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ORIGINAL ARTICLE

Characterization of culturable epiphytic and endophytic bacteria of *Prunus* spp. and their potential for plant growth promotion and antagonistic activity against bacterial canker disease

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Abstract
Bacterial canker disease caused by *Pseudomonas syringae* pv. *syringae* is one of the major limiting factors in the growing and productivity of *Prunus* species in Iran. A total of 293 bacterial strains were purified from the surface and internal tissues of aerial parts of almond (*Prunus dulcis*) and apricot (*Prunus armenica*) trees in East Azerbaijan province, Iran. Based on 16 S rRNA gene sequencing of selected 113 strains, these strains belong to 15 different genera with *Pseudomonas*, *Pantoea*, and *Lysinibacillus* being most abundant. Most genera included strains that were either isolated from both the surface (epiphytes) and internal tissues (endophytes). However, strains of *Rouxiella*, *Escherichia*, and *Curtobacterium* were only isolated from internal tissues and strains of *Arthrobacter*, *Massilia*, *Microbacterium*, *Paenibacillus* and *Kocuria* were only isolated from the surface. Eighteen of the strains showed antagonistic activity under in vitro conditions against *Pseudomonas syringae* pv. *syringae* Pss-170 strain, the causal agent of apricot canker disease. Most of the antagonistic strains belonged to *Pseudomonas fluorescens*, as confirmed by sequencing a fragment of the citrate synthase (*cts*) gene. All antagonistic strains were evaluated for their ability to produce auxin, gibberellin, siderophore, protease, ACC-deaminase, and hydrogen cyanide, as well as phosphate solubilization. Each strain was found to have three or more properties related to plant growth promotion. This study revealed plant growth promoting and biocontrol properties of bacterial strains isolated from almond and apricot trees, which can be further tested for their ability to control bacterial canker disease in the field.

Keywords Bacterial composition · Antagonist · Almond · Apricot · *Pseudomonas syringae* pv. *Syringae*

Introduction

Plants live in association with a diverse array of microorganisms, especially bacteria, on leaf surfaces, referred to as the phyllosphere or phylloplane. Bacteria living epiphytically on healthy host plant species can develop large populations with their taxonomic composition depending both on the plant genotype and on environmental factors (Thapa et al. 2017).

Endophytic bacteria have been defined as bacteria living inside plants for at least part of their life cycle, interacting with cells of the host, taking up secreted metabolites, and releasing plant-growth-promoting (PGP) compounds without causing negative effects on their host (Schulz and Boyle 2006).

Many studies have shown that endophytic bacteria can have the capacity to control phytopathogens via production of compounds such as antibiotics, siderophores, and enzymes, and enhance plant growth through nitrogen fixation and protection of plants from a series of abiotic stresses including drought, low temperature, and salinity (Ali et al. 2014; Bent and Chanway 1998; Sheibani-Tezerji et al. 2015; Subramanian et al. 2015).

Almond and apricot are one of the most important cropped and consumed fruits in the world, including Iran. Few studies so far have investigated epiphytic and endophytic bacteria

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of the aerial parts of almond and apricot for their biocontrol and plant growth promoting potential. Such bacteria could be useful to control bacterial canker disease of stone fruit. This disease is one of the most destructive diseases of *Prunus* species including plums, cherries, peaches, nectarines, apricots, and almonds (Wenneker 2013; Popović et al., 2021). The disease can be caused by either *Pseudomonas syringae* pv. *syringae* (*Pss*), *Ps* pv. *morsprunorum*, *Ps* pv. *avii*, or *Ps* pv. *persicae*. *Pseudomonas syringae* pv. *syringae* belongs to genomospecies I (Young 1991) and is unique in its ability to cause disease in over 180 species belonging to both mono- and di-cotyledonous plants including fruit trees, vegetables, ornamentals, and other annual and perennial species (Bradbury 1986; Gardan et al. 1999; Young et al. 1996). Bacterial canker of stone fruit trees caused by *Pss* is also known as twig blight, blossom blight, gummosis, dieback and spur blight, has a worldwide distribution with causing important economic losses (Kennelly et al. 2007; Kotan and Sahin 2002; Vicente et al. 2004; Wenneker et al. 2012). *Pseudomonas syringae* pv. *syringae* infections on stone fruit trees usually start from blossoms, where the pathogen starts to colonize and then reaches a large population size and from where bacteria enter into plant host tissues. When the infection progresses, blossom infections lead to wood invasion and canker formation. Dormant buds are an overwintering site for the bacterial canker pathogens. The ability of *Pss* to colonize host trees both epiphytically and endophytically limits effective disease management. Also, the absence of effective and specific chemical or biological control measures and poor knowledge of host resistance have made it almost impossible to control bacterial canker disease (Kennelly et al. 2007).

The aim of the present study was to characterize endophytic and epiphytic bacteria associated with aerial parts (e.g. stem, bud, and blossom) of apparently healthy and diseased almond and apricot trees in East Azerbaijan province, Iran, using culture-dependent approaches to evaluate their biological control and plant growth promoting potential.

