HAYATI
Journal of Biosciences
Vol. Vol. 33 No. 4, July 2026 1147-1156
DOI:10.4308/hjb.33.4.1147-1156
ISSN: 1978-3019
EISSN: 2086-4094

Research Article
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OPEN ACCESS

Biopriming Potential Screening of Endophytic Bacteria to Prevent *Amorphophallus muelleri* Blume Agaist Soft Rot Disease Through In-vitro and In-Planta Screening

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ARTICLE INFO

Article history:
Received Nopember 30, 2025
Received in revised form December 29, 2025
Accepted December 30, 2025
Available Online May, 30 2026

KEYWORDS:
Acinetobacter radioresistance,
Bacillus velezensis,
Priestia aryabhattai,
biopriming,
soft rot

ABSTRACT

Soft rot is the serious problem in cultivation and post-harvest of *A. muelleri* Blume Yam (Elephant foot Yam). This experiment aims are to screen and characterize endophytic bacteria that potentially prevent soft rot on porang (*A. muelleri* Blume) through in-vitro and in-planta screening. Screening of the bacteria resulted three isolates i.e., EAP10, EAP17, and *Bacillus velezensis* EG113 could inhibit soft rot caused by *P. carotovorum* B4. The three isolates demonstrated that EAP10, EAP17, Bv EG 113 have anti quorum sensing (AQS) activity against *Chromobacter violaceum* and have capability to decrease the soft rot incidence of porang plantlets artificially infected with *Pectobacterium carotovorum* B4. Compared to the other selected endophytes, *B. velezensis* EG113 showed the best capability to decrease the disease incidence (75%). Physiological characterization of the selected endophytic isolates showed cellulolytic, proteolytic, and IAA production capability. The 16S rRNA sequences homology analysis of endophytic isolates showed similarity to A*cinetobacter radioresistance* (100%) and *Bacillus aryabhattai* (99%) or *Priestia aryabhattai* (revision name) respectively for EAP10 and EAP17 isolates . The results suggested that *B. velezensis* EG113 is potentially to be develop as biopriming agent for early protection of porang.

1. Introduction

Elephant foot yam of *Amorphophallus muelleri* Blume is well known as porang by in Indonesia. Currently, A.muelleri Blume is the most economically potential among the *Amorphophallus* species cultivated in Indonesia due to the highest glucomannan content in its yam (Ashan *et al*. 2023; Fajarini *et al*. 2020; Rohmawati and Ardiarini 2023). Glucomannan is a water-soluble polysaccharide that has diverse application such as in pharmaceutical, food, and cosmetics in industries (Sharma and Wadhwa 2022). Continuous with growth of large-scale cultivation area of porang in Indonesia, the threat of the plant disease is needs to be mitigated and anticipated.

The disease often emerges due to environmental change along with the expantion of the cultivation area and the increasing of intensive monoculture in large scale (Jiang *et al*. 2021). Member of *Pectobacterium* is the phytopathogenic bacteria reported causing "soft rot" the main disease on *Amorphophalus konjag* which has known as the highest glucomannan producing species (Luo *et al*. 2025; Wu *et al*. 2011; Perfileva *et al.2025*; Xiong et al. 2025; Zhanget. al. 2022). Loss of the yield could reach 30% - 50% and in the serious cases it could more than 80 % in very serious cases or even no harvest at all (Jiang et al. 2021). The disease causes severe irreparable damage and serious economic losses during cultivation, transportation, and storage.

