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In vitro efficacy of plant growth-promoting rhizobacteria isolated from ginger (*Zingiber officinale*) rhizosphere for biological control of plant pathogens

Efikasi *in vitro* rhizobacteria pemacu pertumbuhan tanaman yang diisolasi dari rizosfer jahe (*Zingiber officinale*) untuk pengendalian hayati patogen tanaman

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ABSTRACT
The excessive use of synthetic chemical pesticides in controlling plant diseases poses significant challenges to sustainable agriculture. As an environmentally friendly alternative, plant growth-promoting rhizobacteria (PGPR) have shown promise by colonizing plant roots and forming symbiotic relationships that enhance plant growth and suppress plant pathogens. Research on the isolation of PGPR from ginger (*Zingiber officinale*) rhizosphere in Indonesia remains limited, highlighting the need for further exploration to support genetic resource conservation and sustainable agricultural practices. This study aims to evaluate the potential of PGPR isolates from *Z. officinale* rhizosphere in Semarang, Indonesia, as biological control agents. Isolates were characterized based on morphology, biochemical traits, and their antagonistic activity against fungal pathogens, including *Fusarium oxysporum*, *Colletotrichum* sp., and *Alternaria* sp. A total of 14 isolates were obtained, exhibiting diverse morphological, biochemical, and antagonistic properties. Among them, isolate 235A2 demonstrated the highest potential as a biological control agent, with capabilities including phosphate solubilization, nitrogen fixation, protease enzyme production, and significant inhibition rates of *F. oxysporum* (13.79%), *Colletotrichum* sp. (55.56%), and *Alternaria* sp. (35.61%) in vitro. These findings underscore the potential of PGPR as a sustainable alternative for biological control of plant pathogens, supporting both enhanced plant productivity and environmental conservation.

ABSTRAK
Penggunaan pestisida kimia sintetis yang berlebihan dalam pengendalian penyakit tanaman menghadirkan tantangan signifikan bagi pertanian berkelanjutan. Sebagai alternatif yang ramah lingkungan, *plant growth-promoting rhizobacteria* (PGPR) menunjukkan potensi dengan mengolonisasi akar tanaman dan membentuk hubungan simbiosis yang meningkatkan pertumbuhan tanaman sekaligus menekan patogen tanaman. Penelitian tentang isolasi PGPR dari rizosfer jahe (*Zingiber officinale*) di Indonesia masih terbatas, sehingga diperlukan eksplorasi lebih lanjut untuk mendukung konservasi sumber daya genetik dan praktik pertanian berkelanjutan. Penelitian ini bertujuan untuk mengevaluasi potensi isolat PGPR dari rizosfer jahe di Semarang, Indonesia, sebagai agen pengendali hayati. Isolat dikarakterisasi berdasarkan morfologi, sifat biokimia, dan aktivitas antagonistiknya terhadap patogen, termasuk *Fusarium oxysporum*, *Colletotrichum* sp., dan *Alternaria* sp. Sebanyak 14 isolat berhasil diperoleh dengan berbagai karakteristik morfologi, biokimia, dan antagonistik. Di antara isolat tersebut, isolat 235A2 menunjukkan potensi tertinggi sebagai agen pengendali hayati dengan kemampuan melarutkan fosfat, memfiksasi nitrogen, memproduksi enzim protease, serta menghambat *F. oxysporum* (13.79%), *Colletotrichum* sp. (55.56%), dan *Alternaria* sp. (35.61%) secara in vitro. Temuan ini menunjukkan potensi PGPR sebagai alternatif berkelanjutan dalam mengendalikan patogen tanaman secara hayati, serta mendukung produktivitas tanaman dan konservasi lingkungan.

