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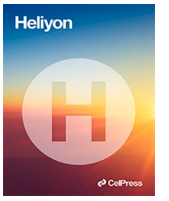

Research article

Screening of rhizobacteria for multi-trait plant growth-promoting ability and antagonism against *B. fabae,* the causative agent of chocolate spot disease of faba bean

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ABSTRACT

This study aimed to isolate and characterize plant growth-promoting rhizobacteria from the faba bean rhizosphere for future inoculum production. For this purpose,127 dissimilar rhizobacterial colonies were isolated. All isolated colonies were tested for plant growth-promoting traits. Based on their multiple plant growth-promoting traits, eight potential isolates were selected and identified GY01, GY03, and GY08 are affiliated with Acinetobacter sp. GY04 and GY05 are affiliated with Chryseobacterium sp. GY06 and GY07 are affiliated with Pseudomonas costantinii and Pseudomonas chlororaphis, respectively; and GY02 is affiliated with the Bacterium strain. All eight isolates were evaluated for their effects on seed germination and vigor index and potential antagonism against Botrytis fabae. Selected isolates showed positive effects on seed germination and vigor index with different potentials. Isolate GY04 resulted in the highest vigor index (501), while isolate GY08 achieved the lowest (218). B. fabae radial growth inhibition was found in all eight isolates. The isolates inhibited the radial growth of the test pathogen with an inhibition efficacy of 72.38 % in GY04 to 25.57 % in GY-03. Generally, the results of this study indicated the potential of these isolates as a microbial inoculant with multiple functions for faba beans.

1. Introduction

The faba bean (Vicia faba L.) is a cool-season legume produced worldwide for food and feed [1]. The major cultivated areas are Mediterranean countries, Ethiopia, Egypt, China, Afghanistan, India, Northern Europe, and Northern Africa [2]. Ethiopia is one of the major producers next to China [3]. It is frequently produced in the country for multiple purposes, including ecological (rotational crop), nutritional, and cash crops [4]. Uptodate, the national average yield is under its potential in the country. According to the report of CSA [5], the national average yield of the country is 2.2 t ha^{-1} in 2019/2020. This is lower than the average yield obtained by other countries, for example, Egypt produces 3.47 t ha^{-1} and the United Kingdom produces 3.83 t ha^{-1} [6]. The low national average yield of faba bean in the country is because of biotic and abiotic factors [7].

To escape from these problems, the development of strategies that help mitigate biotic and abiotic factors that result in low yields of

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faba beans is required. Additionally, the strategy should not harm the environment. The most crucial of these tactics and currently attracting interest around the globe is the application of multi-trait plant-growth-promoting rhizobacteria (PGPR) [8; 53–54]. PGPRs are bacteria that live in or on plant roots and help the host plant through various mechanisms [8].

It improves plant growth directly in the absence of deleterious microorganisms that cause plant disease through biofertilization and photostimulation. Moreover, PGPR increases growth by protecting against phytopathogens through biocontrol mechanisms [9]. Several reports confirmed that the application of PGPR significantly improved plant growth and yield for a variety of crops and vegetables [10,11].

Several rhizobacteria were isolated from faba beans in different regions of the world and improved growth and yield under both stressed and normal conditions [12]; [13–15]. In Ethiopia, few studies were undertaken on faba bean-associated rhizobacteria [15–17]. However, there is a dearth of information on the screening of native rhizobacteria in the study area for growth promotion and disease control. Exploring native rhizobacteria with multiple plant growth-promoting traits may help develop superior inoculants for boosting faba bean and other crop production. Therefore, screening of PGPR indigenous to the study area is critical to the discovery of superior strains that can be utilized to improve growth and phytopathogen suppression. The objectives of the present study were to isolate and characterize multifarious indigenous PGPR species from the faba bean rhizosphere. Another objective was to determine their effects on seed germination and *B. fabae* suppression in vitro.