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Beside of its negative impact on environmental sustainability such as phytopatogen resistance, application of pesticides also has negative impact on human and animal health. Publication of pathogen resistance tends to escalate currently. Some of the *Pectobacterium* species or strains in South Korea were completely or partially resistant to 250 ppm streptomycin and buramycin which are recommended by the manufacturer (Vu *et al*. 2022). Therefore, the effective and safety biocontrol agent is needed as alternative to solve the problem. Considering the negative impact of the pesticides,

Colonization of endophytic microbes in internal tissues can protect plants from disease -causing pathogens and increase plant growth under extreme conditions (Afzal *et al*. 2019; Fadiji and Babalola 2020; Medari *et al*. 2025). Additionally, some endophytes can engage priming plants which elicit a faster and stronger plant defense once pathogens attack. Due to these plant growth -promoting effects, endophytic bacteria are being widely explored for their use in improving crop performance (Aizaz *et al*. 2023; Fiodor *et al* 2023; Hernandez *et al*. 2023).

Various endophytic bacteria isolates have been shown ability to stimulate plant growth by producing growth regulating substances, such as IAA and Cytokinins (Tshikhudo *et al*. 2023). *Bacillus subtilis* strain BSn 5 isolated from callus tissue of *Amorphophallus konjac* can inhibit *Erwinia carotovora* subsp. carotovora (revision name: *Pectobacterium carotovorum*), as phytopathogenic bacteria causing soft rot disease in *Amorphophallus* (Deng *et al*. 2011). The disease is deleterious and limit the plant growth (He 2021).

Exploration and application of porang endophytic bacteria as biopriming agents have not been widely carried out in Indonesia. As a country with the second largest mega-biodiversity in the world, the chances of success are very high in obtaining endophytic bacteria that potential to be developed as effective and safe biopriming agents. Therefore, exploring and selecting endophytic bacteria and assessing their ability as biopriming agents for porang seedlings is Therefore, exploration and selection of endophytic bacteria and assessment of their capabilities as biopriming agents need to be carried out and are expected to provide benefits for safe, environmentally friendly, and sustainable agricultural practices.

2. Materials and Methods

2.1 Microorganisms and Plant

In addition to 114 endophytic bacteria that were isolated from the plantlet and root of healthy *A. muelleri* Blume plant obtain from Leuwikopo Experimental Farm, Bogor, West Java (6°33'46.6"S 106 °43 '35.7"E), in this research we also used the phytopathogenic bacteria *Pectobacterium carotovorum* B4 (Maghfiroh *et al*. 2022), *Bacillus valezencis* EG 113 (collection of Prof. Abdul Munif, Plant Protection Department of IPB), and *C. violaceum* (collection of Dr . Alina Akhdiya). Four to six weeks old of *A. mueller* Blume plantlets and six months old seedlings of the plants which were used for in-vitro and in-planta experiments were obtained from The Division of Plant Cell and Tissue Cultivation of ICABIOGRAD.

2.2 Isolation and Screening of Endophytic Bacteria Isolation

Root of the healthy porang plant were rinse with running water and surface sterilized with 70 % (1 minute), 1% NaOCl (1 minute), and rinsed with sterile distilled water three times (1 minute). The suspension from the maceration was put into a small bottle and then vortexed. A total of 1 mL of suspension was taken with a micropipette for dilution to 10^{-4}. 100 L of bacterial suspension was grown on a 20% NA plates (4.8 g/l NB, 15 g/l Agar) and incubated at room temperature for 48-72 hours. The isolates were purified on NA. The pure isolates were maintained on NA slants and stored in refrigerator (working culture), while the stock culture was maintained in glycerol 25% and store in deep freezer (-20°C).

2.3 Hypersensitivity Test

One loop of bacterial colonies from 24 hours old culture were suspended in sterile distilled water. Half to one milliliter of the suspension (OD600nm=0.2) was infiltrated on the lower surface of the tobacco leaf using sterile syringe (Klement and Goodman 1967). The treatment was repeated three times on different tobacco leaves. Observations were made every day for four days. The presence of necrosis in the area around the inoculation indicates a positive reaction to the hypersensitivity test.

2.4 Hemolytic Test

The endophytic bacterias were spotted on blood agar plate using a sterile toothpick. Incubation was carried out at room temperature for 24–48 hours. Isolates that showed a positive reaction (hemolytic) indicated by the presence of a clear zone around the inoculation point were not used for further tests.