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INTRODUCTION

Globally, crop yield losses due to plant diseases range from 21% to 41% for major crops, underscoring the critical importance of effective plant disease management (Lucas, 2011; Savary et al., 2019; Rizzo et al., 2021). Conventional control practices predominantly rely on synthetic chemical pesticides, which contribute significantly to environmental pollution and disrupt ecological balance (Purnama et al., 2023). Furthermore, widespread use of synthetic pesticides has led to the emergence of pesticide-resistant pathogens, complicating effective disease control (Lucas, 2011). These challenges necessitate a shift toward sustainable solutions, with biological control agents such as plant growth-promoting rhizobacteria (PGPR) emerging as a promising alternative.

PGPR are rhizosphere-colonizing bacteria capable of enhancing plant growth and suppressing pathogens through direct and indirect mechanisms. Direct mechanisms include the production of phytohormones such as auxins, biological nitrogen fixation, solubilization of essential nutrients like phosphorus and potassium, and the sequestration of iron through siderophore production (Cassán et al., 2009; Dewi et al., 2015; Kour et al., 2023). Indirect mechanisms involve biocontrol through systemic resistance induction, quorum sensing disruption in pathogenic bacteria, and the production of antimicrobial compounds (Hartman & Tringe, 2019). Notable genera such as *Acinetobacter*, *Bacillus*, *Pseudomonas*, and *Rhizobium* have been identified as effective PGPR with both plant growth-promoting and biocontrol capabilities (Rodríguez-Sahagún et al., 2020).

Previous research has focused on isolating and characterizing PGPR from various crops, including *Zingiber officinale*, *Capsicum annum*, and *Oryza sativa* (Dinesh et al., 2015; Jasim et al., 2014; Sari et al., 2021; Santosa et al., 2018). PGPR has shown significant potential as a biological control agent. For example, *Bacillus* spp. inhibited the growth of *Fusarium oxysporum*, the causative agent of rhizome rot in *Z. officinale*, by 50–53.3% in vitro (Marwan et al., 2023). The application of formulations containing *Trichoderma* spp. and *Bacillus* spp. to *Z. officinale* rhizomes before planting and 30 days after planting improved plant growth and reduced rhizome rot by 78.57–93.3% (Marwan et al., 2023). Similarly, *Pseudomonas fluorescens* effectively controlled *Phytium* spp., a pathogen causing soft rot in *Z. officinale*, by producing antifungal compounds such as pyoluteorin, phenazine, and pyrrolnitrin (Bhai et al., 2005; Abdelaziz et al., 2023; Balthazar et al., 2022). Moreover, *Bacillus subtilis* MK030136 has been reported to synthesize volatile compounds like siderophores and hydrogen cyanide (HCN), which enhance its biocontrol efficacy (Maghrawy et al., 2020).

Despite these advances, most research on PGPR has been concentrated in regions such as India (Dinesh et al., 2015; Jasim et al., 2014), with limited studies focusing on tropical areas like Southeast Asia, including Indonesia. The scarcity of data on PGPR abundance and diversity in *Z. officinale* rhizosphere soils in Indonesia, particularly in Semarang, Central Java, highlights a significant research gap. Addressing this gap is crucial for developing sustainable agricultural practices and conserving microbial genetic resources. This study aims to isolate and characterize PGPR from the *Z. officinale* rhizosphere in Semarang, Central Java, Indonesia, and to evaluate their potential as biological control agents against fungal pathogens, including *Fusarium oxysporum*, *Colletotrichum* sp., and *Alternaria* sp., under in vitro conditions. The findings are expected to contribute to global efforts in sustainable disease management and genetic resource conservation.

MATERIALS & METHODS

Sample collection

Soil samples were collected from the rhizosphere of *Z. officinale* plants in two sub-districts of Semarang, Central Java: Getasan and Sumowono. These areas were selected based on their status as major *Z. officinale* production regions. Sampling points were identified using a purposive sampling method, considering locations with healthy *Z. officinale* plants exhibiting optimal morphological characteristics. Five sampling points were selected in each sub-district, with three replicates collected per point at a depth of 5–15 cm (Barillot et al., 2013). The samples were placed in sterile plastic bags, stored in a cooler box, and transported to the Laboratory of Plant Protection, Faculty of Agriculture and Business,

Satya Wacana Christian University, for further processing. Details of the sampling locations are presented in Table 1. Table 1 provides the geographical coordinates, location of sampling points, and soil depth for each sub-district. These details highlight the consistency of sampling protocols and ensure reproducibility.