2. Materials and methods

2.1. Rhizospheric soil sample collection

Rhizospheric soil samples were collected from Gondar-Zuria, Wogera, Dabat, and Debark districts in the 2021 main cropping season. A total of 112 faba bean field sites were randomly selected for rhizospheric soil collection, and 4–5 healthy-looking faba bean plants uprooted from a depth of 0–5 cm were sampled from each site. About 50 g of rhizospheric soil collected from each site was placed in a sterile polyethylene bag and brought to the laboratory for further analysis.

2.2. Isolation of rhizobacteria

To isolate rhizobacteria, the serial dilution plate technique was applied [18]. In detail, 10 g of the soil sample was suspended in 90 ml of sterile normal saline solution (0.85 % w/v NaCl) and agitated on an incubator shaker at 150 rpm for 20 min at 30 °C. The suspension was left for 30 min in the laminar air flow hood. Consequently, 1 ml of the supernatant sample was taken and diluted 7-fold in 9 ml of sterile normal saline (0.85 %w/v). One hundred microliters of sample were taken from 10^{-3}, 10^{-5}, and 10^{-7} dilutions and spread on Nutrient Agar (Hi-Media) plates. Inoculated plates were incubated for 72 h at 30 °C. After incubation, morphologically dissimilar colonies were taken and purified by the streak plate method. The purified rhizobacteria isolates were preserved in nutrient agar slants at 4 °C and 30 % glycerol stocks at −80 °C for further studies.

2.3. Source of the test pathogen (B. fabae)

A virulent B. fabae isolate was obtained from the culture collection center of the Biology Department at the University of Gondar. The test pathogen was refreshed on potato dextrose agar (PDA) at 25 °C for 7 days and stored at 4 °C for further investigation.

2.4. Screening of rhizobacteria isolates for multiple plant growth-promoting traits

2.4.1. Phosphate solubilization

To determine the phosphate solubilization ability of the rhizobacteria isolates, one ml of culture was spot-inoculated on the medium, and the plates were incubated at 30 °C for 7 days. The isolate that formed clear halo zones around the colonies was considered positive for phosphate solubilization [19].

2.4.2. Production of indole acetic acid (IAA)

Rhizobacteria isolates were grown in Luria Bertani (LB) broth supplemented with 100 mg/l tryptophan for 5 days at 30 °C on an incubator shaker at 250 rpm. After that, the culture was centrifuged for about 10 min at 10,000 rpm. After centrifugation, 2 ml of the supernatant was taken and mixed with 4 ml of the Salkowaski reagent (150 ml of concentrated H_2SO_4, 250 ml of distilled water and 7.5 ml of 0.5 M $FeCl_3{\cdot}6H_2O$) [20]. Then test tubes were incubated in darkness for 20 min at room temperature to observe the appearance of a pinkish color as an indicator of IAA production.

2.4.3. Production of ammonia

For four days, the rhizobacteria isolates were grown in 5 mL of peptone water at 30 °C. Following incubation, 1 mL of Nessler's reagent was added to the cultures to detect the formation of a yellow color, which indicates ammonia production [21].

2.4.4. Protease activity

A loop full of rhizobacterial cells was streaked on a skim milk agar plate (composition of g/L skim milk 100 g, peptone 5 g, and agar 15 g) and incubated at 28 °C for 48 h. The plates were examined for the formation of a clear halo zone around the colony, which was an

indicator of protease activity [22].

2.4.5. Lipase activity

A rhizobacterial cell loop was streaked on Tween 80 agar medium, which contained g/L peptone 10, agar 20, NaCl 5, $CaCl_2 2H_2O$ 0.1, and 1L distilled water and incubated at 28 °C for 48 h. A white precipitation around the boundary of the colony was indicative of lipase activity [23].

2.4.6. Amylase activity

The amylase production ability of rhizobacterial isolates was studied on a starch agar plate [21]. The inoculated plates were incubated at 28 °C for 48 h. Then the plates were flooded with iodine solution and incubated for 15 min at room temperature. A clear zone around the growth of rhizobacteria indicated amylase activity.

2.4.7. Pectinase activity

Pectinase production was studied by streaking rhizobacteria on pectinase screening agar medium (PSAM) plates and growing for 7 days at 30 °C. Then the plates were flooded with iodine solution and incubated for 15 min at room temperature. A clear zone around the growth of rhizobacteria indicated pectinase activity [24].

