 min in TE buffer. DNA bands were then compared with 1 kb DNA ladder (Promega)[40,41].

The PCR results were purified and sent to the sequencing service company (Bioneer Corporation, Korea). The resulted nucleotide sequences were edited using BioEdit software and analyzed by BLAST (Basic Local Alignment Search Tool-nucleotides) on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov) to determine the

closest kinship level with the bacteria those retrieved from the GenBank nucleotide sequences database and MEGA 7.0[42] for alignment and phylogenetic tree construction. The phylogenetic tree was arranged using the maximum likelihood method, with grouping stability using bootstrap analysis with 1,000 times replications[43,44]. The outgroup species was included for comparative determination.

RESULTS

Exploration and isolation of endophytic bacterial isolates: Bacterial isolates were morphologically characterized through color, size, shape, edges and consistency. Seven isolates with different morphologies are shown in Table 1.

***In vitro* antagonistic activity against *Acidovorax citrulli* and *Fusarium oxysporum* f.sp. *melonis*:** The antagonistic activity of endophytic bacteria against *A. citrulli* that was grown in layers on the YPA medium showed that two of the seven isolates produced inhibitory zones (Table 2). The inhibition zone was indicated by the presence of a clear zone (Fig. 1).

Antagonistic activity assay against *F. oxysporum* f.sp. *melonis* was performed using a dual culture method on the PDA medium, showing that seven isolates could inhibit the growth and development of fungi (Table 2). Figure 2 showed that bacterial isolates have strong inhibition against *F. oxysporum* f.sp. *melonis.* However, the bacterial growth patterns were different. Isolate KP A003 grew only in the streaked line (Fig. 2a), meanwhile isolate KP A004 could grow well with swarm cells on the medium (Fig. 2b). Like KP A003, isolate KP B01 grew only in the streaked line; however, the fungal growth on the opposite direction seemed to be normal (Fig. 2c). The growth inhibition of fungal pathogen was also demonstrated by isolate KP B51 (Fig. 2d) and Klt D01, the

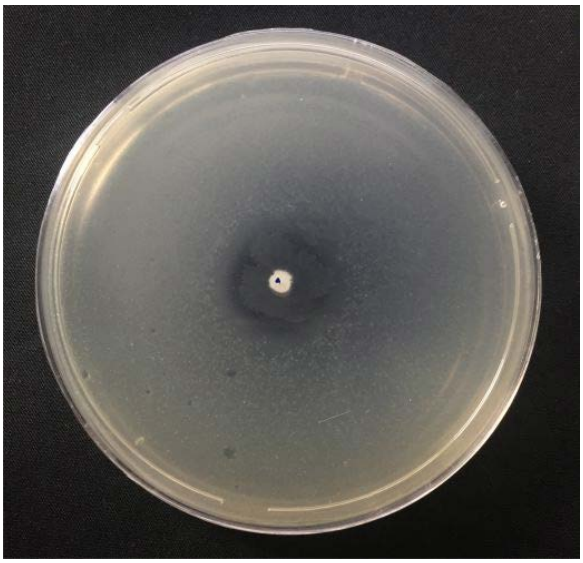

Fig. 1: *In vitro* antagonistic activity of endophytic bacterial isolate, Klt D01
Positive results marked by the formation of inhibitory zones on the lawn of *Acidovorax citrulli*

Table 1: Characteristics of the endophytic bacterial colonies on YPA medium

			Characteristics of the colony on YPA medium after 4 days				
Isolates	Origin	Plant organs	Color	Size (mm)	Shape	Edge/Margin	Consistency
Brb T1	Berbah	Petioles	Milky white	0.5-3	Circular	Entire	Slimy/mucoid
KP A003	Kulon Progo	Roots	Milky white	1-2	Circular	Undulate	Slimy/mucoid
KP A004	Kulon Progo	Roots	Milky white	3	Circular	Lobate	Dry
KP B01	Kulon Progo	Stems	Cream	0.5-2	Circular	Entire	Slimy/mucoid
KP B51	Kulon Progo	Stems	Milky white	0.5-2	Circular	Lobate	Dry
Klt D01	Klaten	Leaves	Brownish white	5-12	Irregular	Lobate	Slimy/mucoid
Klt D04	Klaten	Leaves	Milky white	2	Circular	Lobate	Slimy/mucoid