Table 1. Sampling locations in Getasan and Sumowono, Semarang, Central Java, Indonesia

Sub-District	Sampling Points	Replications per Point	Coordinates (Latitude, Longitude)	Soil Depth (cm)
Getasan	Nogosaren	3	S 07.35988° E 110.41447°	5–15
	Manggian	3	S 07.35909° E 110.44214°	
	Manggian	3	S 07.35997° E 110.44901°	
	Polobogo	3	S 07.35471° E 110.45959°	
	Polobogo	3	S 07.36282° E 110.45908°	
Sumowono	Kebonagung	3	S 07.23233° E 110.29389°	5–15
	Candi Garon	3	S 07.23685° E 110.29134°	
	Candi Garon	3	S 07.23549° E 110.28714°	
	Kemitir	3	S 07.22670° E 110.27414°	
	Ngadikerso	3	S 07.24688° E 110.32033°	

Isolation and purification of rhizobacteria

Rhizobacteria were isolated using a serial dilution method on nutrient agar (NA) medium, followed by incubation at 30°C for 24 hours. Colonies with distinct morphological characteristics were purified by repeated streaking on yeast peptone glucose agar (YPGA) medium (Maya et al., 2022). Pure isolates were stored on YPGA slants at 4°C for subsequent analyses.

Morphological and biochemical characterization

The purified isolates were characterized for colony morphology (shape, edge, elevation, color, and texture). Gram staining was performed using a 3% KOH solution to distinguish Gram-positive and Gram-negative bacteria (Schaad et al., 2001).

Hypersensitivity reaction test

The pathogenic potential of isolates was assessed through a hypersensitivity reaction (HR) test. A bacterial suspension (10^8 CFU/mL) was infiltrated into the leaves of tobacco plants (*Nicotiana tabacum*) using a sterile syringe. The development of necrotic lesions within 72 hours indicated a positive HR, while no lesions indicated non-pathogenicity (Klement & Goodman, 1967).

Phosphate solubilization and nitrogen fixation tests

Phosphate solubilization was tested by inoculating isolates on Pikovskaya's agar and incubating at room temperature for seven days. Clear halo zones around colonies indicated solubilization activity (Gupta & Pandey, 2023). Nitrogen fixation was assessed using Jensen's medium, with positive results indicated by a yellow halo zone after incubation at 30°C for 5–7 days (Prodhan et al., 2023).

Protease activity assay and antagonistic activity against fungal pathogens

Protease activity was tested on skim milk agar (SMA) plates. Clear zones around bacterial colonies after incubation at 30°C for 24 hours indicated protease enzyme production (Chang et al., 2009).

The antagonistic activity of bacterial isolates was tested against *Fusarium oxysporum*, *Colletotrichum* sp., and *Alternaria* sp. using the dual culture method. The bacterial isolates were inoculated on one side of potato dextrose agar (PDA) plates,

and fungal pathogens were inoculated on the opposite side. Plates were incubated at 28°C for seven days. The percentage of growth inhibition was calculated using the formula:

$$I\ (\%) = \frac{(R1-R2)}{R1} \times 100 \quad (1)$$

where *I* is the inhibition percentage, *R*1 is the fungal colony diameter in the control, and *R*2 is the fungal colony diameter in the presence of bacterial isolates (Dikin et al., 2006).

Data analysis

Quantitative data, including antagonistic activity and enzyme assays, were analyzed using one-way analysis of variance (ANOVA) at a 5% significance level to determine treatment effects. Post-hoc multiple comparisons were conducted using Tukey's Honestly Significant Difference (HSD) test to identify significant differences among treatment means. Morphological and biochemical characteristics of bacterial isolates were analyzed descriptively. All statistical analyses were performed using SAS software version 9.3, and results were presented as mean.