2.5. In vitro antagonistic effects of isolated rhizobacteria against the test pathogen (B. fabae)

The dual culture assay was done to evaluate the antagonistic effects of rhizobacteria against the test pathogen (*B. fabae*) [25].A 5-mm mycelia plug of the actively growing pathogen was placed in the center of the PDA modified with 10 % sucrose. The rhizobacterial isolate was streaked 2 cm away on either side of the mycelia plug and incubated at 28 °C for 9 days. After which, the radial growth of *B. fabae* mycelium was measured, and the percent inhibition of growth over control was estimated using the formula. PIRG = [(R1-R2/R1)] × 100, where PIRG-percent inhibition of radial growth, R1 is the radial growth of the control colony, and R2 is the radial growth of the treated colony.

2.6. Effects of rhizobacterial isolates on seed germination and vigor index in faba bean

In this study, we used seeds of the broad bean cultivar Dosha obtained from the Gondar Agricultural Research Center. Healthy seeds of the same size were selected and surface-sterilized with 96 % ethanol for 1 min, then rinsed with a 0.2 % HgCl2 solution for 3 min and washed with 3 changes of sterile distilled water. Rhizobacterial isolates were grown in a volumetric flask containing 50 ml of nutrient broth at 30 °C in an incubator shaker at 120 rpm for 48 h. The culture pellets were separated from the supernatant by centrifugation at 8000 rpm for 10 min. The pellet was diluted in normal saline (0.85 % w/v NaCl) to give a final concentration of 10-8 cfu/ml using the survival plate counting method. Surface-disinfected broad bean seeds were soaked in their respective cell pellet suspensions for 1 h [26].

A total of 10 broad bean seeds inoculated with each isolate were incubated in 9-cm Petri dishes with two layers of moistened filter paper in a completely randomized design with three replicates per isolate. Seeds treated with water instead of bacterial suspension were used as a control treatment. To maintain sufficient humidity for germination, 5 mL of sterile distilled water was added to the Petri dish at 2-day intervals, and the seeds were incubated at 28 °C in an incubator. Germination was considered to have occurred when the radicle was half the seed length. Germination rate was recorded for 7 days. Root and shoot lengths were measured after 7 days, and the germination rate and vigor index were calculated according to ISTA [27].

Germination rate (%) = no. of germinated seeds/total no of seeds × 100 Vigor Index = (Mean of root length + Mean ofshoot length) × % of Seed germination.

2.7. Molecular identification of selected multifarious PGPR isolates

Genomic DNA was extracted using the Gen Elute Bacterial Genomic DNA Kit (Sigma-Aldrich, St, MO) according to the manufacturer's protocol. For 16S rRNA gene amplification, universal primers 27F 5′-AGAGTTTGATCCTGGCTCAG-3′ 1492R 5′ GGTTACCTTGTTACGACTT-3′ were used [28]. The PCR reaction setup and thermal profiling conditions were performed according to Souza et al. [29]. The PCR amplification cycle included an initial 2 min denaturation at 95 °C followed by 10 cycles of denaturation at 95 °C for 1 min, 1 min primer annealing at 65 °C with the annealing temperature decreased by 1 °C with each succeeding cycle, and 1 min elongation at 72 °C.The denaturation phase, annealing, and elongation were conducted for 35 cycles. The amplicon was stained with ethidium bromide on a 1 % agarose gel and visualized under UV light using a gel documentation system. Amplicons were sent to Macrogen, Netherlands, for sequencing. The sequences obtained were blasted against the GenBank database using the Basic Local Alignment Search Tool (BLAST at http://www.ncbi.nlm.nih.gov). The blasted sequences' similarity was determined using the best score of the compared species from the National Centre for Biotechnology Information (NCBI). To align the nucleotide sequences, a Multiple Alignment fast Fourier transform (MAFFT) was used [30]. To construct a phylogenetic tree, Molecular Evolutionary Genetic Analysis (MEGA) version 11 was used.

