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Research article

Characterization of faba bean (*Vicia faba* L.) rhizosphere associating rhizobacteria against *Botrytis fabae* AAUBF-12 and their plant growth-promoting properties

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ABSTRACT

The rhizobacteria are known to protect plants from different pathogens acting as biocontrol agents and promote growth of plants. This study was conducted to isolate, screen and identify faba bean associating rhizobacteria for their antagonistic properties against *Botrytis fabae* AAUBF-12 and plant growth-promoting properties under *in vitro* conditions. In the dual culture assay, the isolates inhibited the mycelia growth of *B. fabae* AAUBF-12 (6–40 %) upon 3 days of incubation, and the inhibition increased to 9–43 %, 16–50 %, and 24–68 % after five, seven and 9 days of incubation, respectively. The inhibitory activity increased from 6 to 82 % using the culture filtrates of the isolates. Isolate AAUB95 displayed the highest mycelial inhibition (27 %) at 5 % concentration of culture filtrate, followed by AAUB146b that exhibited 21 % inhibition at the same concentration. AAUB146b and AAUB100 effectively inhibited *B. fabae* AAUBF-12 by 79 % and 80 % at 20 % concentrations of the culture filtrate. The qualitative study demonstrated 75 % of the isolates positive for protease and 60 % for lipase synthesis. Furthermore, the isolates that showed antagonistic activity against *B. fabae* AAUBF-12, produced IAA and ammonia with 65 % and 60 %, respectively. Moreover, 310–760 μg mL^{-1} and 200–620 μg mL^{-1} of tricalcium phosphate (TCP) was released on the 3rd and 6th days of incubation, respectively, due to rhizobacterial solubilization. Nevertheless, the Pearson's correlation analysis between pH and TCP solubilization revealed an inverse relationship (r = -.422**). Based on 16S rRNA sequences analysis, isolate AAUB95, AAUB146b, AAUB100 and AAUB92 were identified as *B. subtilis* AAUB95, *S. nematodiphila* AAUB146b, *B. tequilensis* AAUB100 and *B. subtilis* AAUB92, respectively. Of the isolates, *B. subtilis* AAUB95 showed best antagonism of *B. fabae* AAUBF-12 with multiple plant growth-promoting properties.

1. Introduction

Faba bean (*Vicia faba* L.) is one of the pulse crops grown in the highlands of Ethiopia. However, abiotic and biotic stresses are implicated with low yield of faba bean (Mulugeta et al., 2019). Chocolate spot that is caused by *Botrytis fabae* is one of the most important fungal disease attacking faba bean. The yield loss by the disease can reach up to 50–100 % in highly susceptible faba bean varieties (Saber et al., 2011). In Ethiopia, the yield losses of about 67.5 % was reported in susceptible faba bean varieties (Sahile et al., 2010). The disease can be managed through the application of fungicides such as Mancozeb and Natura®250 EW. However, the extensive uses of fungicides are undesirable due to health concerns, the hazard effects they inflict on the environment and their high cost. For example, fungicides have been reported to cancer, respiratory and hormonal imbalance diseases in humans (Piel et al., 2019). So as to overcome these problems, biological control (BC) via the application of different microorganisms has drawn attention for the best growth promotion and yield of plants in addition to controlling the pathogens (Glare et al., 2012).

Plant growth-promoting rhizobacteria (PGPR) produce lytic enzymes such as protease, lipase, cellulase, chitinases and induction of systemic resistance against phytopathogens (Thomloudi et al., 2019). They also synthesize phytohormones such as, indole-3-acetic acid (IAA) and solubilize inorganic phosphate to promote plant growth (Gupta et al., 2015). The genera of *Bacillus*, *Pseudomonas*, *Serratia* and *Enterobacter* spp. are the well-studied biocontrol agents (BCAs) of several plant pathogens.

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Pseudomonas fluorescens isolates showed 88 % mycelial growth inhibition of *B. fabae* (Alemu, 2016). *Bacillus* spp. also displayed 23–64 % mycelial growth inhibition of *B. fabae* under *in vitro* conditions (Sahile et al., 2009), Ahmed (2015) and El-Banoby et al. (2013) have reported the inhibitory activity of *B. subtilis* on the mycelial growth of *B. fabae* by 62.6 % and 67.03 % respectively. Similarly, de Senna and Lathrop (2017) showed that *Serratia plymuthica* inhibited the growth of *Botrytis cinerea* by 51 % under *in vitro* study.

The hitherto studies focused on screening of selected isolates such as *Bacillus* and *Pseudomonas* spp. antagonistic to *B. fabae* under laboratory conditions in Ethiopia (Sahile et al., 2009; Alemu, 2016). However, there is a dearth of information on the genetic identification and plant growth-promoting properties of rhizobacteria isolated from the rhizosphere of faba bean. Therefore, the present study was initiated to evaluate the *in vitro* inhibitory potential of rhizosphere bacterial isolates against *B. fabae* AAUBF-12; assess the mechanisms of antagonism and plant growth-promoting properties exhibited by the rhizobacterial isolates.

2. Materials and methods

2.1. Sampling area, sample collection and isolation of rhizobacteria

Rhizospheric soil samples were collected from Arsi zone (DigelunaTijo and Tiyo districts) and Bale zone of Goba district (Figure 1). Ten gram (10 g) of soil sample was suspended in 90 mL of 0.85 % of normal saline solution and further prepared to appropriate dilutions (10^{-1}-10^{-7}), 100 μL was transferred to nutrient agar media containing 100 μg Amphotericin in triplicates, and incubated at 28 ± 2 °C for 24–72 hr. The representative colonies were picked, purified, preserved in 20 % glycerol and incubated at -20 °C for further studies.

2.2. Isolation of the study pathogen (Botrytis fabae) and pathogenecity test

The test pathogen (*Botrytis fabae*) was isolated from faba bean leaves (showing symptoms of chocolate spot) and stored seeds using faba bean dextrose agar (FDA) medium according to Shinde (2016). The identification was made based on the cultural characteristics (i.e. colony color and formation of sclerotia on the potato dextrose agar (PDA)). The purified cultures were preserved at 4 °C using PDA slants and used for pathogenicity test according to Abdel-Aleem et al. (2011) and the isolate that showed the common symptom of chocolate spot was labeled as *Botrytis fabae* AAUBF-12.

2.3. Dual culture assay

The dual culture assay was done according to Zivkovic et al. (2010). A loopful of bacteria from 48 hr. old culture was streaked on one side of the Petri plate containing PDA modified with 10 % sucrose and incubated at 28 ± 2 °C. After 48 hr. of incubation, 5 mm mycelial disk from 9 days old *B. fabae* AAUBF-12 culture was inoculated on the opposite side to the bacteria on the same plate and incubated until the control plates were fully covered by the growth of *B. fabae* AAUBF-12. The measurement of radial/mycelial growth was taken on the 3rd, 5th, 7th and 9th days of incubation. The assay was performed three times in triplicates and the percentage inhibition of radial growth (PIRG) was calculated using the following formula;

$$PIRG = R_1\text{-}R_2/R_1X\ 100\ \%,$$

where, R_1-the radius of *B. fabae* AAUBF-12 in the control and R_2-radial growth of *B. fabae* AAUBF-12 toward the rhizobacterial isolate in the treated Petri plates.