RESULTS & DISCUSSIONS

Isolation and morphological characterization of rhizobacteria

A total of 14 bacterial isolates were successfully obtained from the rhizosphere of *Z. officinale*. However, not all sampling locations yielded isolates due to technical challenges during the isolation process and the subsequent harvesting of *Z. officinale* plants before re-sampling could occur. The isolates were distributed across various villages in Sumowono and Getasan sub-districts, as detailed in Table 2.

Table 2. Sampling locations and rhizobacteria isolates from *Z. officinale* rhizosphere

Sub-district	Village	Hamlet	Coordinates (Latitude, Longitude)	Isolate Codes
Sumowono	Ngadikerso	Ngadikerso	S 07.24688° E 110.32033°	85A
Sumowono	Candi Garon	Bodean	S 07.23549° E 110.28714°	115A, 115BM
Sumowono	Kemitir	Ngoho	S 07.22670° E 110.27414°	124A, 124B
Getasan	Polobogo	Blogoran	S 07.36282° E 110.45908°	234A, 234B, 234C
Getasan	Manggian	Manggian	S 07.35909° E 110.44214°	235B1, 235B2, 235A1, 235A2
Getasan	Manggian	Pendem	S 07.35997° E 110.44901°	244A, 244B

Most farmers practiced intercropping *Z. officinale* with other crops such as cassava, banana, chili, eggplant, and leek, as shown in Figure 1. Intercropping systems often result in higher pesticide use, particularly with crops in the Solanaceae family. This practice can reduce soil microbial abundance and diversity due to pesticide residues, as validated by field observations and discussions with farmers. These findings align with studies by Onwona-Kwakye et al. (2020) and Walder et al. (2022), which reported significant reductions in soil microbial diversity caused by pesticide residues.

The morphological characteristics of bacterial colonies varied significantly in terms of shape, color, edge, and size. Most isolates (92.9%) were Gram-positive, while one isolate (7.1%) was Gram-negative (Table 3). The diversity observed is consistent with previous reports of high microbial diversity in rhizosphere soils of tropical crops (Dinesh et al., 2015; Jasim et al., 2014). The hypersensitivity reaction (HR) test was conducted to assess the pathogenic potential of the rhizobacteria isolates. Of the 14 isolates, 9 Gram-positive isolates exhibited negative HR results, including isolates 85A, 115BM, 124A, 234C, 234A, 235A2, 235B2, 234B, and 244A (Table 3). Negative HR results indicate that these isolates did not induce necrosis on tobacco leaves within 72 hours of inoculation, suggesting they are non-pathogenic and safe for further applications as plant growth-promoting rhizobacteria (PGPR).

Figure 1. Intercropping of *Z. officinale* plants in farmer fields; the red arrow indicates *Z. officinale* plants

Table 3. Morphological characteristics and hypersensitivity reaction of rhizobacteria isolates

Isolate Code	Colony Shape	Color	Size	Edge	Consistency	Gram Reaction	Hypersensitivity Reaction
85A	Circular	Milky white	Large	Undulate	Butyrous	+	-
115A	Circular	Milky white	Small	Entire	Mucoid	-	+
115BM	Circular	Red	Large	Filamentous	Butyrous	+	-
124A	Circular	Transparent	Large	Undulate	Mucoid	+	-
124B	Circular	Cream	Large	Entire	Mucoid	+	+
234A	Circular	Milky white	Medium	Entire	Butyrous	+	-
234B	Circular	Milky white	Large	Entire	Butyrous	+	-
234C	Circular	Cream	Medium	Wavy	Mucoid	+	-
235A1	Circular	Yellow	Medium	Entire	Butyrous	+	+
235A2	Circular	Transparent	Small	Entire	Mucoid	+	-
235B1	Irregular	Cream	Small	Wavy	Butyrous	+	+
235B2	Circular	Cream	Large	Entire	Mucoid	+	-
244A	Circular	Cream	Medium	Entire	Mucoid	+	-
244B	Circular	Milky white	Small	Entire	Butyrous	+	+