Table 2: Characteristic of the endophytic bacterial isolates

	Antagonistic activity					Synthesis of phosphate
Isolates	*A. citrulli*	*F. oxysporum* f.sp. *melonis*	Protease enzyme	Endoglucanase enzyme	IAA	solubility
Brb T1	-	+	+	-	+	-
KP A003	-	++	++	+	+	+
KP A004	-	+	++	+	+	+
KP B01	-	+	++	-	+	+
KP B51	+	++	+++	+	+	+
Klt D01	+	++	++++	++	+	++
Klt D04	-	+	+++	-	+	+

+/-: Able/unable to inhibit pathogen or synthesize protease, endoglucanase, IAA and solubilizing phosphate, number of + indicated degree of pathogen inhibition or ability in synthesizing protease, endoglucanase, IAA and solubilizing phosphate

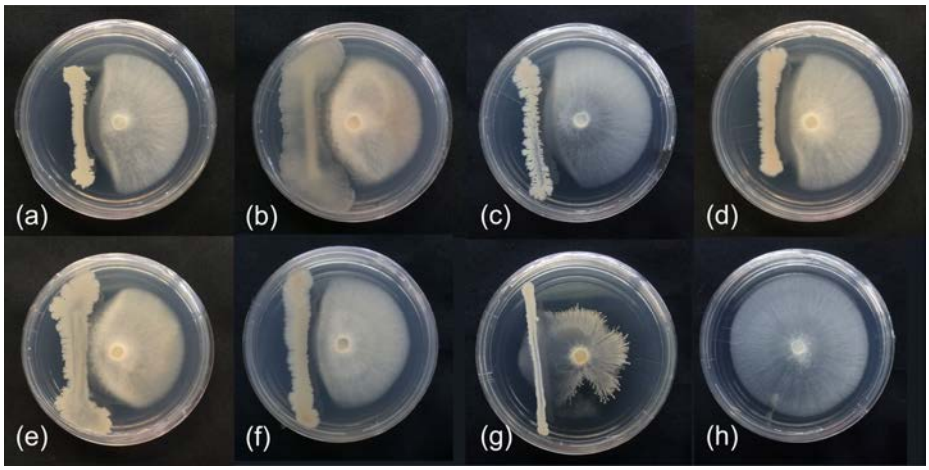

Fig. 2(a-h): *In vitro* antagonistic activity of endophytic bacterial isolates by the dual culture method against *Fusarium oxysporum* f.sp. *melonis*

(a) KP A003, (b) KP A004, (c) KP B01, (d) KP B51, (e) Klt D01, (f) Klt D04, (g) Brb T1 and (h) Control, positive results are indicated by the formation of inhibitory zone, while negative results are characterized by no formation of inhibitory zones

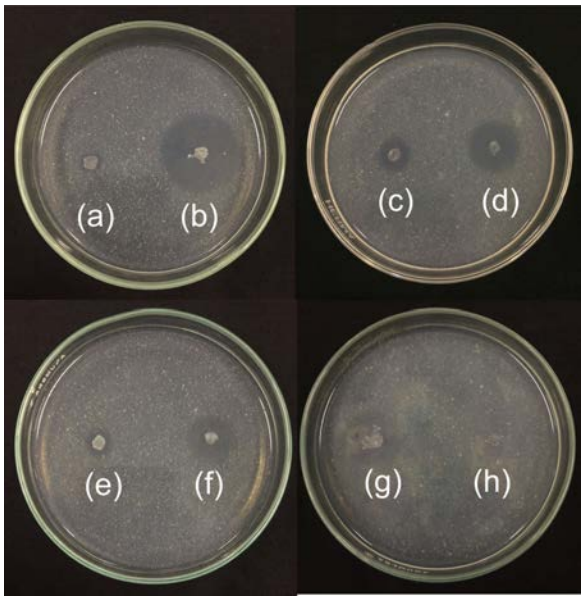

Fig. 3(a-h): Protease enzymes synthesis of bacterial endophytes on Skim Milk Agar

(a) Brb T1, (b) Klt D01, (c) KP A003, (d) Klt D04, (e) KP B01, (f) KP B51, (g) KP A004, showing positive results marked by the formation of a clear zone and (h) Control, negative results marked by no clear zone formation

suppression was also in the opposite direction as shown by dark color of the fungal pathogen (Fig. 2e). Similar inhibition pattern with KP B01 was demonstrated by isolate Klt D04, but the bacterial growth was better in the medium (Fig. 2f). In contrast, isolate Brb T1 could inhibit fungal growth in both directions (Fig. 2g). The intact and normal growth of fungal hyphae was revealed on *F. oxysporum* f.sp. *melonis* under control without any antagonist treatment (Fig. 2h). The formation of inhibitory zones indicated the existence of antimicrobial or antibiotic mechanisms of endophytic bacterial isolates against *A. citrulli* and *F. oxysporum* f.sp. *melonis*.