2.5 Pathogenesis Test on Plantlet

Endophytic bacterial colonies (from 24 hours culture) were dissolved in sterile distilled water with OD600nm =0.2.

A total of 100 L of inoculum was poured over the surface of the media in a culture bottle using a micropipette. Incubation was carried out at a temperature of 25–27°C for four weeks. Observations were made every week based on the presence or absence of disease symptoms in porang plantlets after endophytic bacteria inoculation.

2.6 Anti-Quorum Sensing (AQS) Bioassay

AQS activity of selected bacterial isolates were carried out with the procedure published by Sari *et al.* (2016). A total of 100 µL of culture *Chromobacterium violaceum* (Cv), used as a bioindicator, was spread on NA media and allowed to stand until the media dries. Selected bacterial isolates were grown on it by spotting it with a sterile toothpick. Incubation was carried out at room temperature in the dark for 48–72 hours. The non-purple zone formed around the colony indicates the ability of the tested bacteria to inhibit QS Cv activity.

2.7 Bioassay of Cellulolytic Activity

Endophytic bacterial isolates aged 24 hours were spottted on CMC plate (2.4 g/l NB, 10 g/l CMC, and 15 g/l Agar). The inoculated plates were incubated at room temperature for 4–5 days before flooded with 0.1% Congo red and allowed to stand for 30 minutes, and then rinsed with 1 M NaCl to remove Congo red. Cellulolytic isolates will form a clear zone around the endophytic bacterial colonies.

2.8 Bioassay of Proteolytic Activity

Proteolytic activity was tested by growing endophytic bacterial isolates aged 24 hours on NA medium enriched with skim milk (2.4 g Nutrient broth, 2g agar, 10 mL skim milk, 90 mL distilled water). Incubation at room temperature was carried out for 48 hours. A clear zone will be formed around bacterial colonies capable of producing protease enzymes.

2.9 IAA-like Compound Assay

Bacteria isolates were grown in Luria Bertani (LB) broth on shaking incubator for 24 hours at room temperature for seven days. After incubation, the cultures were centrifuged (Refrigerated Centrifuge, Eppendorf, US) at 10000 rpm, 4°C for 10 min. The supernatant was assayed its IAA content qualitatively using the Salkowski reagent (Glickmann and Dessaux 1995).

2.10 Test of Phosphate Solibilizing Ability

Bacteria isolates were grown by streaking on Pikovskaya's Agar plates and then incubated at room temperature for 3-7 days. Clear zones formed around the bacteria colonies indicated ability of the isolates to dissolve the insoluble phosphate contained in the media.

2.11 Antagonism Test of Endophytic Bacteria against *Pectobacterium carotovorum*

The test was carried out by cross streak method. Percentage of bacterial inhibition (PPB) was calculated using formula:

PPB = (AWG/TSA) x 100%,

PPB = percentage of bacterial inhibition (%),

AWG = length of the scratch area that was not overgrown by bacteria,

TSA = total length of bacterial streak.

2.12 Biopriming Assay of the Selected Isolates

Endophytic bacterial colonies (from 24 hours culture) and Pc B 4 (from 24 hours culture) were suspended separately in sterile distilled water to obtain a suspension with a value of OD 600 nm =0.2. Six weeks 6 weeks after acclimatization (waa) the porang seedlings obtained from BB BIOGEN were transferred to new planting media (mixture of soil, husks charcoal , and sheep manure in a ratio of 1:1:1) in polybag (d: 20 cm). Application of N, P2O5, K2O fertilizers was carried out once every month for three months of observation with successive doses according to Rosdiana dan Santosa (2019). The experiment consisted of 8 treatments i.e : (1) Pc B4 (control), (2) sterile distilled water (control), (3) EAP17+Pc B4, (4) EG 113 +Pc B4, (5) EAP 10+Pc B4, (6) EAP 10, (7) EAP17, (8) EG113. Each treatment replicates 3 times. The disease incidence (DI), plant height, root length, and number of shoots data were recorded during the observation period. At the end of experiment, At the ends of the experiment, stem base of the plants was slices transversely and the tissues slices were observed using a ZEISS Axioscope 5 microscope at 100 X magnification.