In contrast, necrotic lesions were observed in the remaining isolates within 24 hours of inoculation, indicating a positive HR response associated with programmed cell death in plants. This rapid defense mechanism limits pathogen spread and is a critical component of plant immune responses (Li et al., 2016). The HR response is particularly evident in Gram-positive bacteria like *Bacillus subtilis* and *Bacillus amyloliquefaciens*, which are well-known PGPR species that induce systemic resistance in plants while promoting growth (Chowdhury et al., 2015).

Interestingly, some species of *Streptomyces*, another Gram-positive PGPR group, have also been shown to induce HR by producing reactive oxygen species (ROS) and reinforcing cell walls to protect plants from pathogens (Viaene et al., 2016). The ability of PGPR to activate immune responses while remaining non-pathogenic underscores their dual role in promoting plant health and enhancing pathogen defense. These findings provide essential insights into the safety and potential application of the isolates. The 9 non-pathogenic isolates were further evaluated for plant growth-promoting traits and biocontrol potential.

Plant growth-promoting traits

Seven isolates demonstrated phosphate-solubilizing activity, as indicated by clear halo zones on Pikovskaya's agar (Figure 2a). All isolates exhibited nitrogen fixation activity, forming yellow zones on Jensen's medium (Figure 2b). These traits highlight the potential of these isolates as biofertilizers, consistent with the role of phosphate-solubilizing bacteria (PSB) and nitrogen-fixing bacteria in enhancing nutrient availability in the rhizosphere (Rawat et al., 2020).

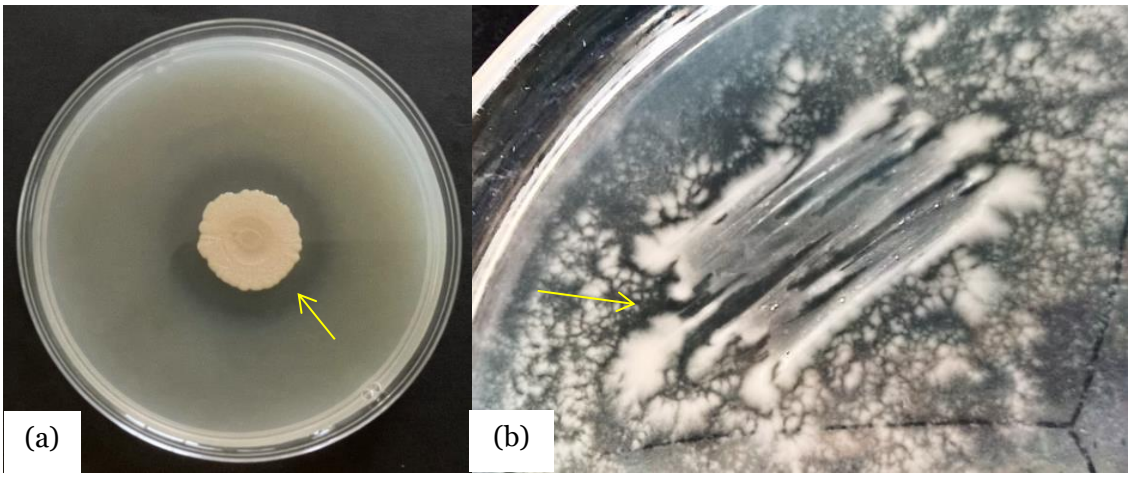

Figure 2. Plant growth-promoting traits of rhizobacteria: (a) phosphate solubilization on pikovskaya's medium by isolate 235a2; (b) nitrogen fixation on jensen's medium by isolate 234b. *Yellow arrows indicate clear zones around colonies.*