Production of protease and endoglucanase enzymes: The Qualitative production of protease enzymes by bacterial isolates was characterized by the formation of clear zones around the colonies on the Skim Milk Agar medium. All endophytic bacterial isolates were able to produce protease enzymes with different clear zone area (Table 2). Figure 3a-h shows clear zones of different isolates. Figure 3a showed smallest clear zone determined by isolate Brb T1, while largest clear zone was shown by isolate Klt D01 (Fig. 3b), followed by Klt D04 (Fig. 3d) and KP B51 (Fig. 3f). Isolates KP A003 (Fig. 3c), KP B01 (Fig. 3e) and KP A004 (Fig. 3g) had similar clear zone. Water was used as control treatment showing no clear zone formation (Fig. 3h). Likewise, the production of endoglucanase enzymes was indicated by the formation of clear zones around the colonies on Carboxymethyl cellulose medium after being reacted with Congo red and washed with 5 M NaCl. The results showed that four of the seven isolates (Fig. 4a-d) were able to produce the endoglucanase enzymes with isolate Klt D01 showed largest clear zone, while isolates Klt D04, Brb T1 and KP B01 had no endoglucanases activity on CMC medium (Fig. 4e-h).

Production of indole-3-acetic acid (IAA) and the ability to solubilize inorganic phosphate: The Qualitative production of IAA was indicated with the color change to brick red when

the bacterial supernatant was reacted with the Salkowski reagent. The result showed that all endophytic bacterial isolates could produce IAA (Table 2). As shown in the Fig. 5a, the isolate Brb T1 could change the color into brick red reflecting its ability to synthesize IAA. The other isolates such as Klt D01 (Fig. 5b), Klt D04 (Fig. 5c), KP A003 (Fig. 5d), KP A004 (Fig. 5e), KP B01 (Fig. 5f) and KP B51 (Fig. 5g) also showed the ability with different color intensity. The higher color intensity was shown by isolates Brb T1, Klt D01, KP A003 and KP A004, while isolates Klt D04 and KP B01 have lower color intensity. The high color intensity was shown by control treatment using *Pectobacterium brasiliense* (Fig. 5h).

The formation of a clear zone around the colony on the Pikovskaya medium indicated the ability of endophytic bacteria to solubilize inorganic phosphate. Qualitatively, six of the seven endophytic isolates had the ability to solubilize phosphate (Table 2). The clear zones were shown in Fig. 6a-i as by Isolates KP A004 (Fig. 6a), KP A003 (Fig. 6b), KP B51 (Fig. 6c), Klt D01 (Fig. 6d), Klt D04 (Fig. 6h) and KP B01 (Fig. 6i). Klt D01 was the highest in the phosphate solubilization activity and the only negative was isolate Brb T1 (Fig. 6e). Positive control using *Pseudomonas fluorescens* also showed positive results (Fig. 6g), while negative controls using sterile water showed negative results (Fig. 6f).

Identification of potential endophytic bacteria: Two of the seven endophytic bacterial isolates that have the potential to be beneficial microbes (Klt D01 and KP B51), showing the largest inhibitory zones against *A. citrulli* and *F. oxysporum* f.sp. *melonis* were molecularly identified. The partial 16S rDNA genes were subjected to DNA sequencing and nucleotide BLAST search showed that KP B51 isolate was identified as