2.8. Data analysis

The statistical analysis was performed by One-Way ANOVA of SPSS version 25. The comparisons among means were done by using Tukey HSD analysis at $\alpha = 0.05$. Values were considered significant at $p < 0.05$.

3. Results

3.1. Isolation and screening of rhizobacteria for multiple plant growth-promoting traits

A total of 127 morphologically distinct rhizobacteria colonies were isolated from faba bean rhizosphere soil. All isolated rhizobacteria were positive for at least one of the plant growth-promoting traits. Out of 127 rhizobacterial isolates, 8 (6.3 %) showed multiple plant growth-promoting traits (≥3) and were selected for further work (Table 1).

3.2. In-vitro antagonistic effects of multifarious plant growth promoting rhizobacteria against the test pathogen (B. fabae)

All eight rhizobacterial isolates showed significant ($p \leq 0.05$) antagonistic activity against *B. fabae* in the dual culture experiment (Table 2). The rhizobacterial isolates inhibit the mycelial growth of *B. fabae* with different potential (Fig. 1. A (control/*B. fabae* only), B (*B. fabae* + GY 04), C (*B. fabae* + GY05), and D (*B. fabae* + GY07)). On 3 days of incubation, the rhizobacterial isolates inhibited the pathogen range from 1.83 cm to 4.36 cm and further increased the inhibition range from 2.13 cm to 5.40 cm in five days, 2.23 cm–5.76 cm in 7 days, and 2.30 cm–6.20 cm in 9 days of incubation. Rhizobacterial isolate GY04 displayed the highest inhibition within a short incubation time (3 days) up to 9 days, showing consistency in their inhibitory activity on the test pathogen. Rhizobacterial isolates GY05, GY06, and GY07 were characterized by mild inhibition consistent throughout the incubation period. Most of the other isolates did show significant antagonism as a function of time. The maximum radial growth inhibition of the pathogen was recorded by isolate GY04 (72.38 %), followed by isolate GY05 (63.62 %) and isolate GY07(61.94 %). The fungal mycelia growth inhibition by isolate GY03 (25.57 %) was lower than others isolates.

3.3. Effects of multifarious plant growth promoting rhizobacteria on seed germination and vigor index in faba bean

When compared to the control treatment, all isolates enhanced the germination of seeds and the vigor index. The isolate GY04 results in maximum seed germination (100 %) and a vigor index of 501. Similar results were also recorded with root and shoot growth. Treatment with GY04 results in maximum root and shoot growth of 2.01 cm and 3.0 cm, followed by GY 07 (1.91 cm, 2.98 cm) and GY05 (1.9 cm, 2.9 cm) (Table 3).

3.4. Molecular identification of multifarious plant growth promoting rhizobacteria isolates

A total of eight rhizobacterial isolates with multiple plant growth promotion traits were used for further identification based on a partial 16S rRNA gene sequence. The sequence of the isolate GY01 revealed 98.35 % resemblance with *Acinetobacter* sp.NEB 394; GY02 had 98.23 % homology with Bacterium OPB5; GY03 showed 99.81 % similarity with *Acinetobacter johnsonii* strain XY27; GY04 showed 99.30 % sequence homology with *Chryseobacterium* sp. strain Zn–C; GY05 showed 99.07 % sequence homology with *Chryseobacterium proteolyticum*; GY06 showed 93.07 % sequence homology with *Pseudomonas costantinii* strain 334.12.1; GY07 GY07showed 97.99 % sequence homology with *Pseudomonas chlororaphis* strain BF2-5; and GY08 showed 99.35 % similarity with *Acinetobacter johnsonii* strain cqsV8. The nucleotide sequences of the bacterial strains were submitted to the NCBI GenBank, and accession numbers were received (Table 4).

4. Discussion and conclusion

The ability of various rhizobacteria to promote plant growth, increase crop yield, improve soil quality, and control phytopathogens

Table 1
Rhizobacteria from faba beans with multiple plant growth promotion traits in vitro.