These results indicate that most rhizosphere bacteria can solubilize phosphate and fix nitrogen, making them promising candidates for biofertilizer development. PGPR not only act as biocontrol agents but also enhance nutrient availability and uptake in the soil, improving overall plant health. Phosphate and nitrogen are essential macronutrients required by plants, and their availability in usable forms is critical for optimal growth. PSBs play a significant role in the phosphorus cycle by breaking down insoluble phosphorus compounds in the soil and converting them into plant-available forms, such as orthophosphate (Rawat et al., 2020). In this study, most isolates exhibited phosphate-solubilizing capabilities. Similar findings were reported by Yu et al. (2022), where the application of *Pseudomonas sp.* strain JP233 on maize increased phosphorus uptake and utilization efficiency.

Table 4. Plant growth-promoting and biocontrol traits of rhizobacteria isolates

Isolate Code	Phosphate Solubilization	Nitrogen Fixation	Protease Activity	Pathogen Inhibition (% Growth Reduction at Day 7)		
				Fusarium oxysporum (%)	*Colletotrichum* sp. (%)	*Alternaria* sp. (%)
85A	+	+	+	0.57 gh	23.33 de	17.85 cd
115BM	+	+	+	7.47 def	45.00 b	25.00 bc
124A	+	+	+	3.44 dfg	33.33 c	17.85 cd
234A	-	+	-	11.49 bcd	8.33 fg	5.37 ef
234B	-	+	-	8.62 cde	8.33 fg	12.50 de
234C	-	+	-	5.17 efg	10.00 f	25.00 bc
235A2	+	+	+	13.79 ab	55.56 a	35.71 a
235B1	+	-	-	10.34 bcd	10.00 f	10.71 de
235B2	+	+	+	0.00 gh	20.00 de	17.86 cd
244A	+	+	-	16.67 a	25.00 cde	32.14 ab

Note: Values in the same column followed by different letters indicate significant differences based on Honestly Significant Difference (HSD) test at α = 5%.

Nitrogen, another essential macronutrient, is often unavailable in its organic form and must be converted into ammonium or nitrate through biological nitrogen fixation. Nitrogen-fixing bacteria, such as *Bacillus* and *Klebsiella* spp., have been identified as PGPR on maize, not only promoting growth through nitrogen fixation but also serving as biocontrol agents against soilborne pathogens (Chaudhary et al., 2021). Table 4 summarizes the plant growth-promoting and biocontrol traits of the rhizobacteria isolates. The table highlights the diverse abilities of the isolates to solubilize phosphate, fix nitrogen, and produce protease enzymes, along with their antagonistic activities against fungal pathogens (*Fusarium oxysporum*, *Colletotrichum* sp., and *Alternaria* sp.).

Protease enzyme activity

Five out of nine isolates tested positive for protease enzyme production (Table 4). The formation of clear zones around colonies on skim milk agar indicated the ability of these microorganisms to produce protease (Figure 3). Notably, isolates producing protease showed strong inhibitory effects on the growth of *Colletotrichum* sp. and *Alternaria* sp. These findings align with the study by Khalil et al. (2022), which reported that most PGPR isolates from faba bean (*Vicia faba*) were capable of producing protease and suppressing soilborne pathogens.

Protease is a crucial enzyme in biological control mechanisms and plant protection. Proteolytic bacteria can hydrolyze proteins by producing extracellular proteinases. Hydrolytic enzymes like protease play an essential role in suppressing pathogens by degrading fungal cell walls, ultimately leading to the lysis of pathogenic fungal cells (Bhattacharyya et al., 2020; Solanki et al., 2021).