2.13 Morphology Observation and Gram Prediction of the Selected Bacterial Isolates

The morphology of bacterial colonies growing on NA media was characterized visually, while the morphology of the cells was observed by using light microscope at 1000X magnification. Prediction of the Gram reaction was carried out using 3% KOH. The formation of sticky mucus in the bacterial cell mass

mass that was dripped with KOH indicated Gram -negative, whereas the absence of sticky mucus indicated Gram-positive.

2.14 Molecular Identification

Bacterial DNA was extracted following the protocol of the PrestoTM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., Taiwan). DNA 16S rRNA was amplified using primer pairs 63 F (5’-CAG GCC TAA CAC ATG CAA GTC-3’) and 1387R (5’-GGG CGG WGT GTA CAA GGC -3’). The PCR reaction used a total volume of 25 L with the composition: 12,5 L GoTaq® Green Master Mix 2x, 1 L forward, 1 L reverse, 1 L DNA template, and 9,5 L ddH2O. Amplification was carried out under thermal PCR conditions (T100 Thermal Cycler, Bio-Rad, US) as published by (Marchesi *et al*. 1998). The PCR product was electrophoresed on 1,5% agarose gel for 40 minutes, 90 V, 400 mA, and visualized under ultraviolet (UV) light. The amplification results were sent to the Genetics Science Indonesia for the sequencing process. The DNA sequences obtained were analyzed for alignment using Bioedit software and compared with the 16 S rRNA DNA sequence database found by GeneBank using the basic local alignment search tool-nucleotides (BLAST-N) program on the https://blast. ncbi.nlm.nih.gov website./blast.cgi. Phylogenetic tree analysis using MEGA X software.

3 Results

3.1 The Endophytic Bacterial Isolates of Porang

A total of 114 bacterial isolates were isolated from plantlets and porang (*A. muelleri* Blume) root. Based on the HR test, 24 of 114 isolates showed a negative reaction indicated by the absence of necrotic symptoms. Ten of the 24 isolates showed no clear zone forming around its colonies that non-hemolytic isolates. Next screening showed that 8 of the 10 isolates caused plantlet wilt. Only the plantlet which artificially inoculated with EAP 10, EAP 17, and aquadest (as control treatment) did not show any disease symptoms at all (Figure 1). The screening result indicated that EAP 10 and EAP 17 were non-pathogenic against *A. muelleri*. Based on three biosafety tests that have been carried out, only two isolates were not potentially pathogenic against mammals as well as against host plants (porang) and non-host plants (tobacco).

3.2 Characterization of Selected Isolates

The endophytic bacteria isolated from planlet and porang roots showed various cell shapes and morphological colonies. EAP10 isolate had short rod or cocco-bacil shape, while the EAP 17 was rod (bacil) in shape with various cell sizes. Both isolate colonies are white. EAP10 and EAP17 colonies grown on solid media containing CMC, skim milk , and unsoluble phosphate (Magnesium tri-silicate) formed clear zones around its colonies which indicated cellulolytic , proteolytic, and P solubilizing activities. Both isolates also showed AQS activity against *C. violaceum* , that were performed by the forming of non-purple zone around the colonies on the bioassay plates that had spreaded with *C. violaceum* . Moreover, supernatant cultures of the both isolates change to pink after reacted with Salkowsky reagent that indicated IAA content in the supernatants (Table 1). Based on 16S rRNA gene sequence analysis, EAP 10 isolate was related to *Acinetobacter radioresistens* OsEp _Plm (100%) and EAP17 was similar to *Priestia aryabhattai* Y-602 (syn. *B. aryabhattai*) (99%) (Table 2).

