BIODIVERSITAS
Volume 25, Number 6, June 2024
Pages: 2556-2564

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250626

Isolation and characterization of plant growth-promoting bacteria from medicinal plants Java cardamom (*Amomum compactum*) and bitter ginger (*Zingiber zerumbet*)

ALMANDO GERALDI[1,2,3,4], CHRISTOPHER CLEMENT[1], MAYA DEWI PERTIWI[1], YESYI LESTARI[1], DHAMMARUCI HEMADHIRA PARINNATA[1], RIFAID NUR ARSAD[1], RAMA ALI SADIKIN[1], ARIF LUQMAN[5,6], HERI SANTOSO[7], ALFINDA NOVI KRISTANTI[4,8], YOSEPHINE SRI WULAN MANUHARA[1,4,♥], ANJAR TRI WIBOWO[1,4,♥♥]

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia. Tel.: +6231-5936501, Fax.: +6231-5936502, ♥email: yosephine-s-w-m@fst.unair.ac.id, ♥♥email: anjar.tri@fst.unair.ac.id
[2]University CoE-Research Center for Bio-Molecule Engineering, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
[3]Institute of Life Science, Technology and Engineering, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
[4]Biotechnology of Tropical Medicinal Plants Research Group, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia
[5]Department of Biology, Institut Teknologi Sepuluh Nopember. Jl. Raya ITS, Keputih, Surabaya 60115, East Java, Indonesia
[6]Institute for Molecular Infection Biology, Julius Maximilians University of Wuerzburg. Wuerzburg, Germany
[7]Generasi Biologi Indonesia (Genbinesia) Foundation. Jl. Swadaya Barat No. 4, Gresik 61171, East Java, Indonesia
[8]Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga. Jl. Dr. Ir. H. Soekarno, Surabaya 60115, East Java, Indonesia

Manuscript received: 28 March 2024. Revision accepted: 19 June 2024.

Abstract. *Geraldi A, Clement C, Pertiwi MD, Lestari Y, Parinnata DH, Arsad RN, Sadikin RA, Luqman A, Santoso H, Kristanti AN, Manuhara YSW, Wibowo AT. 2024. Isolation and characterization of plant growth-promoting bacteria from medicinal plants Java cardamom (*Amomum compactum*) and bitter ginger (*Zingiber zerumbet*). Biodiversitas 25: 2556-2564.* Java cardamom (*Amomum compactum*) and bitter ginger (*Zingiber zerumbet*) are valuable medicinal plants native to Indonesia with significant economic and pharmacological importance. While their pharmacological properties have been extensively studied, little is known about the role of their endophytic bacterial communities in influencing plant growth and development. Understanding the interactions between these plants and their associated bacteria could provide insights into novel strategies to enhance their cultivation. This study investigates the potential of endophytic bacteria associated with *A. compactum* and *Z. zerumbet* in promoting plant growth. Samples were collected from leaf, rhizome, and rhizosphere soil, followed by isolation and characterization of bacterial strains. Molecular identification revealed the presence of *Methylobacterium aquaticum, Paenibacillus tyrfis, Priestia megaterium* strain 1, *Priestia aryabhattai,* and *Microbacterium arthrosphaerae* among the isolated strains from *A. compactum*, while strains isolated from *Z. zerumbet* included *Enterobacter mori, Pr. megaterium* strain 2, and *Pr. megaterium* strain 3. Those results demonstrated that *Pr. megaterium* strains exhibited strong phosphate solubilization, nitrogen fixation, and cellulolytic activity, while *Pr. aryabhattai* and *Pa. tyrfis* showed significant nitrogen fixation and cellulolytic potential. Additionally, *M. aquaticum, Pa. tyrfis,* and *Pr. aryabhattai* exhibited anti-phytopathogenic activity against *Xanthomonas campestris*. These findings not only highlight the diverse beneficial attributes of the isolated bacterial strains but also suggest their potential application as biofertilizers and biocontrol agents in agriculture, offering a promising avenue for sustainable farming practices.

Keywords: *Amomum compactum,* Plant Growth Promoting Bacteria*, Priestia megaterium,* sustainable agriculture, *Zingiber zerumbet*

INTRODUCTION

Java cardamom (*Amomum compactum*) and bitter ginger (*Zingiber zerumbet*) are medicinal plants native to Indonesia with high economic value. Indonesia's Central Bureau of Statistics (BPS) reports that cardamom seeds are the most widely produced non-rhizome biopharmaceutical product in Indonesia, where the production level reaches 81,724 tonnes per year. Bitter ginger is also one of the main pharmaceutical commodities in Indonesia, where the rhizome production level reaches 9,150 tonnes per year (BPS 2018). Both plants are important commodities for the food industry as spices or seasonings and the pharmaceutical industry as raw materials for drugs and medicines. Various studies have shown that Java cardamom seed extract has various pharmacological activities, including, anticancer (Subehan et al. 2006), anti-asthma (Lee et al. 2010), anti-inflammatory (Lee et al. 2012), antifungal (Ujilestari et al. 2019), and antibacterial (Juwitaningsih et al. 2020). Bitter ginger rhizome extract is also known to have various pharmacological activities, including antitumor (Kirana et al. 2003), antiviral (Tan et al. 2006), antibacterial (Yunus et al. 2015), and larvicidal (Sofian et al. 2019). Several studies have focused on cultivating java cardamom and bitter ginger, biochemical properties, and bioactivity. However, there is still limited information available regarding the microorganisms associated with the organs of these two medicinal plants and their effects on plant growth.

Microbes, including bacteria, fungi, viruses, and protists, can inhabit various surfaces and organs of an organism (Do et al. 2022; Nugrahapraja et al. 2022; Luqman et al. 2023). In plants, they are found on the surface of plant organs (epiphytic) and the inside of the organs (endophytic); together, they constitute the overall microbiome of plant compositions (Compant et al. 2019). Various studies have shown that the plant microbiome plays an important role in plant growth, stress response, and environmental adaptation (Liu et al. 2020; Vu et al. 2022; Do et al. 2022). Microorganisms that inhabit the surface and the inside of plant organs while forming symbiotic relationships that benefit plant growth and health are called plant growth-promoting bacteria (PGPB). These bacteria facilitate plant growth through various mechanisms, including nitrogen fixation, phosphate solubilization, phytohormones production, pathogens biocontrol, and nutrient uptake enhancement. By promoting plant growth and improving resistance to environmental stresses, PGPB is crucial for sustainable agriculture and crop production (Khalid 2012; Pathania et al. 2020).

Besides acting as PGPB, recent studies also show that the endophytic microbiome can influence the production of secondary metabolites in various medicinal plants. Research by Das et al. (2012) reported the promising application of the probiotic fungus *Piriformospora indica*, showing its capacity to promote inflorescence development and enhance p-Cymene production within the inflorescence (Das et al. 2012). Similarly, in chamomile (*Chamomilla recutita*), the inoculation of indigenous Gram-positive bacterial strains leads to an increase in the bioactive secondary metabolite apigenin-7-O-glucoside (Schmidt et al. 2014); furthermore, the symbiotic relationship between the fungal endophyte *Gilmaniella* sp. AL12 and the medicinal herb *Atractylodes lancea* lead to improvements in both plant growth and the accumulation of sesquiterpenoids (Yuan et al. 2019). Those findings showed the potential of harnessing beneficial microbial interactions to enhance medicinal plants' growth and secondary metabolite production.