Materials and methods

Plant sampling, bacterial isolation and identification

Stem, bud, and blossom tissues of 27 almond and 32 apricot trees belonging to different cultivars were collected in March and April 2015 from 13 geographic areas within East Azerbaijan Province, Iran. Trees were either symptomless or symptomatic (canker, oozing on woody tissues, blast of blossoms, and spur dieback). Plant samples were placed in paper bags and immediately brought to the laboratory for further analyses.

Epiphytic bacteria were isolated without surface sterilization of plant material while endophytic bacteria were isolated after surface sterilization. At first, the samples were washed with tap water. For epiphytic isolation, 5 g of healthy and infected tissues were suspended in 20 ml of 0.1 M potassium phosphate buffer (PB) for 10 min on a shaker at 150 rpm. No disinfectant was used. For isolation of endophytic strains, 5 g of healthy and infected tissues were surface-sterilized in 0.5% (for bud and blossom tissues) or 5% (for twig and branch tissues) sodium hypochlorite for 1 and 5 min, respectively, followed by rinsing three times in sterile-double distilled water (DDW). One hundred microliters of the final wash were spread on nutrient agar NA to check sterility. Then, sterilized tissue crushed into pieces of 1 cm were then suspended in 20 ml of 0.01 M magnesium buffer (MB) for 120 min on a shaker at 150 rpm. One hundred microliters of the final suspensions were streaked on Nutrient agar (NA) medium (Merck, Germany) and King's medium B agar (Biolife, Italy) amended with cycloheximide (KBC) with three replications. The plates were incubated at 25–28 °C for 3–7 days and observed daily for the growth of bacterial colonies. After incubation, the bacterial population was estimated by counting bacterial colonies.

Pure cultures of randomly selected bacterial colonies with different morphology and pigmentation were obtained by colony subculturing on NA medium and were preliminarily classified based on Gram reaction. Strains were suspended in DDW and maintained at 4 °C for short-term storage. For long-term storage, all bacterial strains were grown in Luria Bertani (LB) (QUELAB, USA) broth medium for 24 h, and maintained in 15% sterile glycerol at − 70 °C.

Hypersensitive reaction and pathogenicity test

Hypersensitive reaction (HR) was evaluated on tobacco, *Nicotiana tabacum*, leaves using both Gram negative and Gram-positive bacterial suspensions in DDW from 48-h-old cultures on NA medium at a concentration of approx. 1×10^7 CFU/ml. Bacterial suspensions were injected using a sterile needleless syringe. DDW was used as a negative control. The appearance of necrosis in the injected sites after 48 h was considered as a positive HR reaction.

Pathogenicity tests were performed using cut, one year-old, green apricot shoots. Bacterial strains were grown for 24 h on NA medium at 28 °C and suspended in DDW at a concentration of approx. 1×10^7 CFU/ml. One ml of bacterial suspensions was injected into the shoots at three sites of leaf germination (Little et al. 1998). DDW was used as a negative control. The inoculated tissues were maintained in high moisture conditions at 28 °C for 14 days. The presence

of black necrotic lesions was recorded as positive pathogenic reaction.

Molecular characterization of bacterial strains

After extracting the genomic DNA of bacterial strains by boiling for 8 and 15 min at 98 °C for Gram positive and Gram negative strains, respectively, 16 S rRNA oligonucleotide primers 16 S-F (5′- CCAGCAGCCGCGGTAATACG-3′)/16S-R (5′- ATCGGYTACCTTGTTACGACTTC-3′) (Lu et al. 2000) provided by Eton Bioscience Inc. (USA) were used to amplify an approximately 1000 bp-long fragment corresponding to an internal region of the 16 S rRNA gene. For further identification of strains in the genera *Pseudomonas* and *Pantoea*/*Erwinia*, amplification of the citrate synthase (*cts*) and gyrase (*gyr*B) genes, respectively, was performed using oligonucleotide primers cts-Fs (5′- CCCGTCGAGCTGCCAATWCTGA-3′)/ cts – Rs (5′- ATCTCGCACGGSGTRTTGAACATC-3′) (Sarkar and Guttman 2004) and gyrB3 (5′-GCGTAAGCGCCCGGGTATGTA – 3′) /gyrB4 (5′-CCGTCGACGTCCGCATCGGTCAT – 3′) (Deletoile et al. 2009) as described in the original papers.

Phylogenetic analyses

PCR products of the 16 S rRNA, *cts*, and *gyr*B genes were Sanger-sequenced by Eton Bioscience Inc. (USA). Phylogenetic analysis by Bayesian Inference (BI) was performed using MrBayes v.3.2.2, and the phylogenetic tree was visualized using the program FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/). The best model of nucleotide substitution was selected under the Akaike Information Criterion (AIC) (Akaike 1974) implemented in MrModeltest v.2.3 (Nylander 2004).