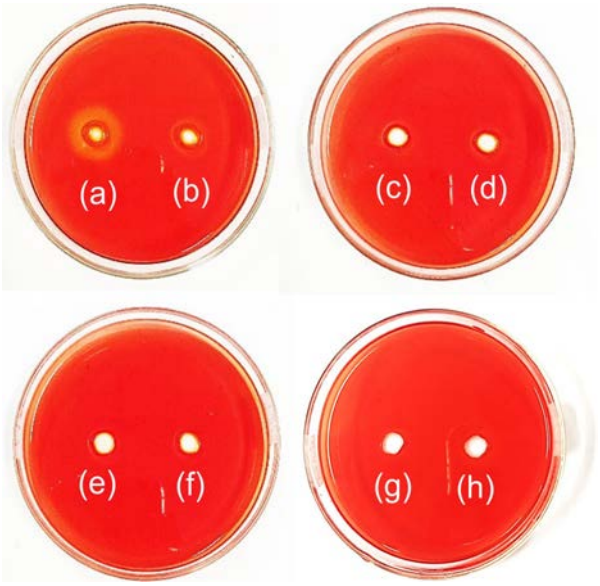

Fig. 4(a-h): Endoglucanase enzymes production of bacterial endophytes on CMC agar medium
(a) Klt D01, (b) KP A003, (c) KP B51, (d) KP A004, (e) Klt D04, (f) Brb T1, (g) KP B01 and (h) Negative control, positive results are marked by the formation of clear zones, while negative results are marked by no clear zones formation

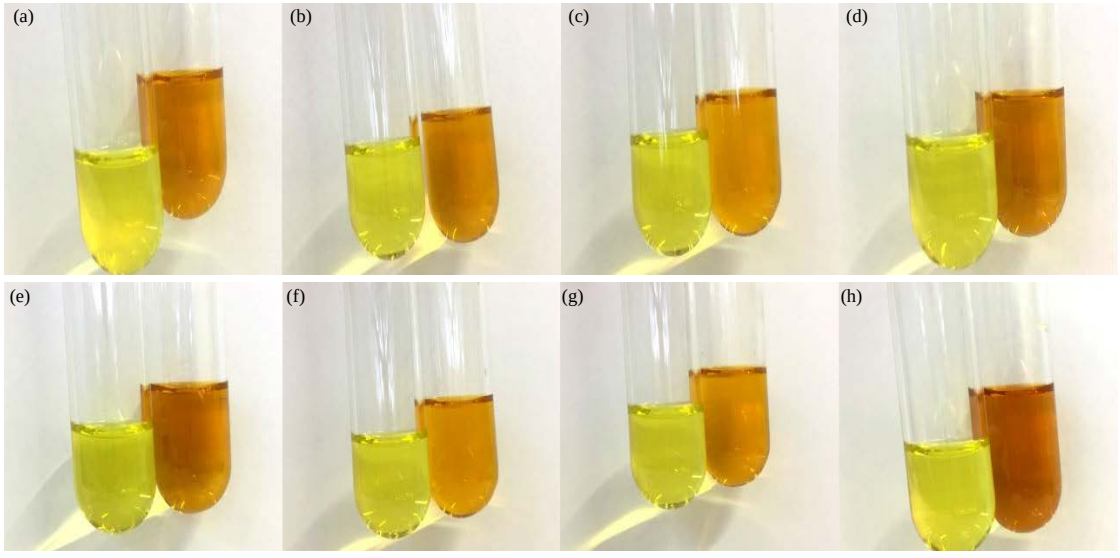

Fig. 5(a-h): Indole-Acetic-Acid (IAA) hormones production by bacterial isolates
(a) Brb T1, (b) Klt D01, (c) Klt D04, (d) KP A003, (e) KP A004, (f) KP B01, (g) KP B51 and (h) Positive control, the color was yellow before being reacted with Salkowski reagent and the change to brick red color shows positive results

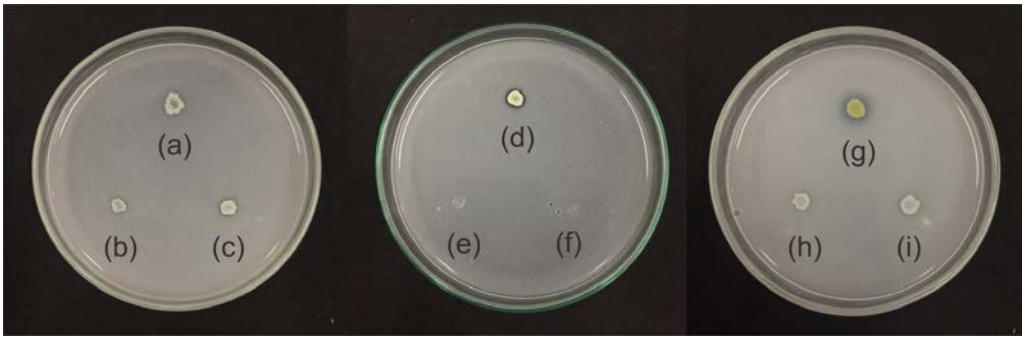

Fig. 6(a-i): Phosphate solubilization ability of bacterial endophytes on Pikovskaya medium
(a) KP A004, (b) KP A003, (c) KP B51, (d) Klt D01, (e) Brb T1, (f) Negative control, (g) Positive control using *Pseudomonas fluorescens*, (h) Isolate Klt D04 and (i) KP B01, positive results are marked by the formation of clear zones, while negative results are marked by no clear zone formation