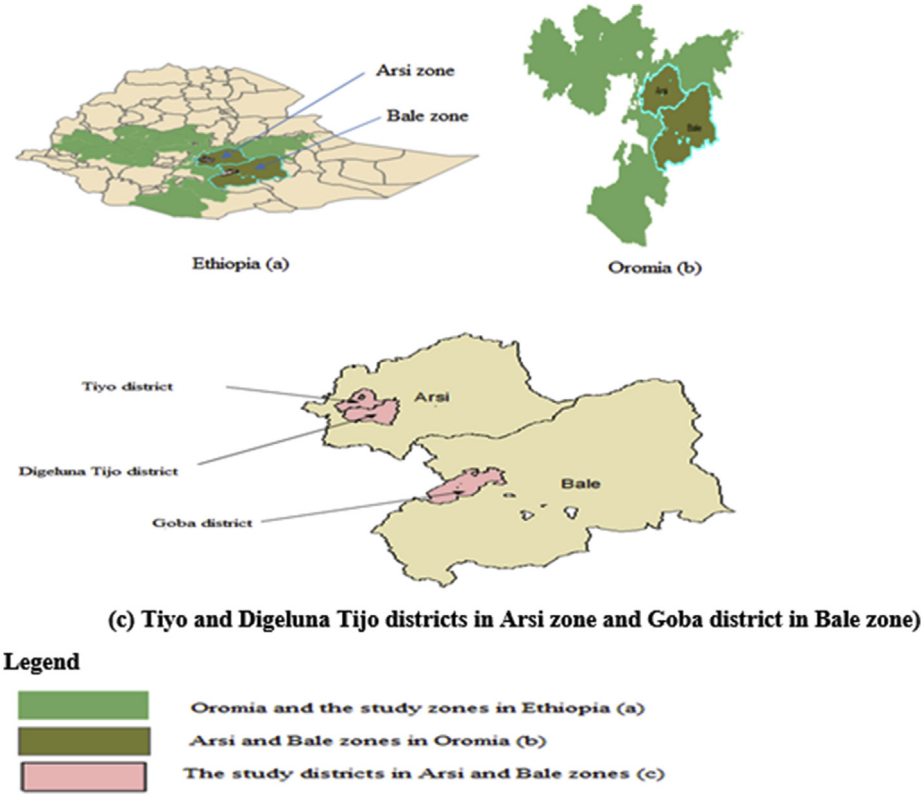

Figure 1. A map showing the study region (Oromia) and zones in Ethiopia (a), zones (b) and selected districts in each zone (c).

2.4. Culture filtrate-none volatile assay

The isolates that inhibited the growth of *B. fabae* AAUBF-12 under dual culture assay were further screened for their antagonistic activity using cell filtrates according to Haggag and El Soud (2012). The filtrates were obtained from 48 hr. old nutrient broth culture grown at 28 ± 2 °C on a rotary shaker (ZJZD-III, Shanghai, China). The broth culture was centrifuged (Centrifuge, Wagtech international, United Kingdom) at 4000 rpm for 30 min, the supernatants were sterilized by passing through sterile filter papers (0.2 mm), the culture filtrates were prepared at the concentrations of 5, 10, 15 and 20 % (v/v) and supplemented to PDA amended with 10 % sucrose. The plates were then centrally inoculated with 5 mm mycelial cut from 7 days old culture of *B. fabae* AAUBF-12 and incubated at 25 ± 2 °C. The experiment was performed three times and three replicates were made for each treatment. The percent inhibition of mycelial growth (PIMG) was calculated using the following formula;

$$PIMG = D_1\text{-}D_2/D_1X100,$$

where, D_1-the diameter of *B. fabae* AAUBF-12 in the control and D_2-the diameter of *B. fabae* AAUBF-12 in the treated Petri plates.

2.5. In vivo-detached leaf assay method

A detached leaf assay was conducted according to Li et al. (2018). A healthy faba bean leaves were sterilized with 5% sodium hypochlorite for 1 min, followed by repeated rinsing in double distilled sterile water and air dried under fume hood. Then, 20 μL of culture suspension (CSS) of antagonistic bacteria (Approx. 1×10^8 CFU/mL) was poured at the center of the leaves and allowed for two hours. Following that, the spore concentrations of *B. fabae* AAUBF-12 was adjusted to 4.5×10^5 spore mL^{-1} using haemocytometer. The inoculated leaves were kept on sterile moistened filter papers in Petri dishes with 85 cm of diameter and kept at 28 °C for 7 days. The size of the lesions was used to calculate the percentage of inhibition relative to the control. The experiment was performed three times in three replicates including the positive control (PC) and the negative control (NC).

2.6. Cultural characterization of rhizobacterial isolates

The isolates were gram stained by taking a loopful of 24 hr. culture on glass slides, heat fixed, flooded with crystal violet for 1 min and rinsed with water. Slides were mordant dyed with Gram's iodine for 1 min, decolorized with 95 % alcohol for 1 min, and counter stained with Safranin for 30 seconds after having been washed with water, air dried and observed under microscope (OLYMPUS-BX51, Germany) of oil immersion to distinguish the gram staining of the cells.

2.7. Extraction of rhizobacterial Deoxyribonucleic acid (DNA)

The extraction of rhizobacterial DNA was conducted according to Souza et al. (2017). The isolates were grown on nutrient agar medium at 25 °C for 48 hr. Genomic DNA was extracted from 2-3 colonies picked from the medium by transferring into 1.5 mL micro centrifuge tubes containing 200 μL of cell lysis buffer (0.05 M NaOH and 0.25 % SDS mixture), heated at 100 °C for 20 min, centrifuged at 10,000 rpm for 3 min and the DNA pellet was diluted 20x in sterile MilliQ water and stored at -20 °C for PCR reactions.

2.8. Primers, polymerase chain reaction (PCR) conditions for DNA amplification (16S rRNA) and identification of rhizobacteria

For PCR reactions, 3 μL of the extracted DNA was used as a template. The PCR reaction was conducted using the primers pair of 8NF (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1429R (5′-ACGGCTACCDTTGTTACGACTT-3′) according to Esikova et al. (2002). The PCR 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 sequences obtained were blasted against the GenBank database using the Basic Local Alignment Search Tool (BLAST at http://www.ncbi.nlm.nih.gov). The sequence similarity was identified from the best score of the compared species and sequences were aligned using multiple alignment fast Fourier transform (Katoh et al., 2002). The phylogenetic tree was constructed by molecular evolutionary genetic analysis (MEGA) version X. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to construct the phylogenetic tree using K2+G+I model.

2.9. Hydrogen cyanide (HCN) and ammonia production

Hydrogen cyanide production was detected according to Bakker and Schippers (1987). The isolates were grown on HCN induction medium for 2–5 days at 28 ± 2 °C. A hundred micro liter (100 μL) of the culture containing 1×10^6 CFU mL^{-1} was spread at the center of the Petri plates whose lids were over layered with filter paper (Whatman No. 1) strips impregnated with picric acid solution. Petri plates were sealed with parafilm and incubated at 28 ± 2 °C for 48–72 hr. Plates having the picric acid impregnated filter paper only were considered as controls. A change in color of the paper strip from yellow to orange-brown was considered positive for HCN production.

The qualitative assay of ammonia production was done according to Cappuccino and Sherman (1992). The isolates were grown in 5 mL peptone water at 28 ± 2 °C for 4 days, 1 mL of Nessler's reagent was added and the development of yellow color was considered positive for ammonia production.