Screening of bacterial strains for antagonistic activity

Antagonistic activity of purified strains was evaluated against the *Pseudomonas syringae* pv. *syringae* 170 (Pss-170) strain, a causal agent of apricot canker disease (Vasebi et al. 2019) using a dual culture procedure. All bacterial strains were grown in Tryptic soy broth (TSB) medium for 24 h at 28 °C on a shaker at 150 rpm. One hundred microliters of the Pss-170 strain were added to the Petri dishes (9 cm diameter) of Tryptic soy agar (TSA) medium (MilliporeSigma, USA), spread with glass spreader to produce a lawn of bacteria and maintained at room temperature under a laminar flow hood for 15 min. Then, 5 µl of each bacterial strain were placed on the pathogen-inoculated Petri dishes. All Petri dishes were maintained at 28 °C for 48 h. Strains surrounded by an inhibition zone without visible growth of pathogen were selected for a complementary dual culture assay. In the complementary dual culture assay, a suspension of antagonistic strains at an optical density at 600 nm (OD_{600}) of 0.1 was used against the pathogen at three different optical densities (0.01, 0.1, and 1) on TSA medium. Cultures were incubated at 28 °C for 48 h and the diameter of inhibition zones of every strain against the Pss-170 strain was measured. The experiment was repeated twice with three replications at all three concentrations for each antagonist. DDW was used as a negative control.

Plant growth promoting properties of bacterial strains

Siderophore production

Qualitative assay. Chrome azurol S (CAS) agar medium was used for evaluation of siderophore production according to Schwyn and Neilands (1987). Ten microliters of 24-h-old pure bacterial suspensions grown on LB were cultured on the CAS agar medium and incubated at 28 °C for up to 4 days. Formation of a yellowish orange halo surrounding inoculated colonies indicated siderophore production. Experiments were performed in triplicate.

Quantitative assay. The CAS- shuttle assay was used for quantitative estimation of siderophore production according to Schwyn and Neilands (1987). Bacterial strains were grown in succinate medium and incubated at 28 °C for 24 h on a rotator shaking incubator at 120 rpm. After incubation, cultures were centrifuged at 5000 *g* at 4 °C for 10 min. Then, the supernatant was filtered using a 0.22 µm filter and the cell- free filtrate was mixed with CAS solution. The equal mixture of CAS solution and uninoculated succinate medium was used as negative control. Color absorbance was determined 20 min after incubation at 630 nm using a spectrophotometer. The percentage of siderophore was estimated using the formula:

[(Ar - As) / Ar] *100.

Ar = the absorbance of the negative control.

As = the absorbance of each treatment.

Phosphate solubilization

Qualitative assay. Qualitative estimation of phosphate solubilization was determined according to Jasim et al. (2014). Ten microliters of 24-h-old bacterial cultures grown in LB medium were sub-cultured on Pikovskaya (PKV) agar (Sigma, USA) medium for 7 days in 28 °C. Formation of a transparent halo around colonies indicated solubilization of phosphate. Experiments were performed in triplicate.

Quantitative assay. Quantitative estimation of phosphate solubilization was done by the spectrophotometric method described by Ruchi et al. (2012). Seventy microliters of a 24-h-old bacterial suspension grown in LB broth medium were cultured in 10 ml PKV broth medium for 7 days at 28 °C on a shaking incubator at 120 rpm. Bacterial suspensions were centrifuged at 4 °C for 20 min at 5000 *g*. Five milliliters ammonium molybdate reagent (7.5 g of ammonium molybdate, 171 ml of HCl, total volume was made up to 500 ml) was added to 5 ml bacterial supernatant and kept at room temperature for 30 min. Absorbance was measured at 470 nm using a UV-VIS spectrophotometer. A corresponding amount of soluble phosphorous of each strain was calculated from a standard curve of potassium dihydrogen phosphate KH_2PO_4 in the range of 0-1000 µg/ml.

Protease production

Skimmed milk agar (SMA) medium was used for determining the protease production according to Sgroy et al. (2009) with some modification. Ten microliters of 24-h-old bacterial cultures grown on LB medium were inoculated on SMA medium and incubated at 28 °C for 4 days. Formation of transparent halos around colonies indicated protease production. Experiments were performed in triplicate.

Hydrogen cyanide (HCN) production

Production of hydrogen cyanide in strains was determined using the method of Alstrom and Burns (1989). Fifty microliters of 24-h-old bacterial cultures grown on LB medium were streaked on NA medium. Whatman paper soaked in picric acid solution including 0.5% picric acid and 2% Na_2CO_3 and placed inside the inoculated Petri dishes' lids. Dishes were sealed with Parafilm and inversely incubated at 28 °C for 7 days. A change in color of the paper from yellow to orange or red indicated HCN production. Experiments were performed in triplicate.