Isolate	Growth promoting traits			Production of hydrolytic enzymes			
	P Solubilization	(IAA)	NH3 Production	Protease	Lipase	Amylase	Pectinase
GY 01	++	++	–	+	–	–	–
GY02	++	++	++	–	–	–	–
GY03	+++	++	++	–	+	–	–
GY04	+	+++	++	+++	–	++	++
GY05	+	+++	++	+++	+++	++	++
GY06	++	–	++	–	++	–	–
GY07	++	+++	+++	++	–	–	–
GY08	++	+	–	++	–	–	–

Note:= not detected, + = low production, ++ = moderate production, +++ = high production.

Table 2
In-vitro antagonistic assay of multifarious rhizobacterial isolates on culture media.

Isolate	Inhibition of radial growth of *Botrytis fabae* upon 3–9 days of incubation in dual culture in cm				Percent of inhibition over control at day(9)
	3 days	5 days	7 days	9 days	
GY01	3.10 ± 0.05	4.16 ± .08b	4.36 ± 0.03bc	4.40 ± 0.05	47.17
GY 02	2.16 ± 0.08[a]	5.10 ± 0.05c	5.51 ± 0.11c	5.83 ± 0.06c	30.01
GY 03	3.26 ± 0.08b	5.40 ± 0.23c	5.76 ± 0.08c	6.20 ± 0.05c	25.57
GY 04	1.83 ± 0.12a	2.13 ± 0.03a	2.23 ± 0.03a	2.30 ± 0.05a	72.38
GY 05	2.02 ± 0.03a	2.73 ± 0.08 ab	2.93 ± 0.08a	3.03 ± 0.08 ab	63.62
GY 06	2.20 ± 0.05a	2.96 ± 0.08 ab	3.13 ± 0.12b	3.86 ± 0.08b	53.66
GY 07	3.10 ± 0.05b	3.130 ± 0.05b	3.17 ± 0.03b	3.17 ± 0.02 ab	61.94
GY 08	4.36 ± 0.05ns	5.10 ± 0.05c	5.24 ± 0.08c	5.36 ± 0.08c	35.78
Control	4.73 ± 0.14	6.26 ± 0 .08	7.20 ± 0.05	8.33 ± 0.03	
LSD($P < 0.05$)	0.31	0.33	0.36	0.38	

Note: Values are mean ± Standard error of the mean of three replications, Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD.

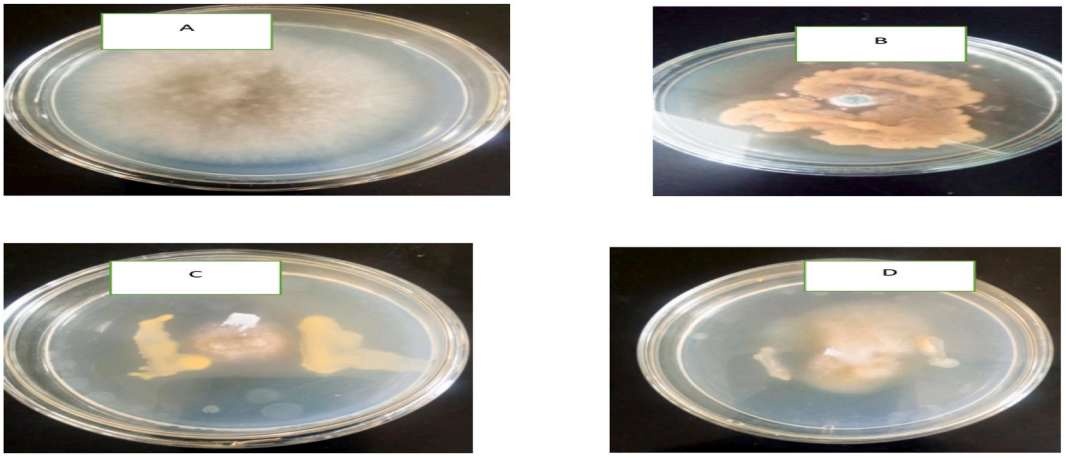

Fig. 1. Some rhizobacterial antagonistic activity against *B. fabae:* A (control/*B. fabae* only), B (*B. fabae* + GY 04), C (*B. fabae* + *GY05*), and D (*B. fabae* + GY07*)*.

in different crops, including faba beans, has been reported by [31–35]. However, the performance of rhizobacteria inoculants in the field was in question. It is because of variations in root exudate composition due to plant genotype, growth stage, and physico-chemical properties of the soil. To avoid the aforementioned issues, it is preferable to use a native PGPR strain rather than an exotic one [36]. Therefore, screening PGPR with multiple PGP traits from rhizosphere soil is critical to selecting the promising isolates used for biocontrol, phytostumlators, and biofertilizers in specific soils and climates.