2.10. Qualitative and quantitative solubilization of tricalcium phosphate (TCP)

The qualitative study of phosphate solubilization was performed by using Pikovskaya's (PVK) agar and quantified according to Nautiyal (1999) using National Botanical Research Institute Phosphate (NBRIP) medium containing tricalcium phosphate as the sole source of phosphate. Phosphate solubilization index (PSI) was calculated according to the following formula;

$$PSI = \frac{\text{Colony diameter} + \text{halo zone diameter}}{\text{Colony diameter}}$$

The concentration of released phosphate was quantified against a standard curve constructed from known quantity of potassium dihydrogen orthophosphate, KH_2PO_4 (μg mL^{-1}). The pH of the supernatant was measured using a digital pH meter (NIG 333, New Delhi, India).

2.11. Qualitative and quantitative assay of indole-3-acetic acid (IAA)

The ability of isolates to produce IAA was checked qualitatively as described by Hartmann et al. (1983). Two hundred micro liter (200 μL) of bacterial culture containing 1×10^8 CFU mL^{-1} were grown in 50 mL nutrient broth amended with tryptophan (1 g L^{-1}), respectively. The cultures were, incubated at 25 °C on an orbital shaker (ZJZD III, Shanghai, China) for 72 hr. whereby uninoculated tubes served as controls. The cultures were centrifuged (Centrifuge, Wagtech international, United Kingdom) at 3000 rpm for 30 min, from which 2 mL of the supernatants were mixed with two drops of orthophosphoric acid and 4 mL of Salkowaski's reagent (50 mL of 70 % per chloric acid and 1 mL of 0.5 M FeCl3 and 49 mL sterilized distilled water), and incubated in dark for 30 min. The development of pink colour was visually checked as an indicator of IAA production and 1 mL of the supernatant was quantified spectrophotometrically (6405 UV/Vis., Jenway, England) at 530 nm. The

Table 1. Identification and gram reaction of rhizobacterial strains obtained from the rhizosphere soils samples of faba bean in Arsi and Bale zones.

Isolate code	The nearest species obtained from GenBank (16S rRNA)	Similarity (%)	Accession number of the nearest strain	Gram staining	Identified strains/species	Accession number of the identified strain
AAUB100	*B. tequilensis* 10b	99.9	HQ223107.1	+	*B. tequilensis* AAUB100	MW879448
AAUB113b	*S. nematodiphila* DZ0503SBS1	99.7	EU036987.1	-	*S. nematodiphila* AAUB113b	MW879456
AAUB146b	*S. nematodiphila* DZ0503SBS1	99.7	EU036987.1	-	*S. nematodiphila* AAUB146b	MW879450
AAUB92	*B. tequilensis* 10b	99.8	AJ276351.1	+	*B. tequilensis* AAUB92	MW879459
AAUB113c	*S. nematodiphila* DZ0503SBS1	99.7	EU036987.1	-	*S. nematodiphila* AAUB113c	MW879454
AAUB53	*E. hormaechei* CIP 103441	99.2	AJ508302.1	-	*E. hormaechei* AAUB53	MW879446
AAUB122a	*B. macroides* LMG 18474	99.2	AJ628749.1	+	*B. macroides* AAUB122a	MW879445
AAUB122b	*B. subtilis* DSM10	99.9	AJ276351.1	+	*B. subtilis* AAUB122b	MW879460
AAUB115	*S. nematodiphila* DZ0503SBS1	99.7	EU036987.1	-	*S. nematodiphila* AAUB115	MW879447
AAUB94	*E. hormaechei* CIP 103441	99.2	AJ508302.1	-	*E. hormaechei* AAUB94	MW879461
AAUB95	*B. subtilis* DSM10	99.9	AJ276351.1	+	*B. subtilis* AAUB95	MW879449
AAUB77	*S. nematodiphila*	99.7	EU036987.1	-	*S. nematodiphila* AAUB77	MW879458
AAUB55	*E. hormaechei* CIP 103441	98.6	AJ508302.1	-	*E. hormaechei* AAUB55	MW879451
AAUB152	-	-	-	-	*Pseudomonas* sp. AAUB152	-
AAUB130b	*A. johnsonii* ATCC 17909T	99.2	Z93440.1	-	*A. johnsonii* AAUB130b	MW879455
AAUB130a	*A. johnsonii* ATCC 17909T	99.2	Z93440.1	-	*A. johnsonii* AAUB130a	MW879452
AAUB47	*E. faecalis*	99.9	AB012212.1	+	*E. faecalis* AAUB47	MW879457
AAUB146a	*A. nicotinovorans* X80743.1	99.7	X80743.1	+	*A. nicotinovorans* AAUB146a	MW879453
AAUB150	-	-	-	+	*Bacillus* sp. AAUB150	-
AAUB113b3	-	-	-	+	*Bacillus* sp. AAUB113b3	-

concentration of IAA produced was calculated in μg mL^{-1} based on the standard curve sketched from known concentrations of IAA.

2.12. Protease and lipase synthesis

The protease producing ability of the isolate was done according to Alnahdi (2012) using skim milk agar medium. The isolates were spot inoculated on the medium and incubated at 28 ± 2 °C for 3–5 days. The occurrence of clear halo zone around the colony was taken as an indicator of protease synthesis.

The lipase producing potential of the isolate was performed using nutrient agar medium amended with egg yolk and incubated at 28 ± 2 °C. The culture was flooded with saturated aqueous solution of copper sulphate ($CuSO_4$) and kept for 10–15 min. to check for the formation of greenish blue colour zones around the colony and taken as a positive of lipase synthesis.

2.13. Data analysis

The statistical analysis was performed by Two-Way ANOVA of SPSS version 24. The comparisons among means were done by Tukey honestly significant difference (HSD) at $\alpha = 0.05$. All the values were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Cultural characterization and molecular identification of the rhizobacterial isolates

Rhizospheric soil samples from faba bean (*Vicia faba* L.) plants were collected from Arsi and Bale Zone in Oromia region to screen bacterial isolates for biological control agents of *Botrytis fabae* AAUBF-12, the causal agents of chocolate spot disease in faba bean and evaluate their antagonistic and plant growth promoting properties. A total of twenty rhizobacterial strains were isolated from the rhizosphere of faba beans from sixteen locations that are found in Arsi and Bale zones (Table 1). Based on the gram reaction, the rhizobacterial strains were grouped into Gram positive (45 %) and Gram negative (55 %). The gram negative strains were grouped into the genera of *Acinetobacter*, *Enterobacter*, *Serratia*, and *Enterococcus*, and the gram positive isolates were the genera of *Bacillus* and *Arthrobacter*. The genera *Bacillus* were the dominant rhizobacteria with eight (8) species representing 40 % of the isolates, followed by the *Serratia* isolates that represented with five (5) species containing 20 % of the isolates. It is interesting to note that the commonest rhizobacteria genus *Pseudomonas* contained only one (1) representative isolate.

The 16S rRNA sequence analysis assigned the isolates into the genera of *Bacillus*, *Serratia*, *Enterobacter*, *Arthrobacter*, *Acinetobacter* and *Enterococcus*. The sequences of the isolates were deposited in NCBI with GenBank accession number listed in Table 1 and the phylogenetic tree of all the isolate is presented below having some related representative of reference strains retrieved from NCB (Figure 2). However, three isolates (AAUB152, AAUB150 and AAUB113b3) were not identified by the sequence analysis, whose DNA amplification failed which might be associated with the primers and/or the PCR conditions employed during DNA amplification. However, these isolates were identified to the genus level through treating at a temperature of 70 °C for 14 min to check their temperature tolerance and an ability to grow when re-streaked on nutrient agar to be considered as *Bacillus* species and inability to grow on King *B medium* for *Pseudomonas* species.