Since the quality and economic value of a medicinal plant are determined by the content and concentration of metabolites in its organs, the endophytic microbiome plays an important role in its cultivation process and economic value. Therefore, isolating and characterizing the microorganisms associated with medicinal plants is important to identify microbes that can improve plant growth and secondary metabolite accumulation. However, research on isolating and characterizing plant growth-promoting bacteria from indigenous Indonesian medicinal plants is limited. In this study, we isolate and characterize various bacterial strains from the leaf, rhizome, and rhizosphere soil of those two medicinal plants to support the cultivation of java cardamom and bitter ginger. Our findings unveil promising attributes among the isolated bacterial strains, including nitrogen fixation, phosphate solubilization, cellulolytic activity, and anti-pathogenic properties. These traits collectively underscore the potential of these bacterial strains as plant growth-promoting agents.

MATERIALS AND METHODS

Sample collection

Rhizome, leaf, and rhizosphere soil samples were collected from *A. compactum* and *Z. zerumbet*, which wildly grow and spread across the forest and mountainous areas of Pacet, Mojokerto, Indonesia. We collect samples from two different plant populations for each species, and for each population, we collect three plant individuals. Rhizome, leaf, and soil samples were also collected from *A. compactum* and *Z. zerumbet* cultivated at Taman Husada Herbal Park, Surabaya, Indonesia.

Isolation of bacterial strains

Bacteria were isolated by mixing 5 g of homogenized samples with 45 mL of sterile distilled water. Next, 1 mL of the resulting suspension is mixed with 9 mL of 0.85% NaCl solution; using the pour plate method, as much as 1 mL of the mixture is inoculated into Reasoner's 2A (R2A) media. The plate was then incubated at 30ºC for 24 to 48 hours. Single bacterial colonies were then inoculated into fresh R2A media using the streak method and incubated at 24-30℃ for 24 to 48 hours (Fatimah et al. 2022).

Macroscopic and microscopic identification of bacterial strains

Macroscopic identification is done by observing the shape, margin, elevation, color, opacity, size, and surface of the bacterial colonies grown on R2A agar plates for 24 to 48 hours. Microscopic identification of endophytic bacteria is done by evaluating the bacteria's cell shape and Gram type under a microscope following Gram's staining.

Molecular identification through 16S rRNA sequencing

According to manufacturer instructions, DNA was extracted from the bacterial culture using the GeneaidTM DNA Isolation Kit. Subsequently, the target region of the 16S rRNA gene at position 27F was amplified using universal primers 27F (5′- AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′- TACGGYTACCTTGTTACGACTT-3′). The PCR product was then purified using a DNA Clean & Concentrator™ kit (Zymo Research, USA) and sent to First Base (Singapore) for sequencing. The resulting 16S rRNA sequences were trimmed using the Bioedit software ver 7.2.5. and similarity analysis of the consensus sequence was performed using NCBI's BLASTn software (Fatimah et al. 2022). Furthermore, the BLAST analysis results were confirmed using 16S-based identification from EZBioCloud (https://www.ezbiocloud.net/).

Phosphate solubilization assay

Bacterial isolates were cultured on liquid Nutrient Broth (NB) media until they reached OD 0.5 at 600 nm. Next, 25 µL of bacterial culture was then inoculated into a 6 mm paper disk, placed on Pikovskaya Agar media, and incubated at 37°C for 72 hours. The clear zone formed around the disk was measured. The diameter of the clear zone indicates the phosphate dissolution index. For each isolate, three replicates were used (Jiang et al. 2018).

Nitrogen fixation assay

Bacterial isolates were inoculated into semi-solid Nitrogen-Free Bromothymol (NFB) media using an inoculating loop and then incubated at 30°C for 10 days. Positive results were indicated by a change in the color of

NFB media from yellow to bluish and the formation of a thin membrane on the media surface (Baldani et al. 2014).

Cellulolytic assay

Bacterial isolates were inoculated into a paper disk placed on CMC (carboxymethyl cellulose) media and incubated at 37°C for 48 hours. The plates were then stained with 1% Congo Red for 15 minutes and 2 to 3 times washing using 1M NaCl for 15 minutes. The formation of a clear zone indicated positive results. The clear zone formed around the disk was measured, and the diameter of the clear zone indicates the cellulolytic index; for each isolate, three replicates were used (Sari et al. 2017; Ardhi et al. 2019).

Anti-plant pathogen assay

Bacterial isolates were cultured in Luria Bertani (LB) Broth media for 24 hours and then centrifuged at 14,000 rpm. Phytopathogenic bacteria *Xanthomonas campestris* were inoculated on Mueller Hinton Agar (MHA) media in three different areas. The first area was placed with a paper disc soaked with bacterial isolate supernatant, the second area was placed with a paper disc soaked with sterile LB Broth (negative control), and the third area was placed with a paper disc soaked with gentamicin 40 mg/mL (positive control). The media is then incubated at 37°C for 24 hours, forming an inhibition zone that indicates the presence of antibacterial activity; for each isolate, three replicates were used (Yemata et al. 2019).

RESULTS AND DISCUSSION

Macroscopic, microscopic, and molecular identification of bacterial isolates

A total of 8 bacterial isolates were obtained from the leaf, rhizome, and rhizosphere soil of *A. compactum* and *Z. zerumbet* (Table 1). Following isolation, macroscopic, microscopic, and molecular identification and characterization were performed for each bacterial strain. Notably, all isolates exhibited rod-shaped morphology except for isolate A, which has pink and opaque colonies. The remaining isolates displayed colonies that were uniformly white and opaque in appearance. Moreover, the bacterial isolates showcased diverse colony characteristics and morphology. Gram staining showed that 5 isolates were categorized as Gram-positive bacteria, while 3 were identified as Gram-negative.