Figure 1 Pathogenicity screening of the bacteria isolates against *A. muelleri* planlets

Table 1 Morphology and some physiological and biochemicals characters of EAP10 and EAP17 isolates

Bacterial strain	Isolates	
	EAP10	EAP17
Colony morphology		
Color	White	White
Shape	Circular	Circular
Margin	Entire	Entire
Elevation	Convex	Raised
Cell shape	Short rod	Bacilli
Physiological and biochemical		
Anti-QS	+	+
Protease	+	+
Cellulase	+	+
IAA	+	-
Phosphate solubilization	+	+

3.3 Bioassay of Biopriming and Suppression of Soft Rot Disease

The analysis of variance showed that the application of endophytic bacteria to porang seedlings had a significant effect on plant height, root length, and the number of roots. The height of porang seedlings treated with Bv EG113 showed the highest average of 10.67 cm, followed by EAP10 treatment of 7.17 cm. In the root length parameter, Bv EG 113 inoculation also showed the highest yield of 23.5 cm, followed by EAP 10, with a length of 19.5 cm. The length of the roots inoculated with Bv EG 113 reached 23.5 cm, higher than the Bv EG113+Pc B4 application, which was 22.3 cm. Inoculation of Bv EG 113 on porang seedlings also caused significant differences in increasing the number of new shoots compared to the control treatment (Table 3). In addition, the application of Bv EG 113 reduced the incidence of disease by up to 50 % compared to the other two treatments. This indicated that the application of endophytes to porang seedlings effectively increased the growth and resistance of porang seedlings to the disease (Figure 3). Microscopic observation results of the basal stems tissues of the infected plants with Pc B 4 showed damage and shrunk cell of the tissues (Figure 4 C). In contrast to the treatment without inoculation of pathogenic bacteria Bv EG 113 + aquades (Figure 4B) and aquades control (Figure 4D), which showed the appearance of tissue with intact, thick, and firm intercellular boundaries. Similarly, tissue samples of inoculated plant with endophyte Bv EG113 either without or infected with Pc B4 showed the shape of the cells that remained intact with firm and thick cell boundaries. The firmness and thickness of the intercellular boundaries in the preparations from samples only inoculated with Bv EG 113 were relatively firmer and thicker than samples from control plants. The graph of disease incidence in porang seedlings is present in Figure 5. The disease incidence in porang seedlings was observed every 4 weeks for 12 weeks. Porang seedlings treated with Pc B 4 showed significant differences against the other 4 treatments: distilled water as control, EAP10+Pc B4, EAP17+Pc B4, and EG 113+Pc B4. It can be seen in the graph that the standard error values of Pc B 4 treatment do not coincide with other treatments. This shows significantly different results at the 4th, 8th, and 12th weeks. The percentage incidence of Pc B4 disease in a row at 4, 8, 12 weeks after bacterial inoculation was 55.56%, 77.78%, dan 88.89%.

4 Discussion

Health and overall wellbeing of the plants are associated crucially with its endophytes (Rutkowska *et al*. 2025). One of the isolates that have been isolated and identified molecularly was known belong to *Priestia* (syn. *Bacillus*). *Bacillus* is the most common type of endophytic bacteria found in plants and promotes plant growth (Azeema *et al*. 2023; Darkazanli *et al*. 2021; Rutkowska *et al*. 2025; Wang *et al*. 2025). Several studies have shown that antagonistic *Bacillus* can suppress several phypathogens (Cui *et al*. 2020), stimulate plant resistance in against drought stress (Gowtham *et al*. 2020), and produce phytohormones (Hernandez *et al.* 2025).