3.2. The antagonistic properties of rhizobacterial isolates against Botrytis fabae AAUBF-12 through dual culture assay

In the present study, the rhizobacterial isolates were evaluated against *B. fabae* AAUBF-12 isolated from chocolate spot infected faba

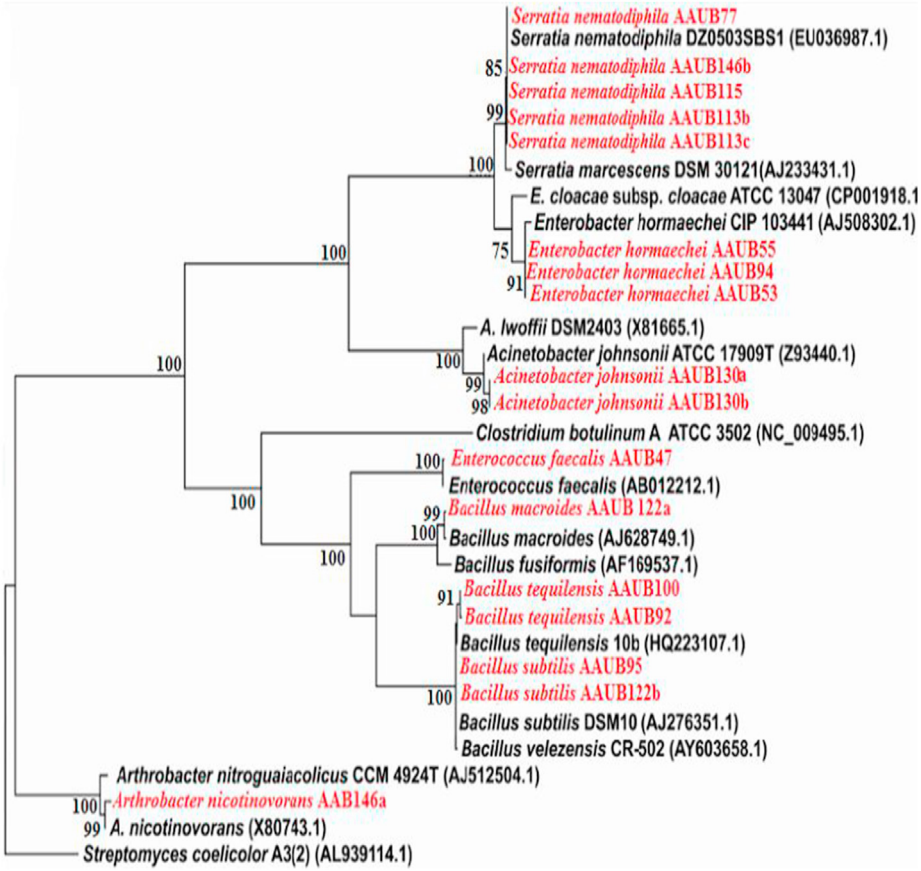

Figure 2. Neighbor-joining tree based on the 16S rRNA sequences analysis of the strains and some related representative of reference strains retrieved from NCBI. The numbers on the tree indicate the percentage of bootstrap based on 1000 replications and are shown for branches with more than 75 % support. The scale bar represents 0.05 nucleotide substitution per sequence position. Strains presented in red color are obtained from this study. *Streptomyces coelicolor* A3(2) (AL939114.1) was used to root the phylogenetic tree.

beans. The isolates showed significant difference to inhibit the test pathogen with the percentage of mycelial growth inhibition that ranged from 6-68 % (Table 2) upon 3–9 days. They differed in their effectiveness to inhibit the mycelial growth of the pathogen by 6–40 %, 9–43 %, 16–50 % and 24–68 % on the 3rd, 5th, 7th and 9th days of incubation, respectively. All the strains showed in the percentage of the pathogen mycelial growth inhibition as the days of incubation increased. Among the identified strains, *Bacillus subtilis* AAUB95, *Bacillus subtilis* AAUB122b and *Bacillus macroides*, AAUB122a exhibited the highest inhibition in all the incubation days (3–9 days). *Bacillus macroides* AAUB122a displayed the highest inhibition (40 %) over the control within the shortest incubation time (3rd days), whereas *Enterobacter hormaechei* AAUB53 showed the least percentage of *B. fabae* AAUBF-12. However, *Bacillus tequilensis* AAUB100, *Serratia nematodiphila* AAUB115, *Bacillus* sp. and AAUB150 showed mild inhibitory activities throughout the incubation days. However, the other isolates did not show significant antagonistic activity as a function of incubation days. In addition, in this study, of all the strains, the *Bacillus* strains were found to show the highest percentage of *B. fabae* AAUBF-12 mycelial growth inhibition compared to the other strains. This might be caused by the ability of *Bacilli* spp. to produce several volatile and non-volatile antifungal compounds to inhibit plant pathogens, indicating their potential use as biocontrol agents to suppress phytopathogenic. For example, the genera of *Bacillus* are known to produce lipopeptides, classes of surfactin, iturin, and fengycin. Microbes that produce such lipopeptides are good biocontrol agents (Malviya et al., 2020).

Moreover, the *Serratia* strains were relatively effective rhizobacteria to inhibit the mycelial growth of *B. fabae* AAUBF-12 compared to others, of which the *Bacillus* strains displayed 10–68 % of inhibition upon 3–9 days of incubation. A study conducted in Ethiopia, Sahile et al. (2009) have reported that *Bacillus* spp. obtained from faba bean leaves indicated 23–64 % of *B. fabae* mycelial growth inhibition upon 5–10 days of incubation. Furthermore, Ahmed (2015) and El-Banoby et al. (2013) have reported that *Bacillus subtilis* inhibited the mycelial growth of *B. fabae* by 62.6 % and 67.03 % within five (5) days) of incubation respectively, which is different from the present study. The difference might be associated with the inherent characteristics of strains used in this study, the inoculum size and/or the experimental conditions used in the studies. Similarly, the different strains of *Serratia nematodiphila* exhibited the percent inhibition of the *B. fabae* AAUBF-12 mycelia that ranged from 8-57 % upon 3–9 days. These strains displayed similar pattern of inhibitory activity with *Serratia plymuthica* that showed 51 % inhibition of *Botrytis cinerea* (de Senna and Lathrop, 2017) and *Serratia marcescens* with 54.81 % inhibition against *Rhizoctonia solani* after seven (7) days (Ahmed, 2016). In this study, only one strain of *Pseudomonas* AAUB152 was found with the inhibitory activity of 13–53 % upon 3–9 days of incubation. *Pseudomonas* species are one of the commonest rhizobacteria that have effective plant growth-promoting and antagonistic properties (Altinok et al., 2016). In contrast, Alemu (2016) has isolated several *Pseudomonas* spp. whereby *P. fluorescens*-10 from the rhizosphere of faba bean showed 88 % mycelial growth inhibition of *B. fabae* on the 7th days of incubation using king *B medium* which is different from the present study. The difference might have associated with the type of medium used to test the antagonistic activity that favors the better performance of *P. fluorescens* than the studied pathogen. However, in our study, potato dextrose agar (PDA) modified with 10 % sucrose was used to study the antagonistic activity of the rhizobacterial strains against *B. fabae* AAUBF-12.