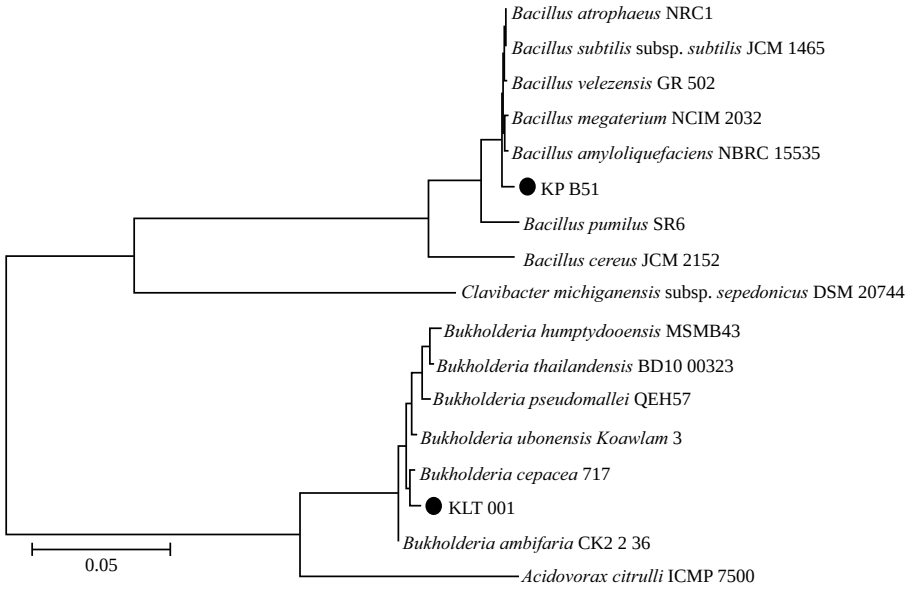

Fig. 7: Phylogenetic tree showing the relationship of the melon bacterial endophytes that have potential as plant growth-promoting bacteria based on 16S rRNA partial gene sequences and the closely related bacterial strains available in the GenBank
Phylogenetic tree was arranged using the maximum likelihood method, with grouping stability using bootstrap analysis with 1,000 times replication, the sequences of *Clavibacter michiganensis* subsp. *sepedonicus* and *Acidovorax citrulli* was used as outgroup species

Bacillus sp. that has close similarities to *Bacillus subtilis* subsp. *subtilis* JCM 1465 and *Bacillus atrophaeus* NRC1 as much as 99.47%, *Bacillus megaterium* NCIM 2032, *Bacillus velezensis* CR-502 and *Bacillus amyloliquefacien* NBRC 15535 as much as 99.36%, *Bacillus pumilus* SR6 as much as 97.41% and *Bacillus cereus* JCM 2152 as much as 93,57%. Meanwhile, Klt D01 isolate was identified as *Burkholderia* sp. that has close similarities to *Burkholderia cepacia* 717 and *Burkholderia ambifaria* CK2 2 36 as much as 99.47%, *Burkholderia ubonensis* Koawlam 3 as much as 99.25%, *Burkholderia thailandensis* BD10-00323 as much as 98.82%, *Burkholderia pseudomallei* QEH57 as much as 98.72% and *Burkholderia humptydooensis* MSMB43 as much 98.39%.

The phylogenetic analysis revealed that KP B51 isolate was in the same clade with that of *B. subtilis* species complex with the percentage identity of more than 99% forming different clade to *B. pumilus and B. cereus* with lower percentage identity, while Klt D01 isolate was included in clade of the *Burkholderia cepacia* species complex. The sequences of *Clavibacter michiganensis* subsp. *sepedonicus* and *Acidovorax citrulli* was used as outgroup species (Fig. 7).