Table 2 Identities of the selected isolates base on 16S rRNA nucleotide sequence homology

Bacterial strains	Species database on Gene Bank	Query cover (%)	E value	Homology (%)	Accesion no.
EAP10	*Acinetobacter radioresistens* OsEpPlm	100	0.0	100	MT367790.1
EAP17	*Priestia aryabhattai* stt55a	100	0.0	99	OL701307.1

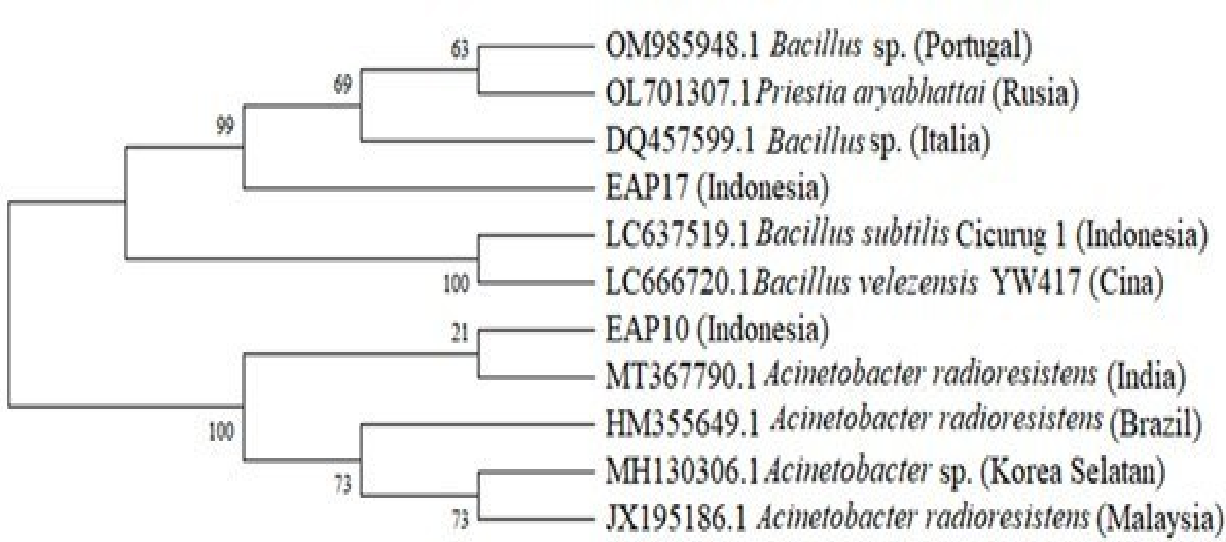

Figure 2 Phylogenetic tree of potential endophytic bacterial isolates EAP10 and EAP17 based on 16S rRNA sequences using the Neighbor-Joining method with 1000× bootstrap

Figure 3 Growth of porang seedlings treated with endophytic bacteria and *P. carotovorum*.

Bacillus isolated from porang also has the ability as AQS. AQS is a process of inhibiting QS, thus preventing the occurrence of QS in pathogenic bacteria.