3.3. The antagonistic activity of the rhizobacterial strains against Botrytis fabae AAUBF-12 using culture filtrates

The antagonistic properties of the rhizobacterial isolates were further screened by using culture filtrates of different concentrations (5–20 %, v/

Table 2. Percent inhibition of radial growth (PIRG) displayed by the antagonistic bacterial isolates against *B. fabae* AAUBF-12 upon 3–9 days of incubation using dual culture method.

Sample	Isolate code	Strains/species	Percent of inhibition over control in days			
			3 days	5 days	7 days	9 days
FRSS-04	AAUB100	*Bacillus tequilensis*	19^{bc}	25^{cd}	41^{abc}	59^{ab}
FRSS-01	AAUB113b	*Serratia nematodiphila*	20^{bc}	32^{bc}	52^{a}	57^{abc}
FRSS-08	AAUB146b	*Serratia nematodiphila*	10^{cd}	17^{de}	30^{bcd}	53^{abc}
FRSS-10	AAUB92	*Bacillus subtilis*	28^{ab}	30^{bc}	40^{abc}	56^{abc}
FRSS-15	AAUB113c	*Serratia nematodiphila*	12^{cd}	17^{de}	22^{cd}	31^{def}
FRSS-02	AAUB53	*Enterobacter hormaechei*	06^{d}	09^{e}	16^{d}	31^{def}
FRSS-13	AAUB122a	*Bacillus macroides*	40^{a}	48^{a}	48^{ab}	68^{a}
FRSS-06	AAUB122b	*Bacillus subtilis*	37^{a}	50^{a}	50^{ab}	55^{abc}
FRSS-03	AAUB115	*Serratia nematodiphila*	20^{bc}	40^{ab}	40^{abc}	46^{bcd}
FRSS-07	AAUB94	*Enterobacter hormaechei*	13^{cd}	23^{cde}	24^{cd}	24^{f}
FRSS-16	AAUB95	*Bacillus subtilis*	30^{ab}	39^{ab}	47^{ab}	68^{a}
FRSS-11	AAUB77	*Serratia nematodiphila*	08^{cd}	15^{de}	25^{cd}	45^{bcd}
FRSS-14	AAUB55	*Enterobacter hormaechei*	12^{cd}	14^{de}	18^{d}	23^{f}
FRSS-08	AAUB152	*Pseudomonas* sp.	13^{cd}	20^{cde}	34^{abcd}	53^{abc}
FRSS-05	AAUB130b	*Arthrobacter johnsonii*	14^{cd}	16^{de}	21^{cd}	25^{f}
FRSS-07	AAUB130a	*Arthrobacter johnsonii*	10^{cd}	14^{de}	23^{cd}	41^{cde}
FRSS-09	AAUB47	*Enterococcus faecalis*	16^{cd}	19^{cde}	20^{cd}	22^{f}
FRSS-12	AAUB146a	*Arthrobacter nicotinovorans*	15^{cd}	19^{cde}	20^{cd}	27^{ef}
FRSS-03	AAUB150	*Bacillus* sp.	13^{cd}	25^{cd}	34^{abcd}	50^{bc}
FRSS-11	AAUB113b3	*Bacillus* sp.	10^{cd}	14^{de}	24^{cd}	31^{def}
CV			0.12	0.10	0.11	0.12

FRSS"- stands for faba bean rhizosphere soil samples with corresponding number and CV-indicates the coefficient of variation among means in the same column. Mean values in the same column labeled with the same letter(s) as superscript are not significantly different ($p > 0.05$) by Tukey honestly significant difference (HSD) analysis of One-Way ANOVA.

v). As noted from the data shown below (Table 3), the culture filtrates showed better mycelial growth inhibition of *B. fabae* AAUBF-12 than observed in the dual culture assay (Table 2). The culture filtrate inhibited the mycelial growth of the test pathogen in the range of 6–82 %. The highest inhibition (27 %) was recorded from the culture filtrate of *Bacillus subtilis* AAUB95 at 5 % of the filtrate concentration. Similarly, in the dual culture assay, the strain exhibited the highest antagonistic activity (68 %) and an inhibition of 82 % at 20 % concentration of its filtrate was

Table 3. Percent inhibition of radial/mycelial growth (PIRG) displayed by the antagonistic bacterial isolates against *B. fabae* AAUBF-12 upon different culture filtrate concentrations (5–20 %).

Strains/species	Inhibition over control at 5–20 % v/v of the culture filtrate			
	5 %	10 %	15 %	20 %
Bacillus tequilensis AAUB100	15^{cdef}	30^{cde}	52^{abcdef}	80^{a}
Serratia nematodiphila AAUB113b	15^{cdef}	27^{cdef}	54^{abcd}	75^{abc}
Serratia nematodiphila AAUB146b	21^{abc}	41^{abc}	60^{abc}	79^{a}
Bacillus subtilis AAUB92	13^{cdefg}	31^{cde}	53^{abcde}	73^{abcd}
Serratia nematodiphila AAUB113c	13^{cdefg}	24^{def}	39^{efg}	63^{defg}
Enterobacter hormaechei AAUB53	18^{bcd}	38^{abcd}	47^{cdef}	53^{gh}
Bacillus macroides AAUB122a	26^{a}	31^{bcde}	46^{cdef}	66^{bcde}
Bacillus subtilis AAUB122b	17^{cde}	41^{abc}	59^{abcd}	77^{ab}
Serratia nematodiphila AAUB115	09^{fg}	35^{bcd}	64^{ab}	65^{cdef}
Enterobacter hormaechei AAUB94	11^{efg}	20^{ef}	47^{cdef}	77^{ab}
Bacillus subtilis AAUB95	27^{a}	47^{ab}	65^{ab}	82^{a}
Serratia nematodiphila AAUB77	17^{cde}	50^{a}	66^{a}	75^{abc}
Enterobacter hormaechei AAUB55	16^{cdef}	31^{cde}	62^{ab}	66^{bcde}
Pseudomonas sp. AAUB152	16^{cdef}	27^{cdef}	50^{bcdef}	72^{abcd}
Arthrobacter johnsonii AAUB130b	25^{ab}	32^{bcde}	38^{efg}	42^{h}
Arthrobacter johnsonii AAUB130a	06^{fg}	32^{bcde}	38^{efg}	59^{efg}
Enterococcus faecalis AAUB47	09^{fg}	15^{f}	27^{g}	29^{i}
Arthrobacter nicotinovorans AAUB146a	26^{ab}	40^{abc}	48^{cdef}	61^{efg}
Bacillus sp. AAUB150	19^{bcd}	33^{bcde}	51^{bcdef}	63^{defg}
Bacillus sp. AAUB113b3	16^{cdef}	27^{cdef}	46^{cdef}	55^{fg}
CV	0.16	0.12	0.15	0.18

Mean values in the same column labeled with the same letter (s) as superscript are not significantly different ($p > 0.05$) by Tukey honestly significant difference (HSD) analysis of One-Way ANOVA. CV-indicates the coefficient of variation among means in the same column.

found after incubation for 9 days. This may indicate the ability of the strains to produce none volatile secondary metabolites that can inhibit the mycelial growth of the phytopathogenic fungi. Thus, further study need to be conducted to identify the compounds that are responsible for the antifungal activity of *B. fabae* AAUBF-12 in the present study.