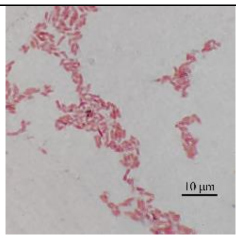
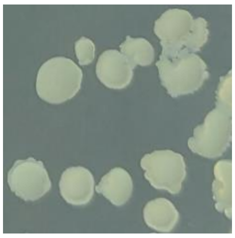
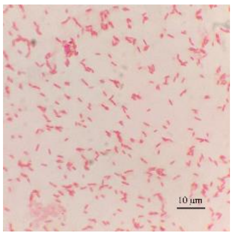
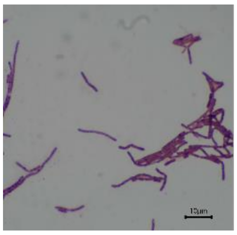
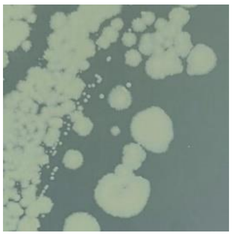
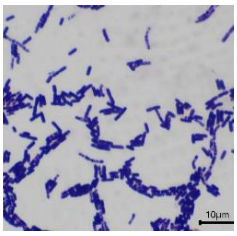
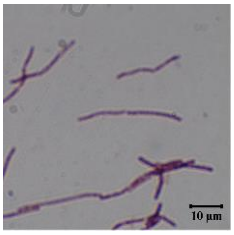
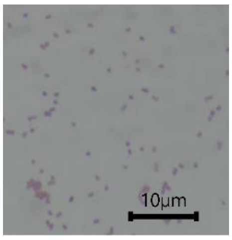
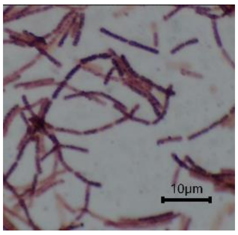
Through molecular identification utilizing 16s rRNA sequencing, our analysis revealed the following bacterial isolates associated with *A. compactum*: *Methylobacterium aquaticum* that is isolated from the leaf, *Paenibacillus tyrfis*, *Priestia megaterium* strain 1, and *Priestia aryabhattai* that isolated from the rhizome, and *Microbacterium arthrosphaerae* that is isolated from the rhizosphere soil (Table 2). Notably, these bacterial species have been previously reported to exhibit plant growth-promoting activities (Lindang et al. 2021; Zhang et al. 2021; Shahid et al. 2022; Jo et al. 2023).

Methylobacterium are among the most prevalent bacterial genera associated with the plant phyllosphere. Studies have shown that inoculation with *M. aquaticum* can effectively promote the growth of model plant *Arabidopsis thaliana*, tobacco (*Nicotiana benthamiana*), and rice (*Oryza sativa*) (Tani et al. 2012; Juma et al. 2022). *Paenibacillus tyrfis* is a soil bacteria previously isolated from forest and peat swamp soil ecosystems. This bacterium exhibits a broad spectrum of antimicrobial activity, including against plant pathogens (Aw et al. 2016). It is also reported to have plant growth-promoting ability and demonstrate high efficacy against phytopathogenic fungi (Tran et al. 2023; 2024).

Various members of the *Priestia* genus have been reported to possess plant growth-promoting activities by facilitating nutrient acquisition by plants and producing compounds that modulate plant growth and defense (Zelaya-Molina et al. 2023). *Priestia megaterium* can be found in diverse habitats, particularly within plants and soil. Various works have reported its beneficial effects on different plant species, including economically significant crops such as tomato (Ibort et al. 2017), maize (Al-Enazy et al. 2017), rice (Feng et al. 2017), bean (*Phaseolus vulgaris*) (Korir et al. 2017), and soybean (*Glycine max*) (Zhou et al. 2017). *Priestia aryabhattai* is a facultative endophytic bacteria reported to be capable of enhancing wheat development by solubilizing mineral nutrients (P, K, and Zn), producing indole-3-acetic acid (IAA), releasing ACC deaminase, ammonia, and siderophores. Furthermore, inoculation of *Pr. aryabhattai* was reported to improve wheat seedling tolerance against salt stress (Shahid et al. 2022). *Microbacterium arthrosphaerae* is a soil bacterium previously isolated from the fecal sample of the pill millipede *Arthrosphaera magna* and the rhizosphere soil of tomatoes. Inoculation of tomatoes with *M. arthrosphaerae* enhanced tomato growth and seed germination, attributed to its ability to produce IAA and salicylic acid (Zazou et al. 2016).

From *Zingiber zerumbet*, we could only isolate bacteria from rhizosphere soil samples; they are identified as *Enterobacter mori*, *Priestia megaterium* strain 2, and *Priestia megaterium* strain 3 (Table 2). Enterobacter are found in diverse environments like water, soil, and the biological systems of animals, humans, and plants, where they exert varying effects on their hosts. While certain strains of *Enterobacter mori* have been reported to induce bacterial wilt in mulberry (*Morus alba*) and kiwi fruit, other strains can function as plant growth-promoting bacteria (Zhu et al. 2011). *Enterobacter mori* isolated from the rhizosphere soil of sorghum has been reported to exhibit plant growth-promoting potential. Additionally, strains isolated from the rhizosphere soil of the hyperaccumulator plant *Sedum* sp. have been found to enhance plant growth and facilitate cadmium (Cd) uptake from Cd-contaminated soil (Liu et al. 2015). While the *Enterobacter mori* strain we isolated exhibited phosphate solubilizing, cellulolytic, and nitrogen-fixing potential, further investigation is necessary to determine if it also possesses pathogenic traits against medicinal plants. This evaluation is crucial to ensure its safety as a biofertilizing agent.

Table 1. Colony and microscopic characterization of bacterial strains isolated from medicinal plants *Amomum compactum* (AC) and *Zingiber zerumbet* (ZZ)

Isolate code	Colony characteristic	Colony morphology	Microscopic characteristic	Cellular morphology
A	Pink and opaque colonies with circular or irregular shapes, raised elevation, entire or undulated margin, smooth surface, and viscid consistency		Rod-shaped, Gram-negative	
B	White and opaque colonies with circular or irregular shape, convex elevation, entire or undulated margin, smooth surface, and viscid consistency		Rod-shaped, Gram-negative	
C	White and opaque colonies with circular shape, raised elevation, entire margin, smooth glistening surface, and viscid consistency		Rod-shaped, Gram-positive	
D	White and opaque colonies with circular or irregular shapes, raised elevation, entire or undulated margin, smooth surface, and viscid consistency		Rod-shaped, Gram-positive	
E	White and opaque colonies with circular shape, raised elevation, entire margin, smooth glistening surface, and viscid consistency		Rod-shaped, Gram-positive	
F	White and opaque colonies with circular shape, raised elevation, entire margin, mucoid glistening surface, and viscid consistency		Rod-shaped, Gram-negative	
G	White and opaque colonies with a circular shape, umbonate elevation, entire margin, rough, dull surface, and viscid consistency		Rod-shaped, Gram-positive	

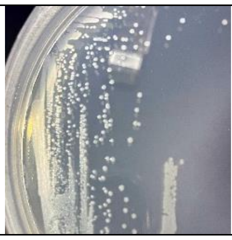
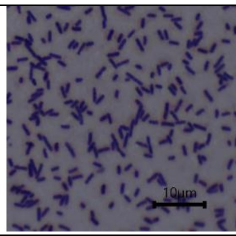
H	White and opaque colonies with punciform shape, pulvinate elevation, entire margin, smooth glistening surface, and viscid consistency		Rod-shaped, Gram-positive	

Table 2. BLASTn similarity results based on 16s rRNA sequence

Plant species	Plant samples	Isolate code	Identification results	Per. ident	Accession
Amomum compactum	Leaf	A	*Methylobacterium aquaticum*	98.53%	NR_025631.1
	Rhizome	B	*Paenibacillus tyrfis*	99.23%	NR_137255.1
		C	*Priestia megaterium*	100%	NR_112636.1
		D	*Priestia aryabhattai*	99.64%	NR_115953.1
	Soil rhizosphere	E	*Microbacterium arthrosphaerae*	98.64%	MN889371.1
Zingiber zerumbet	Soil rhizosphere	F	*Enterobacter mori*	100%	NR_146667.2
		G	*Priestia megaterium*	100%	NR_117473.1
		H	*Priestia megaterium*	99.79%	NR_112636.1

Phosphate solubilization potential of isolated bacterial strains

Bacterial colonies exhibiting clear halo zones in Pikovskaya Agar media encircling microbial growth were identified as phosphate solubilizers (Figure 2.A). Among the isolates, 6 bacterial strains demonstrated phosphate solubilization potential. The average phosphate solubilization index (PSI) value varied among isolates, ranging from 0,34 to 2,66 mm. Pr. megaterium strains 1 and 3 exhibited the highest PSI, followed by Pr. megaterium strain 2, E. mori, M. arthrosphaerae, and Pr. aryabhattai, respectively (Figure 2.B). But overall, the phosphate solubilization activity among all isolates did not differ significantly.