The principle of controlling the pathogenicity of Gram-negative bacteria with AQS is by preventing the accumulation of acyl -homoserine lactone (AHL). Prevention of AHL accumulation is carried out by AHL degradation by endophytic bacteria (Molina *et al*. 2003). Previous research by Sari *et al*. (2017) showed the ability of *B. thuringiensis* SGT 3g to reduce disease symptoms in moon orchids caused by the soft rot bacterium *Dickeya dadantii* . Gram-positive bacteria such as *Bacillus* sp. and *Lysinibacillus*, have high AHL degradation activity and have potential as biocontrol agents (Hartmann *et al*. 2021; Ma *et al*. 2013). Acyl - homoserine lactonase (AHL -lactonase), a hydrolytic enzyme from *Bacillus* sp . that inactivates AHL activity by hydrolysing the lactone bond of AHLs. Role AQS mechanism to protect plants against soft rot and bacterial wilt phytopathogens infection was has been proven previously by significant resistance increasing of the transgenic plants expressing AHL-lactonases gene (Dong *et al*. 2001; Ouyang and Li 2016; Suryanti *et al*. 2022). EAP 10 isolate identified as *A. radioresistens* has high potential as a biological control agent based on physiological and biochemical tests. EAP10 showed a positive reaction on anti-QS activity, cellulolytic, proteolytic , production of IAA-like compounds , and was able to dissolve phosphate. *Acinetobacter* has the potential as a rhizobacterium to promote plant growth and increase the chlorophyll content of plants (Suzuki *et al*. 2014). This is reinforced by the statement of Khanghahi et al. (2021) that *Acinetobacter* have the potential as an effective bio-inoculant in increasing plant growth and health. IAA promotes cell elongation by increasing water permeability into cells, inhibiting or delaying leaf abscission, and inducing flowering and fruiting (Zhao 2010). IAA excreted by *B. thuringiensis* Batista *et al.* (2021) and *A. radioresistens* (Rokhbakhsh-Zamin *et al*. 2011). increased root growth and stimulated plant cell growth elongation. The application of endophytes to seedlings indicated that the cell wall structure at the base of the porang stem became stronger or thicker so that it was more resistant to *P. carotovorum*, which made the middle lamella (cell wall component) a target for degradation. In order to acces the nutrient - rich target niche, Pectobacteriaceae family produces a series of extracellular enzymes that degrade cell walls that play a role in its pathogenicity mechanism. The main constituent of the middle lamella is pectin. The activity of the pectin depolymerization enzyme causes the separation of the cells that make up the tissue so that the tissue becomes soft and rot (Radke *et al*. 2024).

Based on these results, it can be concluded that the application of endophytic bacteria *Bacillus velezensis* EG 113, *Bacillus aryabhatta* i EAP 17, dan *Acinetobacter radioresistens* EAP 10 on porang seedlings provided early protection from *P. carotovorum* attack that cause soft rot . Endophytes share the same ecological niches occupied by phytopathogens (Compant *et al*. 2013). As natural inhabitants of plants, endophytes can be used as biological agents for sustainable food crop cultivation (Verma *et al*. 2021). Applying selected endophytic bacteria as biological agent to seeds or seedlings is a beneficial technique (Srivastava *et al*. 2023). Biopriming which is applied at the early phase so that biological agents colonize firstly and dominate the niche resulting early protection and resistant priming against phytopathogen infections. Biopriming activates metabolism, improves germination, stand formation and stress tolerance in various crop commodities (Habibi *et al*. 2025; Hasanovi'c *et al*. 2025; Mahmood *et al*. 2016; Fiodor *et al*. 2023).

Acknowledgements

The authors would like to grateful Agricultural Biotechnology and Genetic Resources Research and Development, IAARD, MoA Republic of Indonesia for supporting and facilitating the research.