Serratia nematodiphila AAUB146b was also effectively inhibited the test pathogen under this *in vitro* study with the inhibition of 21 % at (5 %) and 79 % at (20 %) culture filtrate concentrations. Although, *Bacillus tequilensis* AAUB100 was relatively weak in mycelial growth inhibition of AAUBF-12 at 5 % of its culture filtrate concentration, it displayed 80 % inhibition with 20 % of the filtrate. In addition, *Serratia nematodiphila* AAUB146b (79 %) was the best antagonistic rhizobacterium against *B. fabae* AAUBF-12 next to *Bacillus subtilis* AAUB95 (82 %) and *Bacillus tequilensis* AAUB100 (80 %) at 20 % concentration of the culture filtrate (Table 3). El Khaldi et al. (2015) have indicated that the culture filtrate of *Serratia marcescens* suppressed the mycelial growth of *Rhizoctonia solani* by 65.6 %, through the application of 100 μL of the culture filtrate after 7 days of incubation. On the other side, *Serratia* sp. C4 displayed antifungal activity with 19.52 % decrease in the mycelial growth of *Fusarium oxysporum* f. sp. *lycoperscisi* (FOL) through dual culture assays. However, the culture filtrate supplemented to PDA medium at 20 % (v/v), slightly improved the inhibition of the pathogen by 23 % at 20 % of concentration on the 5th days of incubation (Aydi-Ben Abdallah et al., 2017). This support our finding that indicated the best inhibition performance by the studied different concentrations of the culture filtrate instead of using the strains directly. This might be due to the early coming together of the metabolites within the filtrate with the study pathogen unlike in the dual culture method of antagonistic activity evaluation/determination.

3.4. Inhibition of *B. fabae* AAUBF-12 on faba bean leaves by antagonistic bacteria and their extracellular culture filtrate-detached leaf assay

The detached leaf assay is a simple *in vitro* method that is used to evaluate the pathogenecity and virulence of pathogenic microbes that cause foliar diseases in plants and that used to screen potential biological control agents for the management of plant pathogens (Cowley et al., 2012). In the present study, the detached leaf assay showed that culture suspension of *B. subtilis* AAUB95 inhibited the occurrence of faba bean leaf lesions by 85 %. In the same way, the culture filtrates (CF) of the strains inhibited leaf lesions by 94. 25 % (Figure 3). This is in agreement with Li et al. (2018) who have reported that the culture filtrate and culture suspension of *B. tequilensis* GYLH001 significantly inhibited the lesions length caused by *M. oryzae* on the detached leaves of rice plants. This result suggested that, the antagonistic rhizobacterial strain (*B. subtilis* AAUB95) can be used as biological control agents of *B. fabae* AAUBF-12. The strain was further studied under field and greenhouse conditions in combination with other plant beneficial fungi (*Trichoderma harzianum* AATU14) against chocolate spot disease, caused by *B. fabae* so as to evaluate the disease severity/incidence and its effect on the growth promotion of faba bean. Our study indicated that, the incidence and severity of chocolate spot was reduced to 23–46 % and 37–48 % in faba beans, respectively (Zewdineh et al., 2020). Further, the study showed 62

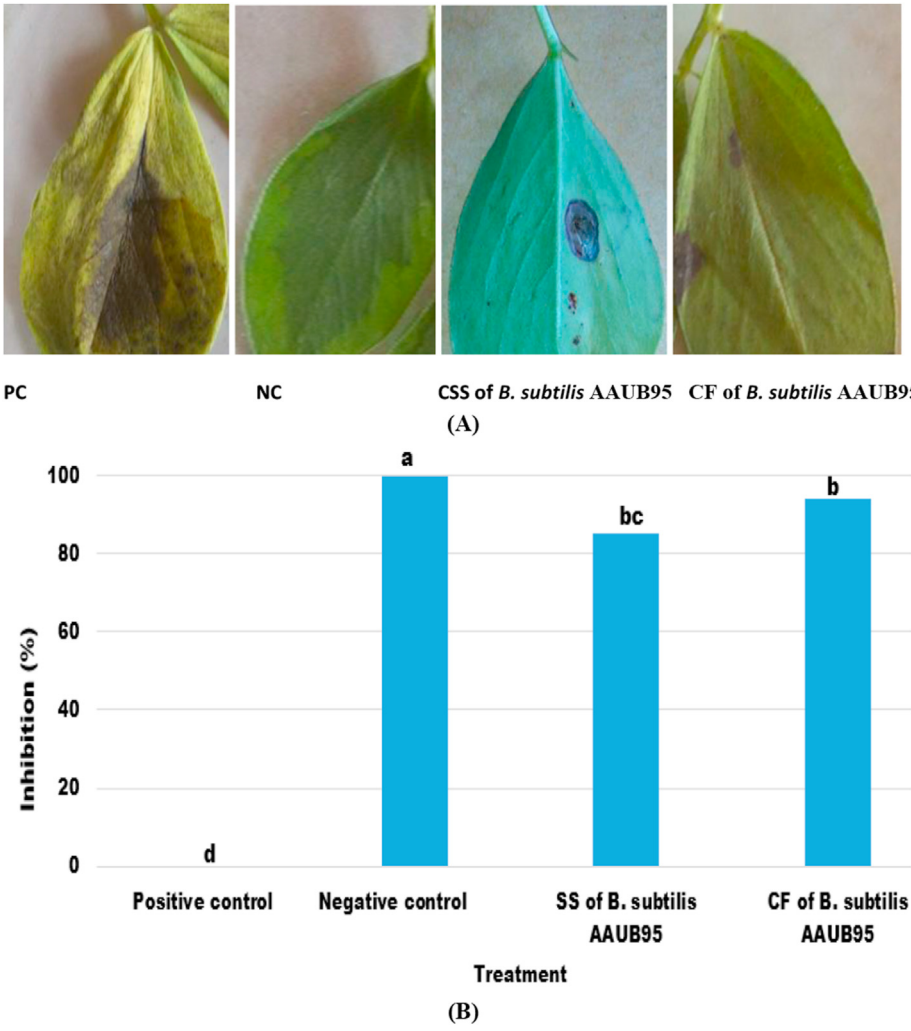

Figure 3. Detached leaf assay for antifungal activity. (A) Effects of antagonistic bacterium culture suspension (CSS) and their extracellular culture filtrate (CF) on faba bean leaves against *Botrytis fabae* AAUBF-12 infection. (B) Disease inhibition (%) relative to the positive control (PC). Data are mean ± SE of triplicates conducted three times for each treatment. Same letters are not significantly different at $p < 0.05$. For the control (C), only the spore suspension (4.5×10^5 spore mL^{-1}) of *B. fabae* AAUBF-12 was applied on faba bean leaves.

Table 4. Plant Growth-promoting and antagonistic properties of rhizobacterial strains from faba bean (*Vicia faba* L.) grown in Arsi and Bale Zones.