Phosphate-solubilizing bacteria (PSB) are crucial for agriculture and the environment due to their ability to solubilize inorganic phosphate, making it available for plant uptake. This is particularly significant because phosphorus is essential for plant growth and development (Pan and Cai 2023). *Priestia megaterium* isolated from the rhizosphere soil of lettuce, paddy field soil, and rhizosphere soil of vegetable fields are reported to have phosphate solubilizing ability (Kang et al. 2014; Damo et al. 2022; Li et al. 2022). Following previous studies, we also found that *P. megaterium* strains 1, 2, and 3 isolated from the rhizome and rhizosphere soil of medicinal plants *A. compactum* and *Z. zerumbet* exhibit strong phosphate-solubilizing ability. These findings underscore the potential application of *P. megaterium* in agriculture and environmental remediation due to its ability to enhance phosphorus availability in soil ecosystems.

Cellulolytic activity of isolated bacterial strains

All bacterial isolates exhibited cellulolytic activity on CMC agar plates following Congo-red staining, indicated by the formation of clear zones around microbial growth (Figure 3.A). *Pr. megaterium* strains 1 and *Pr. aryabhattai* showed the highest cellulolytic index, followed by *M. arthrosphaerae, Pa. tyrfis, Pr. megaterium* strains 3, *Pr. megaterium* strains 2, *M. aquaticum,* and *E. mori*, respectively (Figure 3.B). Nevertheless, the cellulolytic activity among the isolates did not differ significantly.

Cellulolytic bacteria play a vital role in soil and agriculture because they break down cellulose, a major component of plant cell walls, into simpler sugars, making them available for uptake by plants and other soil organisms. This process helps to replenish soil fertility and supports plant growth (Bautista-Cruz et al. 2024). Previous research has reported the ability of both *Pr. megaterium* and *Pr. aryabhattai* strains to produce extracellular cellulase (Kalbarczyk et al. 2018; Shahid et al. 2022; Bamrungpanichtavorn et al. 2023). Additionally, strains of *Pr. aryabhattai* isolated from the leaves and stems of wheat (*Triticum aestivum)* have been reported not only to synthesize cellulase but also protease, amylase, lipase, and pectinase. Furthermore, these strains have demonstrated tolerance to various abiotic stress factors and exhibit a wide range of anti-phytopathogenic activity (Kalbarczyk et al. 2018). Our study demonstrates that *Pr. megaterium* and *Pr. aryabhattai* isolated from the rhizome and rhizosphere soil of *A. compactum* and *Z. zerumbet* exhibit cellulolytic activity on CMC agar plates. These results underline the potential of Priestia genus members as nutrient cyclers within soil ecosystems, particularly in the decomposition of plant residues and organic matter rich in cellulose.

Nitrogen fixation potential of isolated bacterial strains

All bacterial isolates exhibited nitrogen-fixing activity, as indicated by a change in color on NFB media from yellow to a bluish hue after a 10-day incubation period. The isolates demonstrated varying degrees of nitrogen fixation activity, with *Pr. megaterium, Pr. aryabhattai, Pa. tyrfis,* and *E. mori* identified as the bacterial isolates with the highest nitrogen fixation potential (Table 3).

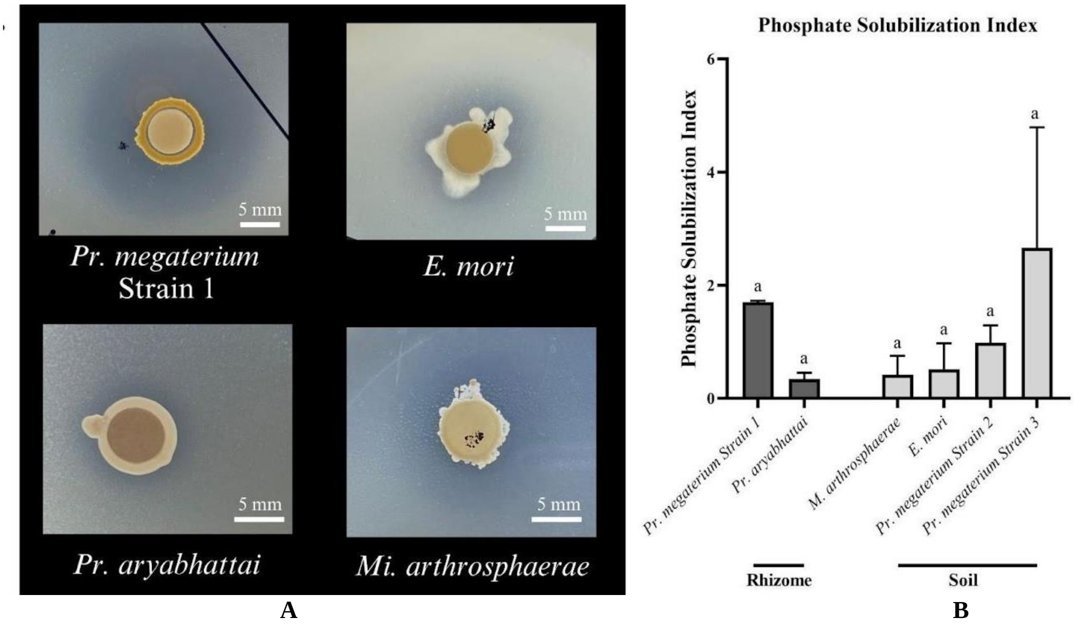


Figure 2. Phosphate solubilizing activity (A) and phosphate solubilization index (PSI) (B) of bacterial strains isolated from medicinal plants *Amomum compactum* and *Zingiber zerumbet*. Data are shown as Mean ±SD (n=3); means followed by different letters indicate significant differences ($p<0.05$) according to the Tukey test