Gibberellic acid (GA) production

Gibberellic acid production was estimated by the method of Holbrook et al. (1961) with slight modifications. Ten microliters of 24-h-old bacterial cultures grown in LB medium were inoculated into Jenson broth media: sucrose, 20 g/l; K_2HPO_4, 1 g/l; $MgSO_4$, 0.5 g/l; NaCl, 0.5 g/l; $FeSO_4$, 0.1 g/l; Na_2MoO_4, 0.005 g/l; $CaCO_3$, 2 g/l) and incubated for 7 days at 28 °C with shaking at 200 rpm. The cultures were then centrifuged at 5000 *g* for 15 min. Two milliliters zinc acetate was added to 15 ml of the supernatant transferred to a separating funnel, kept for 2 min, and then 2 ml of potassium ferrocyanide solution (10.6% in distilled water) was added and centrifuged at 2000 *g* for 15 min. Five milliliters of the supernatant was added to 5 ml of 30% HCl and the mixture was incubated at 20 °C for 75 min. Five milliliters of 5% HCI was used as blank. Jenson broth medium without bacterial inoculant was used as negative control. Absorbance was measured at 254 nm in a UV-VIS spectrophotometer. Concentration of gibberellins produced by each strain was calculated by a preparing standard curve by using pure gibberellic acid (Merck, Frankfurt, Germany) in the range of 0-1000 µg/ml.

Indole acetic acid (IAA) production

Production of auxin indole-3-acetic acid by bacteria was tested using LB medium and Salkowski reagent (Rahman et al. 2010). Briefly, bacterial strains were grown in LB medium containing 0.2% (v/v) of sterile L-tryptophan and without L-tryptophan and incubated at 28 °C with shaking at 180 rpm. After growth for 7 days, the cultures were harvested by centrifugation at 5000 *g* for 10 min. One ml of supernatant was mixed with 2 ml Salkowski's reagent (150 ml H_2SO_4, 250 ml distilled water, 7.5 ml $FeCl_3.6H_2O$ 0.5 m) and incubated at room temperature in the dark for 30 min. The intensity of pink color of the mixture indicating IAA production was read at 530 nm using a spectrophotometer pre-calibrated with the same media. Concentration of indole acetic acid was estimated by preparing a standard curve using pure IAA (Merck, Frankfurt, Germany) in the range of 0-300 µg/ml.

1-aminocyclopropane-1-carboxylate (ACC) production

ACC-deaminase activity was determined according to the method of Glick et al. (1995). Ten microliters of 24-h-old bacterial cultures grown in LB medium were inoculated on NFb medium containing 1-aminocyclopropane-1-carboxylate (5.0 g/l) as unique nitrogen source. Plates were incubated for 4 days at 28 °C to allow colony formation. Colonies were re-inoculated and incubated at 28 °C for 4 days. Newly formed colonies on NFb + ACC medium were considered positive for ACC-deaminase activity.

Statistical analysis

The MSTATC software was used for data analysis, and the comparison of means was carried out using the Duncan test at the 5% probability level for plant growth promotion and biocontrol assays. Graphs were plotted using Excel software.

Results

Strain isolation and characterization

A total of 2867 and 125 bacterial colonies were grown on NA and KBC media, respectively. Two hundred ninety-three of 2992 morphologically different bacterial colonies including 150 Gram negative and 143 Gram positive strains were purified. About 52% and 48% of the purified strains were isolated from almond and apricot trees, respectively. 44% and 56% of the purified strains were isolated endophytically and epiphytically, respectively. One hundred thirteen of 293 purified strains including 81 Gram negative and 32 Gram positive strains were randomly selected for further identification. Among the selected strains, 51% and 49% were isolated from almond and apricot and 44% and 56% were isolated endophytically and epiphytically, respectively. Except for five isolates (Pss-26, Pss-82, Pss-170, Pss-174, and Pss-176) that were later identified as *Pss* (Vasebi et al. 2019), none of the 288 isolates showed an HR on tobacco leaves or pathogenicity on apricot twigs.

Based on 16 S rRNA sequencing followed by BLAST searches at NCBI, we found that these epiphytic and endophytic bacteria associated with almond and apricot trees belonged to four bacterial classes including Gammaproteobacteria (70.4%), Betaproteobacteria (0.9%), Bacilli (25.1%), and Actinobacteria (3.6%).

Within the Gammaproteobacteria, the four families Pseudomonadaceae, Enterobacteriaceae, Xanthomonadaceae, and Moraxellaceae were found. Bacteria belonged to the following genera: *Pseudomonas* (26%), *Pantoea* (26%), *Erwinia* (9.8%), *Stenotrophomonas* (5%), *Acinetobacter* (1.8%), *Rouxiella* (0.9%), and *Escherichia* (0.9%). Nine tenths percent of bacteria were identified as members of the genus *Massilia*, which belongs to the family Oxalobacteraceae within the Betaproteobacteria. Within the Bacilli, the genera *Bacillus* (9%), *Lysinibacillus* (14.3%), and *Paenibacillus* (1.8%) in the Bacillaceae family were identified. Within *Actinomycetales*, members in the genera *Curtobacterium* (0.9%) and *Microbacterium* (0.9%) in the family Microbacteriaceae, and *Kocuria* (0.9%) and *Arthrobacter* (0.9%) in the family Micrococcaceae were identified (Fig. 1). Strains isolated from healthy trees just belonged to the four *Pseudomonas* (31%), *Lysinibacillus* (31%), *Pantoea* (25%), and *Bacillus* (13%) genera. While 16 genera belonged to four bacterial classes including *Pseudomonas* (26.9%), *Pantoea* (24.8%), *Erwinia* (11.4%), *Lysinibacillus* (11.4%), *Bacillus* (8.2%), *Stenotrophomonas* (5.1%), *Paenibacillus* (2%), *Acinetobacter* (2%), *Rouxiella* (1%), *Escherichia* (1%), *Massilia* (1%), *Curtobacterium* (1%), *Microbacterium* (1%), *Kocuria* (1%), and *Arthrobacter* (1%) were isolated from diseased trees. The 16 S rRNA gene sequences obtained in this study were deposited in GenBank under the accession numbers listed in Table 1.