In this regard, a total of 127 rhizobacteria were isolated from the faba bean plant rhizosphere soils collected from different altitudes, growth stages, and varieties. From the total, eight isolates showing multiple PGP traits were selected for this research (Table 1). The effectiveness of PGPR is directly related to the presence of multiple plant growth-promoting mechanisms in the microorganisms. The result of this study is in harmony with the result of [37], who reported that a single PGPR may have multiple modes of action, including biological control. A single rhizobacterium can have multiple PGP traits [38]. Rhizobacteria with multiple PGP characteristics can benefit plants in a variety of ways, including improving root function, suppressing disease, and accelerating growth and development.

Results presented in Table 1 revealed that all eight selected isolates were capable of solubilizing insoluble phosphate. The formation of a clear hallo zone on Pikovskaya is caused by the release of organic acids. The released organic acids are used to chelate calcium associated with phosphate and thus make phosphorus more available [39]. The use of phosphate-solubilizing bacteria (PSB) as biofertilizers for crop production is one solution to the problem of plant phosphate inadequacy. Therefore, our isolate may be good for future inoculant production.

From the selected eight isolates, except for isolate GY06, seven were able to produce IAA. The ability of rhizosphere bacterial strains to produce auxin is a promising tool to study the profound effect of these strains on plant growth [34,35,40]. Indole-3-acetic acid (IAA) has a positive effect on the root length and increases root branches, root laterals, and root hair, thereby enhancing the nutrient uptake capacity from the surrounding environment [41]. Therefore, our isolate may be good for future inoculant production.

Six of our isolates showed ammonia production ability. The ability of rhizosphere bacterial strains to produce ammonia is one of the mechanisms for enhancing the growth of the host plants. Ammonia may also boost root and shoot length and improve plant resistance