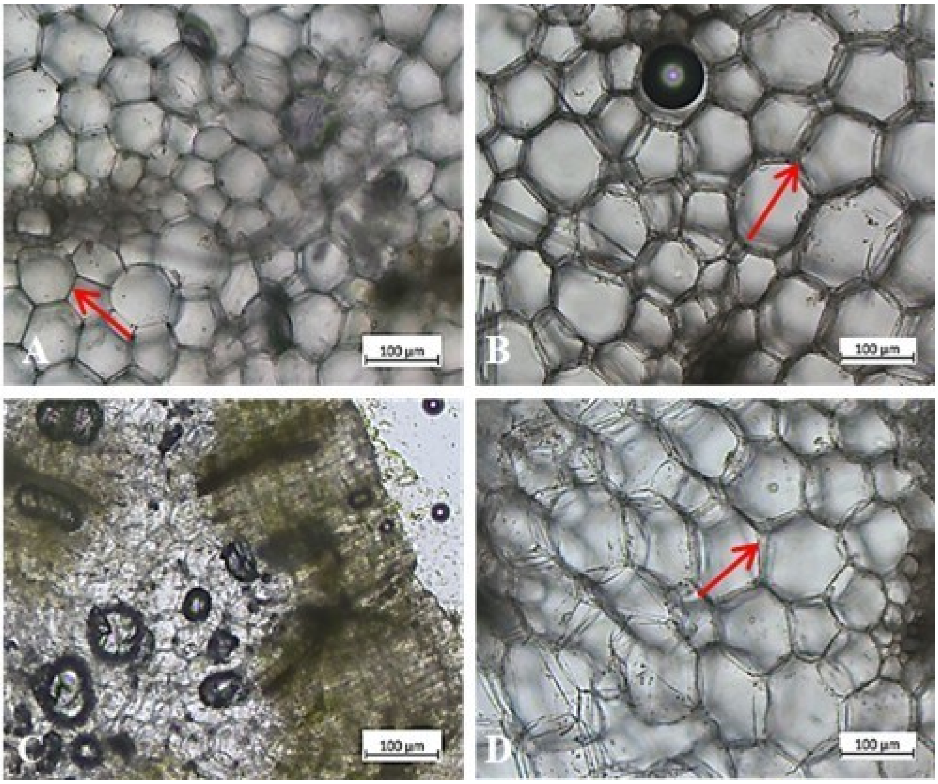

Figure 4 Micrographs of transversal slices of porang stem base tissue treated with : (A) *Bacillus velezensis* (Bv) EG113+*Pectobacterium carotovorum* (Pc) B4; (B) Bv EG113+aquades ;(C) Pc B4+aquades ; and (D) distilled water at a magnification of 100×

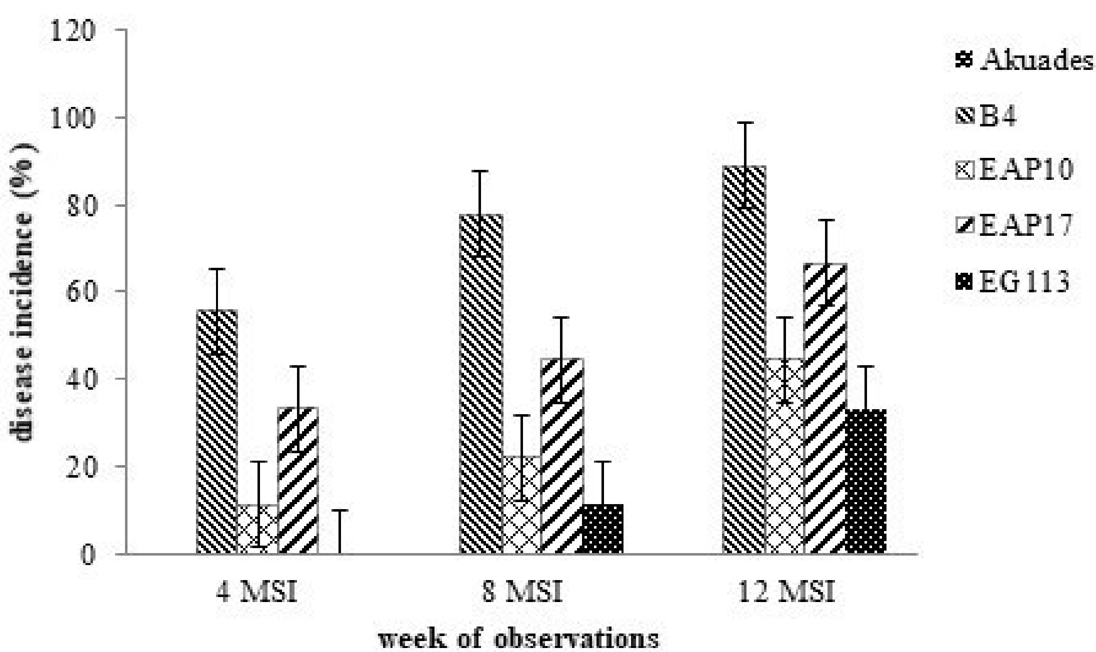

Figure 5 Disease incidence in porang seedlings treated with the endophytic isolates and infected with *P . carotovorum* B4.

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