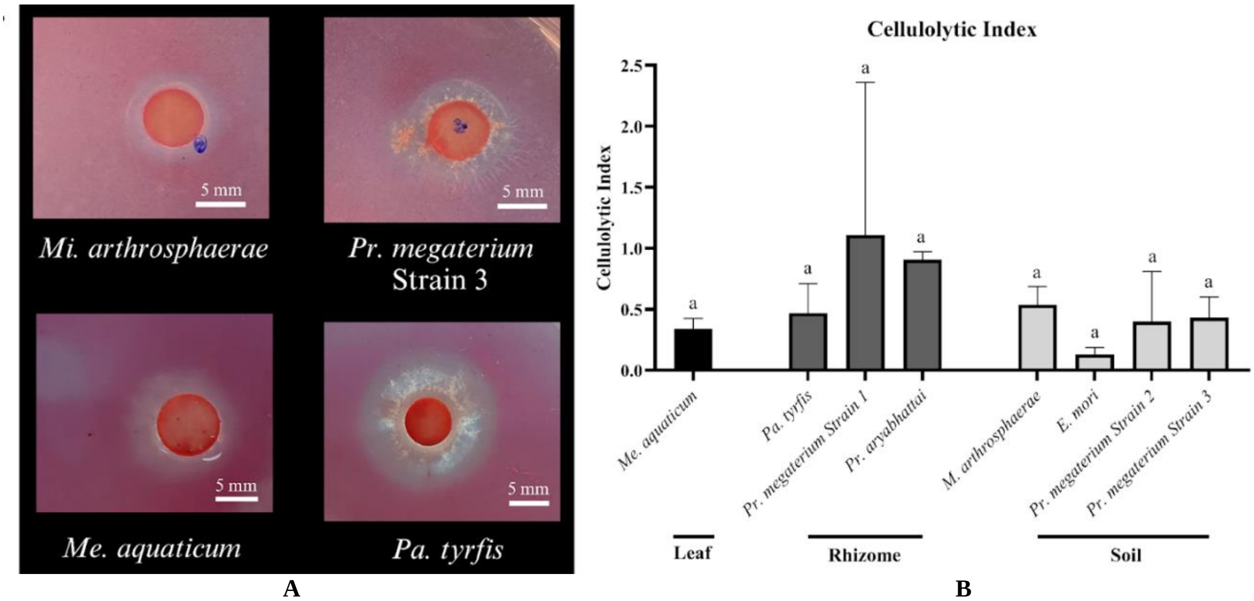


Figure 3. Cellulolytic activity (A) and cellulolytic index (PSI) (B) of bacterial strains isolated from medicinal plants *Amomum compactum* and *Zingiber zerumbet*. Data are shown as Mean ±SD (n=3); means followed by different letters indicate significant differences ($p<0.05$) according to the Tukey test

Nitrogen is an essential nutrient for plants, necessary for synthesizing proteins, enzymes, and chlorophyll; however, it often has low bioavailability in soil. Nitrogen-fixing bacteria play a crucial role in soil and agriculture due to their ability to convert atmospheric nitrogen (N_2) into a form that plants can readily utilize, typically ammonia (NH_3) or nitrate (NO^{-}_3) through the nitrogen enzyme. Harnessing their biological nitrogen fixation capabilities can help reduce dependence on synthetic fertilizers and promote overall soil health and fertility (Sepp et al. 2023). *Priestia megaterium* strain isolated from rhizosphere soil of sugarcane and *Pr. aryabhattai* strain isolated from the soil of maize field has been identified as an effective nitrogen-fixing rhizobacterium (Singh et al. 2020; Deng et al. 2022).

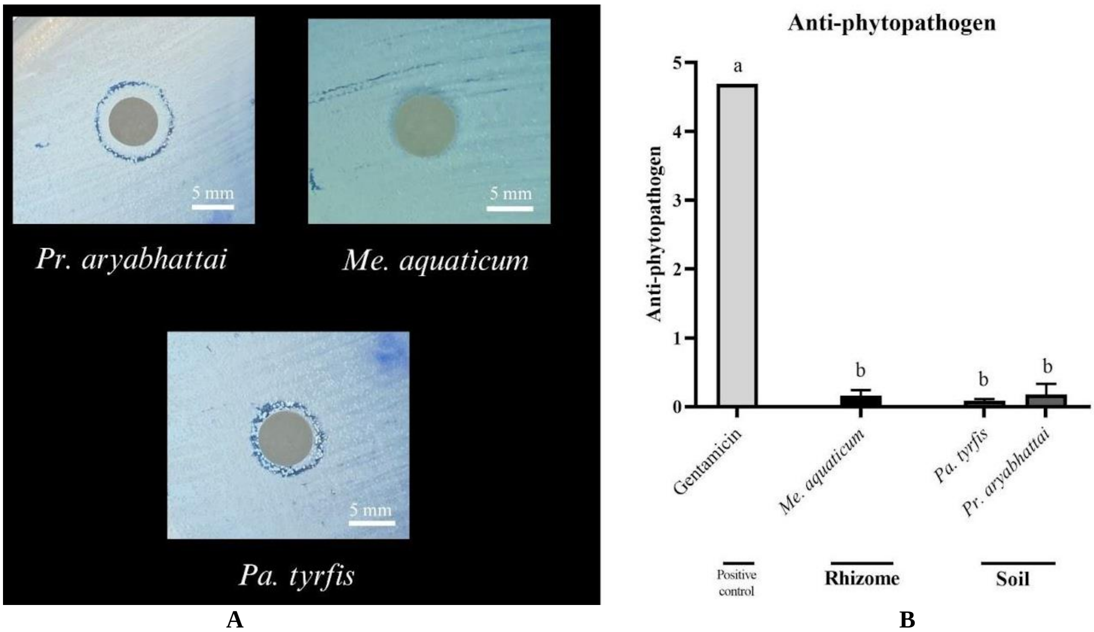


Figure 4. Anti-phytopathogenic activity (A) and diameter of inhibition zone (B) of bacterial strains isolated from medicinal plants *Amomum compactum* and *Zingiber zerumbet* against *Xanthomonas campestris*. Data are shown as Mean ±SD (n=3); means followed by different letters indicate significant differences ($p<0.05$) according to the Tukey test

Table 3. Nitrogen fixation activity of bacterial strains isolated from medicinal plants *Amomum compactum* (AC) and *Zingiber zerumbet* (ZZ)

Samples	Bacteria name (species)	Nitrogen fixing
Leaves	*Me. aquaticum*	++
Rhizome	*Pa. tyrfis*	+++
	Pr. megaterium Strain 1	+++
	Pr. aryabhattai	+++
Soil	*M. arthrosphaerae*	+
	E. mori	+++
	Pr. megaterium Strain 2	+++
	Pr. megaterium Strain 3	+++

Note: +++ (High), ++ (Medium), + (Low), the scoring was based on the intensity of the blue color produced on NFB media following a 10-day bacterial culture period

Similarly, members of the *Paenibacillus* genus isolated from maize farmland and *Enterobacter mori* strain isolated from rhizosphere soil of sorghum have been documented to possess nitrogen fixation potential (Liu et al. 2019; Fadiji et al. 2023). Consistent with prior findings, our study also observed robust nitrogen fixation activity from *Pr. megaterium, Pr. aryabhattai, Pa. tyrfis,* and *E. mori* isolated from rhizome and rhizosphere soil of *A. compactum* and *Z. zerumbet.* These results suggest the potential utility of nitrogen-fixing bacteria from the *Priestia, Paenibacillus*, and *Enterobacter* genera as biofertilizer agents to reduce dependency on synthetic nitrogen fertilizers.