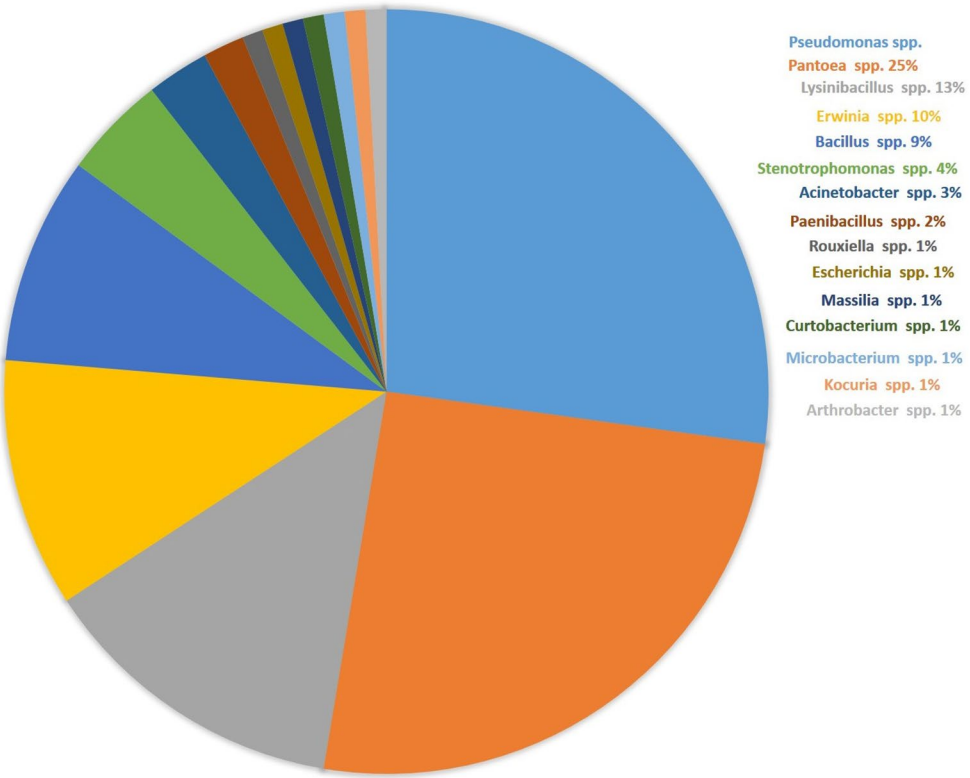

Fig. 1 Phylogenetic tree of partial *cts* gene sequences of *Pseudomnas* spp. isolated from almond and apricot trees constructed by Bayesian inference using the GTR + I + G model. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes obtained for 100,000,000 replicates