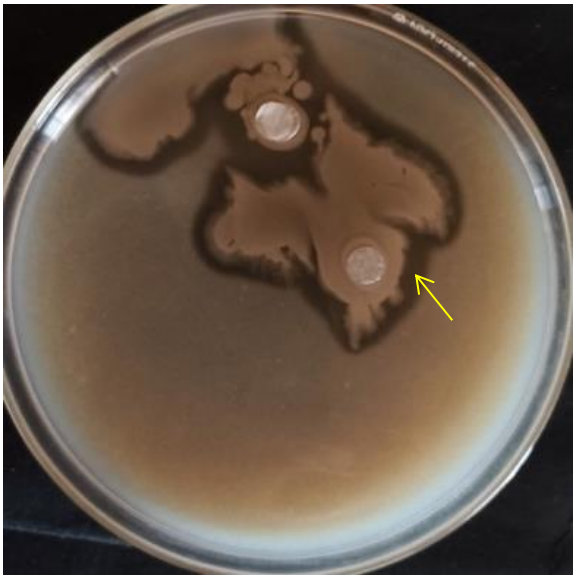

Figure 3. Protease activity of isolate 235a2 on skim milk agar**.** Yellow arrows indicate clear zones around the colony.

Several bacterial genera, including *Pseudomonas fluorescens* and *Enterobacter cloacae*, have been reported to reduce plant pathogens by producing protease enzymes. For example, these bacteria have been shown to inhibit the growth of *Phytophthora capsici* (Admassie et al., 2022). Similarly, *Bacillus* spp. producing protease were found to control *Fusarium oxysporum* f.sp. *lycopersici* and *Ralstonia solanacearum* (Jangir et al., 2018; Prihatiningsih et al., 2021). In addition, Hu et al. (2020) reported that one of the potential mechanisms employed by *Bacillus cereus* BCM2 to infect and kill *Meloidogyne incognita*, a root-knot nematode, was the production of extracellular hydrolytic enzymes, particularly protease and chitinase. These enzymes not only enhance the biocontrol efficacy of bacteria but also contribute to plant growth by protecting against pathogens.

Antagonistic activity against fungal pathogens

The dual culture assay revealed varying degrees of inhibition by rhizobacterial isolates against fungal pathogens, including *Fusarium oxysporum*, *Colletotrichum* sp., and *Alternaria* sp. (Table 4). Among the isolates, 235A2 exhibited the highest inhibition rates, achieving 13.79% inhibition of *F. oxysporum*, 55.56% inhibition of *Colletotrichum* sp., and 35.71% inhibition of *Alternaria* sp. (Figure 3a). Isolate 244A also demonstrated notable inhibition against *F. oxysporum* (16.67%). The strong antagonistic activity can be attributed to the production of antimicrobial compounds and competition for nutrients in the rhizosphere, as previously reported by Sayyed (2019).

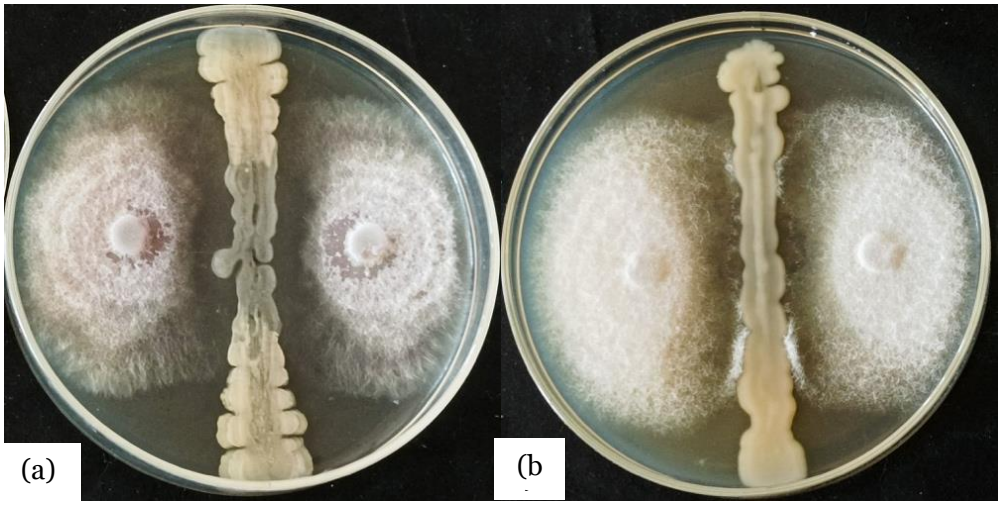

Figure 4. Antagonistic activity of rhizobacteria isolates: (a) dual culture of isolate 235A2 with *Fusarium oxysporum*; (b) dual culture of isolate 234C with *Colletotrichum* sp. The clear zones indicate fungal inhibition.