DISCUSSION

Of the seven isolates of endophytic bacteria isolated, KP B51 and Klt D01 isolates have the potential to be biological control agents, shown by their ability to inhibit the growth of pathogens, to produce extracellular enzymes, to produce IAA hormones and to dissolve phosphate (Table 2). KP B51 and Klt D01 isolates have a higher ability to inhibit the growth of *A. citrulli* and *F. oxysporum* f.sp. *melonis* compared to other isolates. Masanto *et al.*[27] also reported that the potential antagonist bacteria isolated from healthy cacao consistently inhibited the growth of *Phytophthora palmivora*. Observation under a scanning electron microscope revealed that these bacterial endophytes caused abnormal morphology as well as shrinking and lysis of *P. palmivora* hyphae. The ability of endophytic bacteria to inhibit or control the growth of pathogens is due to the activity of producing antibiotics and or antimicrobial substances. Antibiotics are compounds that are resulted from the metabolism of microorganisms, which in low concentrations, can inhibit other microorganisms[45]. The role of endophytic bacteria as biological control agents is not only in terms of their ability to produce antibiotics or antimicrobials. The ability of bacteria as a biological control agent is also supported by their ability to produce extracellular enzymes. A β-glucanase, together with protease, are the main weapons for microbial-producing β-glucanase to degrade fungal cell walls[32]. IAA is a plant hormone that is important in controlling various growth processes[33]. A previous study by Sessitch *et al.*[46] revealed that all corresponding genes responsible for plant growth enhancements were detected in the endophyte metagenome for IAA production even for the three pathways: the indole-3-acetamide, indole- 3-pyruvate and tryptamine pathways. Our results showed that most of endophytic bacteria could solubilize phosphate. Phosphate solubilization revealed the activity of phosphatase enzymes. Plants absorb phosphate in the form of $H_2PO_4^-$, HPO_4^- and PO_4^-. Generally, the form of $H_2PO_4^-$ is more available to plants than HPO_4^- and PO_4^-. Phosphate solubilized microorganisms generally convert the insoluble phosphate into a soluble form through the process of acidification, chelation and exchange reactions[47].

Molecular identification based on the phylogenetic trees shows that both endophytic bacteria in this study belong to the genera *Bacillus* and *Burkholderia*. *Bacillus* is a genus that has been widely studied, consisting of 377 species and 7 subspecies, including synonyms[48]. Some *Bacillus* has potential in agro-biotechnology sector, including as the plant growth promoter[49] and for crop protection[50]. *Burkholderia* as the endophytic bacterium is also potential to be used for PGPR and biological control agents. A previous study by Weilharter *et al.*[22] suggested that *Burkholderia phytofirmans* PsJN strains have been investigated to have strong properties as plant growth-promoting bacteria that resistant to biotic and abiotic stresses and have also been reported to increase plant vigor. Identification using universal 16S rDNA primers in our study gave the less specific results on both isolates, thus only resulted in the genus level. Poretsky *et al.*[51] argued that the slow evolutionary rate of 16S rRNA gene limits its use in differentiating closely related species. The other housekeeping genes are required for bacterial identification in species level. For instances, the *gyrA* gene was reported as a reliable molecular marker for the detection of twelve strains of *Bacillus*[52]. The *gyrB* gene was also reported as a prominent phylogenetic marker for discrimination of *B. velezensis* from another *B. subtilis* species complex[53]. Likewise, the identification of the *Burkholderia cepacia* complex, including the species of *B. cepacia* genomovar I, *B. multivorans, B. cenocepacia, B. stabilis, B. vietnamiensis, B. dolosa, B. ambifaria, B. anthina* and *B. pyrrocinia* was more accurately done using the *recA* gene as a molecular marker[53]. In addition to *recA* gene-derived species-specific primers, a *recA* gene-based RFLP approach, enabling the recognition of multiple types within each genomovar of *B. cepacia.* The *recA* gene showed 94-95% similarity between the different genomovars and typically 98-99% similarity can be found within the genomovars[54].

CONCLUSION

Two endophytic bacteria in melon plants have been identified as *Bacillus* and *Burkholderia*. Both bacteria have the potential to plant growth-promoting bacteria and biological agents that control important diseases in melons.

SIGNIFICANCE STATEMENT

This research described the isolation of endophytic bacteria from healthy melon plant tissues collected from three locations in Yogyakarta and Central Java. This study is conducted to determine the ability of isolated bacteria as plant growth promoters and antagonists for *A. citrulli* and *F. oxysporum* f.sp. *melonis* as well. Furthermore, the representative isolates were then molecularly identified using DNA sequencing of 16S rRNA gene. Thus, a new theory on these beneficial bacterial applications may be arrived at.

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