Table 1 Some properties of selected bacterial strains used in this study

Number of isolates	16 S rRNA gene Accession number in NCBI	Isolate code	Best Blast match (similarity)	Host	Isolation source	Endophytic / Epiphytic isolation	Area of isolation
1	MH717251	9 – 3	*Bacillus amyloliquefaciens* SCDB1439 (96.36%)	Almond	Bud/diseased	Epi	Ajabshir
2	MH717252	11 – 1	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Bud/healthy	Endo	Ajabshir
3	MH717253	14 – 3	*Bacillus pumilus* m414 (100%)	Almond	Stem/diseased	Epi	Ajabshir
4	MH717255	23 – 1	*Lysinibacillus fusiformis* L6aM (98.18%)	Apricot	Bud/healthy	Endo	Ajabshir
5	MH717256	28 – 2	*Pantoea ananatis* PNA 97-1R (92.44%)	Apricot	Stem/diseased	Endo	Ajabshir
6	MH717257	29-k-2	*Bacillus pumilus* m414 (100%)	Almond	Bud/healthy	Epi	Ajabshir
7	MH717258	31 – 3	*Lysinibacillus fusiformis* L6aM (98.18%)	Almond	Bud/healthy	Endo	Ajabshir
8	MH717259	34 – 4	*Erwinia billingiae* TH88 (99.04%)	Almond	Stem/diseased	Epi	Ajabshir
9	MH717260	34 – 2	*Pantoea agglomerans* BBPE8284 (99.37%)	Almond	Stem/diseased	Epi	Ajabshir
10	MH717261	35 – 4	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Bud/diseased	Endo	Ajabshir
11	MH717262	44 – 2	*Lysinibacillus fusiformis* RB-21 (96.63%)	Almond	Stem/diseased	Endo	Azarshahr
12	MH717263	44-k-1	*Lysinibacillus fusiformis* ZLynn800-25 (96.13%)	Almond	Stem/ diseased	Endo	Azarshahr
13	MH717264	55 – 1	*Pantoea* sp. BAV3342 (90.01%)	Almond	Bud/ diseased	Endo	Azarshahr
14	MH717265	69 – 4	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Bud/ diseased	Epi	Ilkhchi
15	MH717266	73 – 3	*Lysinibacillus fusiformis* L6aM (98.18%)	Apricot	Bud/healthy	Epi	Ilkhchi
16	MH717267	88 – 5	*Lysinibacillus fusiformis* WS1-3 (97.14%)	Apricot	Stem/ diseased	Endo	Marand
17	MH717268	88 – 7	*Curtobacterium flaccumfaciens* pv. *flaccumfaciens* Cff1037 (91.19%)	Apricot	Stem/ diseased	Endo	Marand
18	MH717270	117-3	*Bacillus pumilus* O19 (95.83%)	Almond	Bud/ diseased	Epi	Shabestar
19	MH717271	119-2	*Lysinibacillus fusiformis* RB-21 (98.69%)	Almond	Bud/ diseased	Endo	Shabestar
20	MH717272	126-3	*Paenibacillus* sp. HA2 (97.10%)	Almond	Stem/ diseased	Epi	Shabestar
21	MH717273	135-1	*Pantoea agglomerans* UAEU18 (99.37%)	Apricot	Bud/ diseased	Endo	Shabestar
22	MH717274	185-2	*Paenibacillus polymyxa* DBB1709 (94.74%)	Apricot	Bud/ diseased	Epi	Sardroud
23	MH717275	190-2	*Pseudomonas fluorescens* A506 (99.57%)	Apricot	Stem/ diseased	Epi	Sardroud
24	MH717276	199-1	*Lysinibacillus fusiformis* VC-1 (96.71%)	Almond	Bud/ diseased	Endo	Sardroud
25	MH717277	205-4	*Lysinibacillus fusiformis* PgKB25 (99.35%)	Apricot	Bud/healthy	Epi	Zinjanab
26	MH717278	210-3	*Kocuria rhizophila* FDAARGOS_302 (98.44%)	Apricot	Stem/ diseased	Epi	Zinjanab
27	MH717279	213-4	*Pantoea ananatis* BAV3525 (94.55%)	Apricot	Bud/ diseased	Epi	Ilkhchi
28	MH717280	215-2	*Bacillus cereus* UIS0839 (98.65%)	Apricot	Bud/ diseased	Endo	Ilkhchi
29	MH717281	225-4	*Pantoea agglomerans* UAEU18 (98.85%)	Almond	Bud/healthy	Epi	Ilkhchi
30	MH717282	226-2	*Lysinibacillus fusiformis* L6aM (98.18%)	Almond	Stem/healthy	Epi	Ilkhchi
31	MH717283	229-k-3	*Acinetobacter johnsonii* M19 (97.58%)	Apricot	Bud/ diseased	Epi	Basmenj
32	MH717284	232-k-1	*Lysinibacillus fusiformis* NBRC 15,717 (T) (97.75%)	Apricot	Stem/ diseased	Endo	Basmenj
33	MH717285	35 – 1	*Pseudomonas fluorescens* A506 (99.57%)	Apricot	Bud/ diseased	endo	Shabestar
34	MH717286	136-2	*Pseudomonas fluorescens* R3-54 (99.58%)	Apricot	Stem/ diseased	Endo	Shabestar
35	MH717287	141-2	*Pseudomonas fluorescens* A506 (99.57%)	Apricot	Bud/healthy	Epi	Khosroshahr
36	MH717288	88 – 9	*Lysinibacillus* sp. SJ2SN2 (82.39%)	Apricot	Stem/ diseased	Endo	Marand
37	MH717289	69 – 5	*Arthrobacter* sp. FRA12P410 (98.97%)	Almond	Bud/ diseased	Epi	Ilkhchi
38	MH717290	4 – 2	*Escherichia fergusonii* SS1-1 (100%)	Almond	Stem/ diseased	Endo	Ajabshir
39	MH717292	125-2	*Pseudomonas azotoformans* B26 (99.47%)	Almond	Bud/ diseased	Epi	Shabestar
40	MH717293	2–2	*Bacillus subtilis* HUSS-4AG (98.59%)	Almond	Stem/ diseased	Epi	Ajabshir
41	MH717294	2–3	*Erwinia billingiae* TH88 (98.66%)	Almond	Stem/ diseased	Epi	Ajabshir
42	MH717295	5 – 2	*Pseudomonas graminis* IHBB 9249 (99.68%)	Almond	Bud/healthy	Epi	Ajabshir
43	MH717296	14 – 1	*Pseudomonas* sp. J380 (99.89%)	Almond	Stem/ diseased	Epi	Ajabshir
44	MH717297	14 – 5	*Erwinia* sp. KM16 (98%)	Almond	Stem/ diseased	Epi	Ajabshir
45	MH717298	14-k-1	*Bacillus subtilis* SRCM102750 (97.45%)	Almond	Stem/ diseased	Epi	Ajabshir
46	MH717299	18-k-2	*Pseudomonas graminis* IHBB 9249 (99.57%)	Apricot	Stem/ diseased	Epi	Ajabshir
47	MH717300	19 – 2	*Pseudomonas graminis* IHBB 9249 (99.47%)	Apricot	Bud/ diseased	Endo	Ajabshir