The inhibition of fungal growth by rhizobacterial isolates can be attributed to multiple mechanisms, including competition for nutrients, production of secondary metabolites, and release of hydrolytic enzymes such as protease. For instance, Figure 4a illustrates the antagonistic activity of isolate 235A2 against *Fusarium oxysporum*, demonstrating a clear inhibition zone between the fungal pathogen and the bacterial colony. Similarly, Figure 4b shows the antagonistic effect of isolate 234C on *Colletotrichum* sp., which also highlights the presence of inhibition zones.

Previous studies have shown that *Pseudomonas* sp. isolated from the rhizosphere of *Capparis spinosa* inhibited *F. oxysporum* and *Sclerotinia sclerotiorum* by 50–70% under in vitro conditions (El-Sayed et al., 2014). Similarly, *Bacillus amyloliquefaciens* isolated from the bamboo rhizosphere inhibited *Alternaria alternata* by 65.66% and *Botrytis pelargonii* by 69.50% (Kazerooni et al., 2021). The relatively lower inhibition rates observed against *F. oxysporum* (0.57–16.67%) compared to *Colletotrichum* sp. (8.33–55.56%) and *Alternaria* sp. (5.37–35.71%) suggest differences in fungal susceptibility to the metabolites or enzymatic activity produced by the isolates. Secondary metabolites such as antibiotics and other antimicrobial compounds are crucial in biocontrol mechanisms, as previously reported by Sayyed (2019).

In addition, the ability to produce protease enzymes appears to play a critical role in the inhibition of fungal pathogens. Protease-producing isolates demonstrated stronger antagonistic activity against *Colletotrichum* sp. and *Alternaria* sp. than those lacking this enzymatic capability. Protease enzymes degrade fungal cell walls, leading to pathogen lysis and enhanced biocontrol effectiveness (Bhattacharyya et al., 2020; Solanki et al., 2021). This finding reinforces the dual role of PGPR in promoting plant growth and protecting against pathogens. The ability of isolate 235A2 to inhibit multiple fungal pathogens, combined with its other plant growth-promoting traits, underscores its potential as a biocontrol agent. Further studies are recommended to investigate its efficacy under field conditions and identify the specific metabolites responsible for its antagonistic activity.

CONCLUSION

This study highlights the potential of plant growth-promoting rhizobacteria (PGPR) isolated from the ginger (*Zingiber officinale*) rhizosphere as both biofertilizers and biocontrol agents. A total of 14 isolates were obtained, with diverse morphological and biochemical characteristics. Among these, isolate 235A2 demonstrated superior capabilities, including phosphate solubilization, nitrogen fixation, protease enzyme production, and strong antagonistic activity against *Fusarium oxysporum* (13.79%), *Colletotrichum* sp. (55.56%), and *Alternaria* sp. (35.71%). The findings confirm the dual role of PGPR in enhancing plant nutrient uptake and suppressing fungal pathogens through mechanisms such as competition, secondary metabolite production, and enzymatic degradation of fungal cell walls. These traits make the isolates, particularly 235A2, promising candidates for sustainable agricultural practices aimed at reducing dependence on synthetic fertilizers and pesticides. Future research should focus on field trials to validate the efficacy of these isolates under natural conditions and explore the molecular pathways involved in their biocontrol and plant growth-promoting activities. These efforts will contribute to the development of environmentally friendly strategies for improving crop productivity and resilience in the face of agricultural challenges.

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