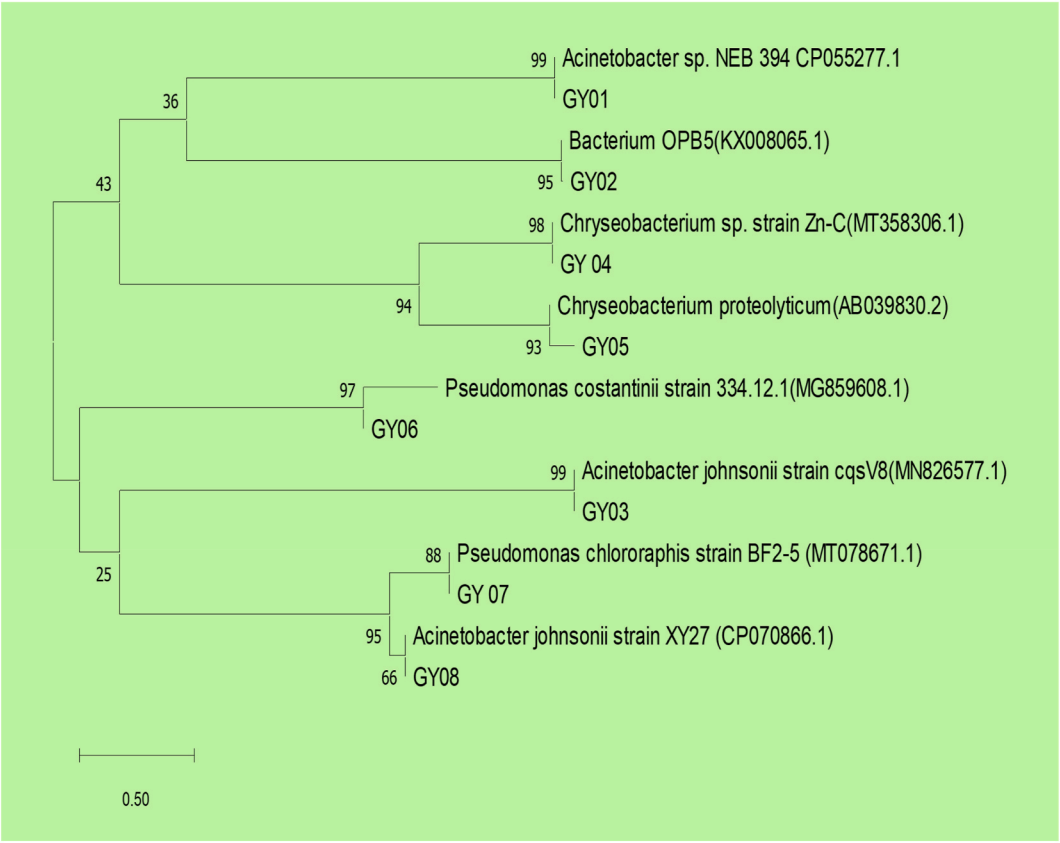

Fig. 2. Neighbor-joining phylogenetic tree based on sequences of the 16S rRNA gene shows the relationships between multifarious plant growth-promoting Rhizobacteria isolates and some related representative reference strains retrieved from NCBI. The numbers on the tree indicate the percentage of bootstrap based on 1000 replications. The scale bar represents 0.05 nucleotide substitutions per sequence position.

Table 3
Effect of bacterial isolates on the seed germination and Vigor Index of cultivars Dosha of faba bean.

Treatment	Seed germination (%)	Mean shoot length (cm)	Mean root length(cm)	Vigor Index
GY 01	98	2.40 ± 0. 08b*	1.40 ± 0.10b*	372.40
GY02	97	2.70 ± 0. 05 ab*	1.50 ± 0.15b*	407.40
GY03	96	2.40 ± 0. 05b*	1.40 ± 0.05b*	364.80
GY 04	100	3.00 ± 0. 05a*	2.01 ± 0.04a*	501.00
GY05	99	2.90 ± 0. 05a*	1.90 ± 0.05a*	475.20
GY06	97	1.46 ± 0.03ns	0.80 ± 0.10c*	219.22
GY 07	99	2.98 ± 0.01a*	1.91 ± 0.08a*	479.16
GY08	98	1.53 ± 0.03ns	0.70 ± 0.05ns	218.00
Control (Dw)	96	1.46 ± 0.08	0.53 ± 0.06	191.04
LSD($P < 0.05$)		6.93	6.92	

Note: Values are mean ± Standard error of the mean of three replications, Means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD, CV, coefficient of variance, DW distilled water.

to phytopathogen attacks [42].

Plant growth-promoting rhizobacteria defend plants from phytopathogens via a variety of mechanisms, including antibiosis, competition, and the production of lytic enzymes. All eight rhizobacteria isolates produced at least one hydrolytic enzyme (Table 1). Hydrolytic enzymes are involved in the lysis of fungal cell walls by deforming the cell wall components of fungal pathogens [43].

Results in Table 4 revealed that rhizobacteria isolates GY01, GY03, and GY08 are closely related to *Acinetobacter* sp., isolate GY04 and GY05 are closely related to *Chryseobacterium* sp., isolate GY06 and GY07 are closely related to *Pseudomonas*, and GY02 is identified as Bacterium strain GY02. Earlier reports also isolated and identified similar PGP bacteria from different plant rhizospheric soils [17, 34,35,44–47].

Table 2 clearly shows that all eight rhizobacteria isolates inhibited the mycelial growth of *B. fabae* in dual culture experiments. However, the antagonizing potential varied among isolates. The observed differences could be attributed to variations in the isolate's

Table 4
Molecular identification of rhizobacteria isolates with multiple plant growth promotion traits.