Table 1 (continued)

Number of isolates	16 S rRNA gene Accession number in NCBI	Isolate code	Best Blast match (similarity)	Host	Isolation source	Endophytic / Epiphytic isolation	Area of isolation
48	MH717301	23 – 3	*Pantoea agglomerans* UAEU18 (99.36%)	Apricot	Bud/healthy	Endo	Ajabshir
49	MH717302	25 – 1	*Erwinia billingiae* TH88 (99.79%)	Apricot	Bud/ diseased	Epi	Ajabshir
50	MH717303	26 – 1	*Pantoea agglomerans* UAEU18 (99.68%)	Apricot	Stem/ diseased	Epi	Ajabshir
51	MH717304	28 – 1	*Pantoea agglomerans* BBPE8284 (99.47%)	Apricot	Stem/ diseased	Endo	Ajabshir
52	MH717305	29 – 2	*Pantoea agglomerans* BBPE8284 (99.27%)	Almond	Bud/healthy	Epi	Ajabshir
53	MH717306	35-k-1	*Lysinibacillus fusiformis* PgKB25 (98.53%)	Almond	Bud/ diseased	Endo	Ajabshir
54	MH717307	41 – 2	*Pantoea agglomerans* P19 (99.58%)	Almond	Bud/ diseased	Epi	Azarshahr
55	MH717308	42 – 1	*Pantoea agglomerans* BBPE8284 (99.37%)	Almond	Stem/diseased	Epi	Azarshahr
56	MH717309	42 – 4	*Pantoea agglomerans* UAEU18 (99.36%)	Almond	Stem/ diseased	Epi	Azarshahr
57	MH717310	49 – 1	*Pantoea agglomerans* P19 (99.58%)	Apricot	Bud/ diseased	Epi	Azarshahr
58	MH717311	54 – 1	*Pantoea agglomerans* ACBP2 (96.17%)	Almond	Stem/ diseased	Epi	Azarshahr
59	MH717312	55 – 2	*Pantoea agglomerans* Az-2 (98.09%)	Almond	Bud/ diseased	Endo	Azarshahr
60	MH717313	56-k	*Pantoea agglomerans* UAEU18 (97.94%)	Almond	Stem/ diseased	Endo	Azarshahr
61	MH717314	56-k-2	*Pantoea agglomerans* KABNA4 (99.26%)	Almond	Stem/ diseased	Endo	Azarshahr
62	MH717315	58 – 1	*Pantoea agglomerans* Az-2 (99.68%)	Almond	Stem/ diseased	Epi	Ilkhchi
63	MH717316	69 – 2	*Stenotrophomonas chelatiphaga* CCUG 56,889 (99.36%)	Almond	Bud/ diseased	Epi	Ilkhchi
64	MH717317	73 – 4	*Bacillus safensis* BN-2 (97.52%)	Apricot	Bud/healthy	Epi	Ilkhchi
65	MH717318	81 – 1	*Pantoea agglomerans* UAEU18 (99.68%)	Apricot	Bud/ diseased	Epi	Marand
66	MH717319	84 – 1	*Rouxiella chamberiensis* 130,333 (99.15%)	Apricot	Stem/ diseased	Endo	Marand
67	MH717320	84 – 2	*Pseudomonas graminis* IHBB 9249 (99.68%)	Apricot	Stem/ diseased	Endo	Marand
68	MH717321	84 – 3	*Bacillus pumilus* IHBB 9209 (98.50%)	Apricot	Stem/ diseased	Endo	Marand
69	MH717322	88-k-1	*Erwinia billingiae* TH88 (98.66%)	Apricot	Stem/ diseased	Endo	Marand
70	MH717323	85-k-2	*Pseudomonas graminis* IHBB 9249 (99.75%)	Apricot	Stem/ diseased	Epi	Marand
71	MH717324	94-k	*Pseudomonas putida* R2-62 (99.26%)	Apricot	Stem/ diseased	Epi	Esfahlan
72	MH717325	96 – 1	*Erwinia* sp. MJJ-R3 (99.68%)	Apricot	Stem/ diseased	Endo	Esfahlan
73	MH717326	96 – 2	*Stenotrophomonas chelatiphaga* CCUG 56,889 (99.47%)	Apricot	Stem/ diseased	Endo	Esfahlan
74	MH717327	96-k-1	*Pseudomonas graminis* IHBB 9249 (99.57%)	Apricot	Stem/ diseased	Endo	Esfahlan
75	MH717328	102-k-1	*Pantoea agglomerans* BBPE8284 (99.69%)	Apricot	Stem/ diseased	Epi	Esfahlan
76	MH717329	102-k-2	*Erwinia billingiae* TH88 (98.66%)	Apricot	Stem/ diseased	Epi	Esfahlan
77	MH717330	104-1	*Lysinibacillus fusiformis* NBRC 15717T.106 (98.77%)	Apricot	Stem/ diseased	Endo	Esfahlan
78	MH717331	106-k	*Stenotrophomonas chelatiphaga* CCUG 56,889 (98.93%)	Almond	Stem/ diseased	Epi	Esfahlan
79	MH717332	108-2	*Pantoea agglomerans* UAEU18 (99.47%)	Almond	Stem/ diseased	Endo	Esfahlan
80	MH717333	108-k	*Pantoea agglomerans* BBPE8284 (99.68%)	Almond	Stem/ diseased	Endo	Esfahlan
81	MH717334	119-3	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Bud/ diseased	Endo	Shabestar
82	MH717335	120-3	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Stem/ diseased	Endo	Shabestar
83	MH717336	124-1	*Pseudomonas fluorescens* A506 (99.47%)	Almond	Stem/ diseased	Endo	Shabestar
84	MH717337	126-2	*Stenotrophomonas chelatiphaga* CCUG 56,889 (98.93%)	Almond	Stem/ diseased	Epi	Shabestar
85	MH717338	132-1	*Pantoea agglomerans* UAEU18 (98.85%)	Apricot	Stem/ diseased	Endo	Shabestar
86	MH717339	146-k-1	*Pseudomonas fluorescens* A506 (99.36%)	Apricot	Stem/ diseased	Epi	Khosroshahr
87	MH717340	150-k-1	*Pseudomonas brassicacearum* S-1 (99.68%)	Apricot	Stem/ diseased	Epi	Khosroshahr
88	MH717341	159-1	*Pantoea agglomerans* UAEU18 (99.37%)	Almond	Bud/ diseased	Endo	Khosroshahr
89	MH717342	159-5	*Pseudomonas fluorescens* A506 (99.57%)	Almond	Bud/ diseased	Endo	Khosroshahr
90	MH717343	130-k-2	*Pseudomonas graminis* IHBB 9249 (99.68%)	Apricot	Stem/ diseased	Epi	Esfahlan
91	MH717344	149-1	*Pantoea agglomerans* Az-2 (99.68%)	Apricot	Bud/ diseased	Epi	Khosroshahr
92	MH717345	158-k-1	*Lysinibacillus fusiformis* L6aM (98.18%)	Almond	Stem/ diseased	Epi	Khosroshahr
93	MH717346	160-1	*Pantoea agglomerans* BBPE8284 (99.36%)	Almond	Stem/ diseased	Endo	Khosroshahr
94	MH717347	160-k-1	*Pseudomonas graminis* IHBB 9249 (99.68%)	Almond	Stem/ diseased	Endo	Khosroshahr