Anti-phytopathogenic potential of isolated bacterial strains

Moreover, the study revealed out of the 8 isolates, only 3 - *M. aquaticum, Pa. tyrfis,* and *Pr. aryabhattai* - exhibited anti-phytopathogenic potential against the phytopathogenic bacteria *Xanthomonas campestris*. Nevertheless, the antibacterial activity demonstrated by these three strains is relatively weak compared to the well-known antibiotic gentamicin (Figure 4).

Anti-phytopathogenic bacteria are essential to sustainable agriculture because they can hinder plant diseases caused by pathogenic microorganisms. These bacteria offer environmentally friendly alternatives to chemical pesticides to control plant diseases (Christakis et al. 2021). This is the first report on the antibacterial activity of *M. aquaticum, Pa. tyrfis,* and *Pr. aryabhattai* against the renowned phytopathogen *X. campestris*, which is responsible for inducing black rot disease in Brassicaceae plants (Liu et al. 2022). These results suggest the potential of *M. aquaticum, Pa. tyrfis,* and *Pr. aryabhattai* as biological control agents for mitigating *X. campestris* infections.

Overall, our findings demonstrate that bacteria isolated from the leaf, rhizome, and rhizosphere soil of medicinal plants *A. compactum* and *Z. zerumbet* possess the potential to act as plant growth-promoting bacteria. This underscores the agricultural and environmental importance of *Priestia, Paenibacillus,* and *Enterobacter* bacteria associated with *A. compactum* and *Z. zerumbet.* These microbes exhibit valuable traits such as phosphate solubilization, cellulolytic activity, and nitrogen fixation, which can enhance soil fertility, support nutrient cycling, and reduce reliance on synthetic fertilizers. They also demonstrated anti-phytopathogenic activity, highlighting their potential as biocontrol agents against plant pathogens. Further research is needed to understand the molecular mechanisms behind these beneficial traits fully. Moreover, the efficacy of the isolated bacterial strains as biofertilizers and biocontrol agents can be influenced by regional climatic factors, soil properties, and microbial diversity. Depending on the environmental conditions and plant species, these strains may exhibit either beneficial or detrimental effects on plants' growth and ecosystem. Therefore, to address this variability and fully explore the potential of these bacterial strains, future research should focus on conducting field trials in diverse regions with varying environmental conditions. Further investigation, including microbiome analysis of the treated plants and soil, will be essential in elucidating both the advantageous and deleterious effects of these bacterial strains on plants and soil ecosystems.

ACKNOWLEDGEMENTS

This study was funded by *Pendanaan Penelitian Skema Penelitian Dasar*, Directorate General of Higher Education, Research, and Technology, Indonesia, with grant number 795/UN3.15/PT/2022.

REFERENCES

Al-Enazy AAR, Al-Oud SS, Al-Barakah FN, Usman ARA. 2017. Role of microbial inoculation and industrial by-product phosphogypsum in growth and nutrient uptake of maize (*Zea mays* L.) grown in calcareous soil. J Sci Food Agri 97: 3665-3374. DOI: 10.1002/jsfa.8226.

Ardhi A, Ahmad KC, Novrianti H, Husna EY, Yulis M, Pratiwi NW, Saryono S. 2019. Hydrolytic enzymes-producing ability of species of actinomycetes and bacteria associated with wilted banana plants (*Musa* sp.). Biodiversitas 20: 1147-1153. DOI: 10.13057/biodiv/d200429.

Aw YK, Ong KS, Lee LH, Cheow YL, Yule CM, Lee SM. 2016. Newly isolated *Paenibacillus tyrfis* sp. Nov., from Malaysian tropical peat swamp soil with broad spectrum antimicrobial activity. Front Microbiol 7: 219. DOI: 10.3389/fmicb.2016.00219.

Baldani JI, Reis VM, Videira SS, Boddey LH, Baldani VLD. 2014. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using n-free semi-solid media: A practical guide for microbiologists. Plant and Soil 384 (1): 413-431. DOI: 10.1007/s11104-014-2186-6.

Bamrungpanichtavorn T, Ungwiwatkul S, Boontanom P, Chantarasiri A. 2023. Diversity and cellulolytic activity of cellulase-producing bacteria isolated from the soils of two mangrove forests in Eastern Thailand. Biodiversitas 24: 3891-3902. DOI: 10.13057/biodiv/d240728.

Bautista-Cruz A, Aquino-Bolaños T, Hernández-Canseco J, Quiñones-Aguilar EE. 2024. Cellulolytic aerobic bacteria isolated from agricultural and forest soils: An overview. Biology 13: 102. DOI: 10.3390/biology13020102.

BPS [Badan Pusat Statistik]. 2018. Statistik Tanaman Biofarmaka Indonesia 2017. [Indonesian]

Christakis CA, Daskalogiannis G, Chatzaki A, Markakis EA, Mermigka G, Sagia A, Rizzo GF, Catara V, Lagkouvardos I, Studholme DJ, Sarris PF. 2021. Endophytic bacterial isolates from halophytes demonstrate phytopathogen biocontrol and plant growth promotion under high salinity. Front Microbiol 12: 681567. DOI: 10.3389/fmicb.2021.681567.

Compant S, Samad A, Faist H, Sessitsch A. 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. J Adv Res 19: 29-37. DOI: 10.1016/j.jare.2019.03.004.

Damo JLC, Ramirez MDA, Agake S, Pedro M, Brown M, Sekimoto H, Yokoyama T, Sugihara S, Okazaki S, Ohkama-Ohtsu N. 2022. Isolation and characterization of phosphate solubilizing bacteria from paddy field soils in Japan. Microbes Environ 37: ME21085. DOI: 10.1264/jsme2.ME21085.

Das A, Kamal S, Shakil NA, Sherameti I, Oelmüller R, Dua M, Tuteja N, Johri AK, Varma A. 2012. The root endophyte fungus *Piriformospora indica* leads to early flowering, higher biomass and altered secondary metabolites of the medicinal plant, *Coleus forskohlii*. Plant Signal Behav 7: 103-112. DOI: 10.4161/psb.7.1.18472.

Deng C, Zhang N, Liang X, Huang T, Li B. 2022. *Bacillus aryabhattai* LAD impacts rhizosphere bacterial community structure and promotes maize plant growth. J Sci Food Agri 102 (14): 6650-6657. DOI: 10.1002/jsfa.12032.

Do HDK, Luqman A, Vu MT, Nguyen HD, Putro YK, Rofiqa EA, Santoso H, Kristanti AN, Hariyanto S, Bui LM, Manuhara YSW, Wibowo AT. 2022. Differences in bacterial composition between vascular epiphyte and parasitic plants living on the same host plants. Biodiversitas 23: 5798-5805. DOI: 10.13057/biodiv/d231133.

Fadiji AE, Ayangbenro AS, Babalola OO. 2023. Genomic assessment of Enterobacter mori AYS9: A potential plant growth-promoting drought-resistant rhizobacteria. Span J Soil Sci 13: 11302. DOI: 10.3389/sjss.2023.11302.