Isolates	The nearest species obtained from GenBank (16S rRNA)	Similarity (%)	The accession number of our isolates
GY01	*Acinetobacter* sp. NEB 394	98.35	OQ248105
GY02	*Bacterium* OPB5	98.23	OQ248106
GY03	*Acinetobacter johnsonii strain XY27*	99.81	OQ248107
GY04	*Chryseobacterium* sp. *strain Zn–C*	99.30	OQ248108
GY05	*Chryseobacteriumproteolyticum*	99.07	OQ248109
GY06	*Pseudomonas costantinii strain 334.12.1*	93.07	OQ248110
GY07	*Pseudomonas chlororaphis strain BF2-5*	97.99	OQ248111
GY08	*Acinetobacter johnsonii strain cqsV8*	99.35	OQ248112

A phylogenetic tree was drawn from the aligned sequences using Mega 11 software (Fig. 2). All the isolates showed high persent similarity amongst each other and were found forming six clusters.

ability to produce the hydrolytic enzyme. Similarly, Zewudinh [17] found variation in the percent inhibition of radial growth potential by different PGPR isolates against *B. fabae.*

The rhizobacteria isolates GY 04 and GY 05 are positive for several hydrolytic enzymes compared to other isolates (Table 1). The maximum *B. fabae* radial growth inhibition percent was also recorded on the plate treated with GY 04 and GY 05, 72.38 and 63.62 %, respectively. These isolates enhance seed germination and vigor index compared to the control and other isolates. Isolates GY04 and GY05 are affiliated with *Chryseobacterium* sp. *Chryseobacterium* sp. is a plant-growth-promoting rhizobacterium that suppresses a wide range of plant pathogens [45,48–50]. To the best of our knowledge, this is the first report of *Chryseobacterium* sp. having antagonistic potential against *B. fabae* and improving seed germination and the vigor index of faba beans.

In this study, isolates GY06 and GY07 showed multiple plant growth-promoting properties (Table 1) and hindered the radial growth of *B. fabae* (Table 2). Previously, various studies have demonstrated that rhizospheric *Pseudomonas* strains could control plant diseases caused by a variety of pathogens and promote plant growth [34,35,45,46,51]. These strains show multiple growth-promoting properties, including IAA production, siderophore production, hydrogen cyanide production, and phosphate solubilization [52]. To the best of our knowledge, this is the first report of *Pseudomonas chlororaphis* and *Pseudomonas costantinii* having antagonistic potential against the *B. fabae* causative agent of faba bean chocolate spot disease, and improving seed germination and the vigor index of faba bean.

Isolates GY01, GY03, and GY08 showed multiple plant growth-promoting traits and inhibited the growth of *B. fabae* in vitro. Rokhbakhsh-Zamin [44] reported that *Acinetobacter* significantly enhanced the growth of pearl millet seedlings as well as the in vitro inhibition of *Fusarium oxysporum*. Similarly, Zewudinh [17] reported that *Acinetobacter* was isolated from the faba bean rhizosphere and can inhibit the radial growth of *B. fabae*.

Because of their ability to fix nitrogen in collaboration with rhizobia, legumes play an important role in agricultural production. Many legume species, including faba beans, have shown increased plant growth and seed yields after being inoculated with nodule bacteria known as rhizobia. However, both rhizobia and legumes are severely harmed by a variety of abiotic and biotic factors. The PGPR inoculation plays a significant role in overcoming these issues [53,54].

The present study showed the presence of a diverse group of rhizobacteria with multiple PGP traits in the rhizosphere of faba beans grown in northwestern Ethiopia. We have obtained rhizobacteria with multiple PGP traits among the natural community of rhizobacteria. These isolates enhance seed germination and vigor index and inhibit the growth of *B. fabae*, the causative agent of the chocolate spot disease of the faba bean. Generally, the results of this study indicated the potential of these isolates as a microbial inoculant with multiple functions for faba beans.

CRediT authorship contribution statement

Gebeyehu Yibeltie Mengstie: Writing - review & editing, Writing - original draft, Investigation, Conceptualization. **Zewdu Teshome Awlachew:** Writing - review & editing, Supervision. **Atsede Muleta Degefa:** Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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