Strains/species	HCN	IAA (μg mL^{-1})	Ammonia	Protease	Lipase
Control	-	0.00^{j}	-	-	-
Bacillus tequilensis 100 AAUB	-	-	+	+	+
Serratia nematodiphila AAUB113b	+	1.19 ^{ghi}	-	+	+
Serratia nematodiphila AAUB146b	-	-	+	+	+
Bacillus subtilis AAUB92	+	15.51^{a}	+	+	-
Serratia nematodiphila AAUB113c	-	-	+	+	+
Enterobacter hormaechei AAUB53	-	8.12^{f}	-	+	-
Bacillus macroides AAUB122a	+	13.93^{b}	+	+	+
Bacillus subtilis AAUB122b	-	9.13 ^{ef}	+	+	+
Serratia nematodiphila AAUB115	+	0.27 ^{hi}	+	-	+
Enterobacter hormaechei AAUB94	-	0.18 ^{hi}	-	+	-
Bacillus subtilis AAUB95	+	10.52 ^{cd}	+	+	+
Serratia nematodiphila AAUB77	-	11.62^{c}	-	-	+
Enterobacter hormaechei AAUB55	-	1.21 ^{ghi}	+	-	-
Pseudomonas sp. AAUB152	+	-	+	+	+
Arthrobacter johnsonii AAUB130b	-	-	-	-	+
Arthrobacter johnsonii AAUB130a	+	-	+	+	-
Enterococcus faecalis AAUB47	-	-	-	+	-
Arthrobacter nicotinovorans AAUB146a	-	2.11^{g}	-	-	-
Bacillus sp. AAUB150	+	1.32 ^{gh}	+	+	-
Bacillus sp. AAUB113b3	-	10.21 ^{de}	-	+	+
CV		0.08	-	-	-

HCN-Hydrogen cyanide, IAA-Indole-3-acetic acid, and μg mL^{-1}- Microgram per milliliter. Mean values in the same column labeled with the same letter (s) as superscript are not significantly different ($p > 0.05$) by Student-Newman-Keuls (S–N–K) of One-way ANOVA. CV-indicates the coefficient of variation among means in the same column.

and 34 % increment of hundred seed dry weight and grain yield respectively over the uninoculated controls under the field study. Under greenhouse study, the same study revealed 50 % reduction of chocolate spot disease incidence in Ashebeka variety of faba beans. Thus this study could suggest that, *B. subtilis* AAUB95, can be re-tested against chocolate disease, formulated and used as bioinoculant for the control of chocolate spot disease in faba beans. In this regard, *Bacillus* spp. based products, such as Kodiak (*Bacillus subtilis* GB03), Quantum-400 (*B. subtilis* GB03), Rhizovital (*Bacillus amyloliquefaciens* FZB42), Serenade (*B. subtilis* QST713), and YIB (*Bacillus* spp.), have also been commercialized for improving crop production (Cawoy et al.,2011).

3.5. The antagonistic and plant growth-promoting properties displayed by the rhizobacterial strains

The antagonistic rhizobacterial strains were characterized for their possible exerted mechanisms associated with the control of *B. fabae* AAUBF-12 and plant growth-promoting traits. The qualitative assay demonstrated that 75 % and 60 % of the strains were positive for protease and lipase synthesis, respectively (Table 4). These are the possible antagonistic features displayed by the strains against *B. fabae* AAUBF-12 to control its mycelial growth as observed in our present study. Enzymes such as cellulase, protease, and chitinase are reported to limit the growth and an abnormal hyphal morphology of several plant pathogens (Shrestha et al., 2015). Therefore, in our study, protease and lipase secreted by the strains can cause the hyphal deformation and growth suppression of *B. fabae* AAUBF-12 which need to be confirmed by extra studies. Of the studied strains, 100 % and 75 % of the *Bacillus* were positive for protease and lipase secretion, respectively. It has been revealed that, the *Bacillus* species has great potential in biotechnology as they can produce a large number of commercially hydrolytic enzymes ab bioactive substances which are beneficial for plant growth health (Ali et al., 2020). In addition, *Pseudomonas* sp. AAUB152, *Bacillus macroides* AAUB122a and *Bacillus subtilis* AAUB95 produced HCN that could have implied for their highest inhibitory activities of *B. fabae* AAUBF-12. Kaur et al. (2018) have implicated the production of HCN by *Bacillus* spp. with the mycelial growth inhibition of *Fusarium oxysporum* growth under *in vitro* study. In the present study, *Serratia nematodiphila* AAUB113b and *Serratia nematodiphila* AAUB115 produced HCN against the test pathogen (*B. fabae* AAUBF-12). The production of HCN by the antagonistic *Serratia nematodiphila* NII-0928 obtained from the rhizosphere of black pepper (*Piper nigrum* L) was also reported by Dastager et al. (2010). In addition, the author reported the biocontrol related metabolite (siderophore) production by the studied *Serratia nematodiphila* NII-0928 strains. Hence, the antagonistic property of the *Serratia nematodiphila* strains against *B. fabae* AAUBF-12 might be assisted by the HCN production and the other none tested metabolites such as the production of siderophore in the present study too. The variability in the inhibition potential of the study strains against *B. fabae* AAUBF-12 was attributed to the synthesis of lytic enzymes (protease and lipase) together with HCN production. Although the antagonistic potential of *Arthrobacter nicotinovorans* AAUB146a was 26–61 % using the culture filtrate assay at different concentrations. The strain did not produce either HCN and ammonia and/or enzymes (protease and lipase). This may indicate that the antagonistic mechanisms acquired by the strain might be different from the antagonistic property investigated in this study. Plant growth promoting rhizobacterial isolates produces enzymes that degrade fungal cellular components including chitinase, β-1,3 glucanase, and are cellulolytic (Heydari and Pessarakli, 2010) which can cause the loss of structural integrity, lysis, fragmentation and perforations of hyphae, and sclerotial degradation in phytopathogenic fungi (Sharma and Dubey, 2017).

In addition, 65 % and 60 %, of the study strains produced IAA and ammonia, respectively (Table 4). The production of IAA was in the range of 0.18–15.51 μg mL^{-1}. Kaur et al. (2018) have reported that different plant growth-promoting bacterial isolates to produce IAA that varied from 5 to 24 μg mL^{-1}. In our study, *Bacillus subtilis* AAUB92 produced the highest IAA (15.51 μg mL^{-1}), followed by *Bacillus macroides* AAUB122a (13.93 μg mL^{-1}), *Serratia nematodiphila* AAUB77 (11.62 μg mL^{-1}) and *Bacillus subtilis* AAUB95 (10.52 μg mL^{-1}). The study also designated as

Table 5. Phosphate solubilization efficiency of different Rhizobacterial strains obtained from faba bean (*Vicia faba* L.) rhizosphere soil; the amount of phosphorus released (μg mL^{-1}) (NBRIP liquid) (from inorganic tricalcium phosphate based upon days (3–6) of incubation (for strains with PSI >2) and change in the initial pH of the medium (pHo = 7).