Fatimah, Suroiyah F, Solikha N, Rahayuningtyas ND, Surtiningsih T, Nurhariyati T, Ni'matuzahroh, Affandi M, Geraldi A, Thontowi A. 2022. Antimicrobial activity of actinomycetes isolated from mangrove soil in Tuban, East Java, Indonesia. Biodiversitas 23 (6): 2957-2965. DOI: 10.13057/biodiv/d230622.

Feng F, Ge J, Li Y, He S, Zhong J, Liu X, Yu X. 2017. Enhanced degradation of chlorpyrifos in rice (*Oryza sativa* L.) by five strains of endophytic bacteria and their plant growth promotional ability. Chemosphere 184: 505-513. DOI: 10.1016/j.chemosphere.2017.05.178.

Ibort P, Molina S, Núñez R, Zamarreño ÁM, García-Mina JM, Ruiz-Lozano JM, Orozco-Mosqueda MDC, Glick BR, Aroca R. 2017. Tomato ethylene sensitivity determines interaction with plant growth-promoting bacteria. Ann Bot 120: 101-22. DOI: 10.1093/aob/mcx052.

Jiang H, Qi P, Wang T, Wang M, Chen M, Chen N, Pan L, Chi X. 2018. Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. 3 Biotech 8 (11): 461. DOI: 10.1007/s13205-018-1485-7.

Jo HW, Lim K, Ibal JC, Kim MC, Kim HB, Baek C, Heo YM, Lee H, Kang S, Lee DG, Shin JH. 2023. Growth increase in the herbaceous plant *Centella asiatica* by the plant growth-promoting rhizobacteria *Priestia megaterium* HyangYak-01. Plants 12: 2398. DOI: 10.3390/plants12132398.

Juma PO, Fujitani Y, Alessa O, Oyama T, Yurimoto H, Sakai Y, Tani A. 2022. Siderophore for lanthanide and iron uptake for methylotrophy and plant growth promotion in *Methylobacterium aquaticum* strain 22A. Front Microbiol 13: 921635. DOI: 10.3389/fmicb.2022.921635.

Juwitaningsih T, Jahro IS, Sari SA. 2020. Evaluation of North Sumatera cardamom seed (*Amomum compactum*) extract as antibacterial and anticancer. J Physics: Conf Ser 1485: 012019. DOI: 10.1088/1742-6596/1485/1/012019.

Kalbarczyk KZ, Mazeau EJ, Rapp KM, Marchand N, Koffas MAG, Collins CH. 2018. Engineering *Bacillus megaterium* strains to secrete cellulases for synergistic cellulose degradation in a microbial community. ACS Synthetic Biol 7: 2413-2422. DOI: 10.1021/acssynbio.8b00186.

Kang SM, Ramalingam R, You YH, Joo GJ, Lee IJ, Lee KO, Kim JH. 2014. Phosphate solubilizing *Bacillus megaterium* mj1212 regulates endogenous plant carbohydrates and amino acids contents to promote mustard plant growth. Indian J Microbiol 54 (4): 427-433. DOI: 10.1007/s12088-014-0476-6.

Khalid KA. 2012. Review: Biological fertilization and its effect on medicinal and aromatic plants. Nusantara Biosci 4: 124-133. DOI: 10.13057/nusbiosci/n040307.

Kirana C, McIntosh GH, Record IR, Jones GP. 2003. Antitumor activity of extract of *Zingiber aromaticum* and its bioactive sesquiterpenoid zerumbone. Nutr Cancer 45 (2): 218-225. DOI: 10.1207/S15327914NC4502_12.

Korir H, Mungai NW, Thuita M, Hamba Y, Masso C. 2017. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front Plant Sci 8: 141. DOI: 10.3389/fpls.2017.00141.

Lee JA, Lee MY, Seo CS, Jung DY, Lee NH, Kim JH, Ha HK, Shin HK. 2010. Anti-asthmatic effects of an *Amomum compactum* extract on an ovalbumin (OVA)-induced murine asthma model. Biosci Biotechnol Biochem 74 (9): 1814-1818. DOI: 10.1271/bbb.100177.

Lee JA, Lee MY, Shin IS, Seo CS, Ha HK, and Shin HK. 2012. Anti-inflammatory effects of *Amomum compactum* on RAW 264.7 cells via induction of heme oxygenase-1. Arch. Pharmacal Res 35 (4): 739-746. DOI: 10.1007/s12272-012-0419-x.

Lindang HU, Subbiah VK, Rodrigues KF, Budiman C. 2021. Isolation, identification, and characterization of phosphate solubilizing bacteria, *Paenibacillus* sp., from the soil of danum valley tropical rainforest, Sabah, Malaysia. Biodiversitas 22 (10): 4370-4378. DOI: 10.13057/biodiv/d221030.

Li Q, Hou Z, Zhou D, Jia M, Lu S, Yu J. 2022. A plant growth-promoting bacteria *Priestia megaterium* JR48 induces plant resistance to the crucifer black rot via a salicylic acid-dependent signaling pathway. Front Plant Sci 13: 1046181. DOI: 10.3389/fpls.2022.1046181.

Liu H, Brettell LE, Qiu Z, Singh BK. 2020. Microbiome-mediated stress resistance in plants. Trends Plant Sci 25 (8): 733-743. DOI: 10.1016/j.tplants.2020.03.014.

Liu W, Wang B, Wang B, Hou J, Luo Y, Tang C, Franks AE. 2015. Plant growth-promoting rhizobacteria enhance the growth and Cd uptake of sedum plumbizincicola in a Cd-contaminated soil. J Soils Sediments 15 (5): 1191-1199. DOI: 10.1007/s11368-015-1067-9.

Liu X, Li Q, Li Y, Guan G, Chen S. 2019. *Paenibacillus* strains with nitrogen fixation and multiple beneficial properties for promoting plant growth. PeerJ 7: e7445. DOI: 10.7717/peerj.7445.

Liu Z, Wang Z, Wang J, Jian Lv, Xie B, Luo S, Wang S. 2022. Physical, chemical, and biological control of black rot of brassicaceae vegetables: A review. Front Microbiol 13: 1023826. DOI: 10.3389/fmicb.2022.1023826.

Luqman A, Jongkon S, Prasetya Y, Ammanath AV, Andini, Amala SN, Zulaika E, Kuswytasari ND, Goetz F, Wibowo AT. 2023. Detection of vancomycin resistant genes in intrinsically antibiotic resistant bacteria from the gut microbiota of Indonesian individuals. Iran J Med Sci 49 (5): 302-312. DOI: 10.30476/IJMS.2023.98767.3087.

Nugrahapraja H, Wicaksono PW, Putri BQ, Ni'matuzahroh, Fatimah, Huang L, Hafza N. 2022. Effects of microplastic on human gut microbiome: Detection of plastic-degrading genes in human gut exposed to microplastics-preliminary study. Environments 9 (11): 140. DOI: 10.3390/environments9110140.

Pan L, Can B. 2023. Phosphate-solubilizing bacteria: Advances in their physiology, molecular mechanisms and microbial community effects. Microorganisms 11: 2904. DOI: 10.3390/microorganisms11122904.