Strains/species	PVKA (solid) PSI	NBRIP (liquid) (μg mL^{-1}) in days		Change in pH (3–6 days)	
		Day 3	Day 6	Day 3	Day 6
Control	1.00^{e}	55^{c}	58^{e}	6.40^{a}	6.90^{a}
Bacillus tequilensis 100 AAUB	2.67^{a}	760^{a}	600^{a}	5.00^{de}	5.44^{ef}
Serratia nematodiphila AAUB113b	2.21^{cd}	410^{b}	340^{c}	4.50^{f}	5.69^{de}
Serratia nematodiphila AAUB146b	2.24^{bcd}	650^{a}	440^{bc}	5.01^{de}	5.00^{f}
Bacillus subtilis AAUB92	2.51^{abc}	75^{a}	600^{a}	5.33^{c}	5.49^{ef}
Serratia nematodiphila AAUB113c	2.16^{cd}	740^{a}	460^{b}	5.91^{b}	6.24^{bc}
Bacillus macroides AAUB122a	2.64^{ab}	700^{a}	620^{a}	4.98^{de}	6.50^{ab}
Bacillus subtilis AAUB122b	2.14^{cd}	650^{a}	450^{bc}	4.90^{de}	6.18^{bcd}
Serratia nematodiphila AAUB115	2.18^{cd}	600^{a}	440^{bc}	5.19^{cd}	6.06^{bcd}
Bacillus subtilis AAUB95	2.09^{cd}	410^{b}	370^{bc}	5.18^{cd}	6.18^{bcd}
Serratia nematodiphila AAUB77	2.43^{abcd}	440^{b}	350^{bc}	4.94^{de}	5.75^{cde}
Pseudomonas sp. AAUB152	2.06^{cd}	310^{b}	200^{d}	5.00^{de}	5.44^{ef}
CV	0.12	0.15	0.10	0.18	0.22

PVKA- Pikovskaya's agar, NBRIP- National Botanical Research Institute Phosphate, PSI- Phosphate solubilization index and μg mL^{-1}- Microgram per milliliter. Mean values in the same column labeled with the same letter (s) as superscript are not significantly different ($p > 0.05$) by Tukey honestly significant difference (HSD) analysis of One-way ANOVA. CV-indicates the coefficient of variation among means in the same column.

Bacillus strains produced the highest quantity of IAA compared to the other strains. Similarly, Nabti et al. (2013) have reported *B. licheniformis* and *Bacillus* sp. as the best producers of IAA, 78 and 101 mg^{-1}, respectively, in addition to their ability to induce lytic enzyme and solubilize inorganic phosphates under *in vitro* conditions. Similarly, different strains of *Serratia nematodiphila* produced IAA that varied from 0.27-11.62 μg mL^{-1}). This was much lower than the IAA concentration of 64.75 μg mL^{-1} and 56.60 μg mL^{-1} produced by *S. marcescens* subsp. *marcescens* strain KB01 and *S. marcescens* subsp. *marcescens* strain KB05, respectively (Hasuty et al., 2017). The production of IAA by the studied strains can promote faba bean growth through reducing the adverse effects of ethylene and plant growth-promoting rhizobacteria can improve plant disease resistance (Li et al., 2018). Bashan and de-Bashan (2005) stated that mechanisms including nitrogen fixation, solubilization of phosphate, production of phytohormones such as Auxins, cytokinins and gibberellins, production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote direct plant growth.

3.6. The potential of rhizobacterial strains to solubilize tricalcium phosphate (TCP)

Phosphorus (P) is the most important nutrient that plants need at an adequate rate from the early stages of their development. This nutrient plays key roles in root development, root traits anatomy modifications and root hair density with a significant contribution in increasing yield of crops and plants resistance against multiple diseases (Ma et al., 2001). Phosphate solubilizing bacteria (PSB), as a group of PGPR, facilitate the hydrolysis of a wide range of phosphorus compounds leading to higher crops yields and reduce chemical hazards to the environment (Malboobi et al., 2009). In the present study, 55 % of the rhizobacterial strains were able to solubilize TCP with a PSI >1. The quantitative assay of TCP solubilization demonstrated that the rhizobacterial strains in this study solubilized 310–760 μg mL^{-1} and 200–620 μg mL^{-1} of phosphorus from TCP on the 3rd and 6th days of inoculation, respectively, indicating solubilization reduction after 3rd days (Table 5). The TCP solubilization revealed 4.6–12.81 and 2.7–9.7 times more phosphorus than the uninoculated controls on the 3rd and 6th days, respectively. A decrease in the pattern of TCP solubilization by rhizobacteria as a function of time was reported by several authors (Bhoopander et al., 2005; Yasmin and Bano, 2011; Jida et al., 2016). The reduction in the amount of solubilized and released TCP could be due to the depletion of nutrients needed for the production of organic acids, the depletion of available and soluble TCP in the medium and/or the excretion of toxic product as a waste may also responsible for the decline of TCP solubilization (Walpola and Yoon, 2013). In this study, the ability of the rhizobacterial strains to solubilize TCP is an indicator for the indirect growth-promoting properties of faba beans through enhancing the amount of phosphorus that is bioavailable. Microorganisms isolated from rhizospheric soil may be better adapted to crop plants and provide better growth and disease control than organisms isolated from other sources such as composts or harsh environments as the formers have been already closely associated with the plant system and adapted to the local environment as well (Bakhshandeh et al., 2014).

The change in the pH of the medium was related to TCP solubilization and the drop in pH value was seen from the 3rd to 6th days (Table 5). A reduction of pH from the initial ($pH_0 = 7$) to 4.5 was observed in *Serratia nematodiphila* AAUB113b on the 3rd days and a pH of 5 was recorded by *Serratia nematodiphila* AAUB146b on day six (6). However, the solubilized phosphate (TCP) by *Serratia nematodiphila* AAUB113b was lower (410 μg mL^{-1}) on the 3rd days and 440 μg mL^{-1} by *Serratia nematodiphila* AAUB146b on the 6th days. This may imply as the drop of pH is not the only factor that is responsible for TCP solubilization. Jida et al. (2016) and Yasmin and Bano (2011) have reported similar results of pH reduction in phosphate solubilizing rhizobacteria obtained from different host plants.

The Pearson's correlation analysis between pH and TCP solubilization revealed an inverse relationship (r = -.422**) (Table 6). The inverse

Table 6. The Pearson's correlation analysis between phosphate solubilization and the change of pH.

Correlations			
		solubilized phosphate	pH change
solubilized phosphate	Pearson Correlation	1	-.422*
	Sig. (2-tailed)		.040
	N	24	24
pH. change	Pearson Correlation	-.422*	1
	Sig. (2-tailed)	.040	
	N	24	24

*Correlation is significant at the 0.05 level (2-tailed).

relationship observed between the pH and the solubilized TCP concentration may indicate the organic acids production by the strains that can play a crucial role in the acidification of the medium (lowering the pH of the medium) and that facilitate the TCP solubilization process (Reena et al., 2013). Thus, this study revealed out the role of medium acidification in phosphate solubilization, as the highest concentration of solubilized TCP was obtained at the time of pH reduction from neutral ($pH_0 = 7$) to acidic conditions ($pH < 7$) on the 3rd days.

4. Conclusion

The present study, pointed out *Bacillus* and *Serratia* species as the potential isolates in showing the best inhibitory activity against *Botrytis fabae* AAUBF-12 along with the antagonistic and plant growth-promoting traits. Of the *Bacillus* strains, *B. subtilis* AAUB95 showed the best antagonistic feature against *B. fabae* AAUBF-12 with plant growth-promoting traits production and thus can be utilized as biofungicides either under greenhouse and/or field conditions after testing the strains several times. Even though, the strains of *Serratia* showed better performance against *B. fabae* AAUBF-12 and plant growth-promoting traits, further study should be conducted on the safety aspects of the strains prior to greenhouse and/or field application of the strains.

Declarations

Author contribution statement

Larissa Maia: conceived and designed the experiments.

Zewdineh Firdu: Performed the experiments; Wrote the paper.

Jorge Teodoro: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Fassil Assefa: Analyzed and interpreted the data.

Tesfaye Alemu: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at NCBI with GenBank under the accession number.

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Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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