Pathania P, Ankita R, Poonam CS, Ranjana B. 2020. Role of plant growth-promoting bacteria in sustainable agriculture. Biocatal Agric Biotechnol 30: 101842. DOI: 10.1016/j.bcab.2020.101842.

Sari NW, Safika, Darmawi, Fahrimal Y. 2017. Isolation and identification of a cellulolytic *Enterobacter* from rumen of Aceh cattle. Vet World 10 (12): 1515-1520. DOI: 10.14202/vetworld.2017.1515-1520.

Schmidt R, Köberl M, Mostafa A, Elshahat MR, Monschein M, Jensen KB, Bauer R, Berg G. 2014. Effects of bacterial inoculants on the indigenous microbiome and secondary metabolites of chamomile plants. Front Microbiol 5: 64. DOI: 10.3389/fmicb.2014.00064.

Sepp SK, Vasar M, Davison J, Oja J, Anslan S, Al-Quraishy S, Bahram M, Bueno CG, Cantero JJ, Fabiano EC, et al. 2023. Global diversity and distribution of nitrogen-fixing bacteria in the soil. Front Plant Sci 14: 1100235. DOI: 10.3389/fpls.2023.100235.

Shahid M, Zeyad MT, Syed A, Singh UB, Mohamed A, Bahkali AH, Elgorban AM, Pichtel J. 2022. Stress-tolerant endophytic isolate *Priestia aryabhattai* BPR-9 modulates physio-biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. Intl J Environ Res Public Health 19: 10883. DOI: 10.3390/ijerph191710883.

Singh RK, Singh P, Li HB, Song QQ, Guo DJ, Solanki MK, Verma KK, Malviya MK, Song, XP, Lakshmanan P, Yang LT, Li YR. 2020. Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: A comprehensive study of plant-microbe interactions for growth enhancement in *Saccharum* spp.. BMC Plant Biol 20 (1): 220. DOI: 10.1186/s12870-020-02400-9.

Sofian FF, Pambayun GW, Runadi D, Susilawati Y, Tjitraresmi A, Herdiana Y, Astuti EP. 2019. Larvicidal activity of ethanol extract and essential oil from *Zingiber aromaticum* Val. rhizome against *Aedes aegypti* larvae. Res J Pharm Biol Chem Sci 11 (1): 11-14.

Subehan, Usia T, Iwata H, Kadota S, Tezuka Y. 2006. Mechanism-based inhibition of CYP3A4 and CYP2D6 by Indonesian medicinal plants. J Ethnopharmacol 105 (3): 449-455. DOI: 10.1016/j.jep.2005.12.001.

Tani A, Takai Y, Suzukawa I, Akita M, Murase H, Kimbara K. 2012. Practical application of methanol-mediated mutualistic symbiosis between *Methylobacterium* species and a roof greening moss, *Racomitrium japonicum*. PloS One 7: e33800. DOI: 10.1371/journal.pone.0033800.

Tan SK, Pippen R, Yusof R, Rahman NA, Ibrahim H, Khalid N. 2006. Screening of selected Zingiberaceae extracts for dengue-2 virus protease inhibitory activities. Sunway Academic J 3: 1-7.

Tran DM, Do TO, Nguyen QV. 2024. Whole genome sequence data of Paenibacillus tyrfis YSS-72.2.G2, a chitinolytic bacterium newly isolated from a National Park of Vietnam. Data in Brief 53: 110087. DOI: 10.1016/j.dib.2024.110087.

Tran DM, Huynh TO, Nguyen TH, Do TO, Nguyen QV, Nguyen AD. 2023. Novel resources of chitinolytic bacteria isolated from Yok Don National Park, Vietnam. J Appl Microbiol 134: lxad141. DOI: 10.1093/jambio/lxad141.

Ujilestari T, Martien R, Ariyadi B, Dono ND, Zuprizal. 2019. Antibacterial effects of essential oils of *Cymbopogon citratus* and *Amomum compactum* under self-nanoemulsifying drug delivery system (SNEDDS). IOP Conf Ser: Earth Environ Sci 387: 012071. DOI: 10.1088/1755-1315/387/1/012071.

Vu MT, Geraldi A, Do HDK, Luqman A, Nguyen HD, Fauzia FN, Amalludin FI, Sadila AY, Wijaya NH, Santoso H, Manuhara YSW, Bui LM, Hariyanto S, Wibowo AT. 2022. Soil mineral composition and salinity are the main factors regulating the bacterial community associated with the roots of coastal sand dune halophytes. Biology 11 (5): 695. DOI: 10.3390/biology11050695.

Yemata G, Desta B, Fetene M. 2019. In vitro antibacterial activity of traditionally used medicinal plants against *Xanthomonas campestris* pv. *musacearum* in Ethiopia. Biodiversitas 20 (2): 555-561. DOI: 10.13057/biodiv/d200235.

Yuan J, Zhang W, Sun K, Tang MJ, Chen PX, Li X, Dai CC. 2019. Comparative transcriptomics and proteomics of *Atractylodes lancea* in response to endophytic fungus *Gilmaniella* sp. AL12 reveals regulation in plant metabolism. Front Microbiol 10: 1208. DOI: 10.3389/fmicb.2019.01208.

Yunus Y, Suprapti TJW. 2015. Antibacterial activity of the essensial oils of lempuyang wangi (*Zingiber aromaticum* Val.), lempuyang gajah (*Zingiber zerumbet* Sm), and lempuyang emprit (*Zingiber amaricans* BI.) on the Gram negative bacteria. Asian J Appl Sci 3 (2): 290-293.

Zazou K, Tifrit A, Chama Z, Kaldi A, Tounssi S, Abbouni B. 2016. Isolation and characterization of *Microbacterium arthrosphaerae* and its effect on tomato culture growth. Pharm Lett 8 (18): 1-8.

Zelaya-Molina LX, Guerra-Camacho JE, Ortiz-Alvarez JM, Vigueras-Cortés JM, Villa-Tanaca L, Hernández-Rodríguez C. 2023. Plant growth-promoting and heavy metal-resistant *Priestia* and *Bacillus* strains associated with pioneer plants from mine tailings. Arch Microbiol 205 (9): 318. DOI: 10.1007/s00203-023-03650-5.

Zhang C, Wang MY, Khan N, Tan TT, Yang S. 2021. Potentials, utilization, and bioengineering of plant growth-promoting *Methylobacterium* for sustainable agriculture. Sustainability 13 (7): 3941. DOI: 10.3390/su13073941.

Zhou Y, Wang Y, Zhu X, Liu R, Xiang P, Chen J, Liu X, Duan Y, Chen L. 2017. Management of the soybean cyst nematode *Heterodera glycines* with combinations of different rhizobacterial strains on soybean. PLoS One 12: e0182654. DOI: 10.1371/journal.pone.0182654.

Zhu B, Lou MM, Xie GL, Wang GF, Zhou Q, Wang F, Fang Y, Su T, Li B, Duan YP. 2011. *Enterobacter mori* sp. nov., associated with bacterial wilt on *Morus alba* L. Intl J Syst Evol Microbiol 61: 2769-2774. DOI: 10.1099/ijs.0.028613-0.