Table 1 (continued)

Number of isolates	16 S rRNA gene Accession number in NCBI	Isolate code	Best Blast match (similarity)	Host	Isolation source	Endophytic / Epiphytic isolation	Area of isolation
95	MH717348	164-1	*Pseudomonas graminis* IHBB 9249 (99.68%)	Almond	Stem/ diseased	Endo	Khosroshahr
96	MH717349	169-2	*Erwinia billingiae* TH88 (99.58%)	Apricot	Bud/ diseased	Epi	Sepidan
97	MH717350	173-1	*Microbacterium paraoxydans* CL-9.11a (99.68%)	Apricot	Bud/ diseased	Epi	Sepidan
98	MH717351	176-1	*Erwinia billingiae* TH88 (99.68%)	Apricot	Stem/ diseased	Endo	Sepidan
99	MH717352	177-1	*Erwinia billingiae* TH88 (99.79%)	Almond	Bud/ diseased	Epi	Sepidan
100	MH717353	178-2	*Erwinia billingiae* TH88 (99.79%)	Almond	Stem/ diseased	Epi	Sepidan
101	MH717354	178-k-1	*Pseudomonas graminis* IHBB 9249 (99.68%)	Almond	Stem/ diseased	Epi	Sepidan
102	MH717355	181-2	*Pantoea agglomerans* Az-2 (99.48%)	Apricot	Bud/healthy	Epi	Sardroud
103	MH717356	185-1	*Massilia* sp. 51Ha (99.89%)	Apricot	Bud/ diseased	Epi	Sardroud
104	-	188-1	*Pseudomonas fluorescens* A506 (99.07%)	Apricot	Stem/ diseased	Endo	Sardroud
105	MH717357	193-2	*Pseudomonas* sp. J380 (99.79%)	Almond	Bud/healthy	Epi	Sardroud
106	MH717358	199-3	*Pseudomonas fluorescens* A506 (99.36%)	Almond	Bud/ diseased	Endo	Sardroud
107	MH717359	210-1	*Stenotrophomonas* sp. NJ1024 (99.05%)	Apricot	Stem/ diseased	Epi	Zinjanab
108	MH717360	212-k-1	*Bacillus pumilus* EE106-P1 (99.53%)	Apricot	Stem/ diseased	Endo	Zinjanab
109	MH717361	213-k-3	*Lysinibacillus fusiformis* PgKB25 (99.42%)	Apricot	Bud/ diseased	Epi	Ilkhchi
110	MH717362	214-k-1	*Acinetobacter* sp. NEB 394 (99.79%)	Apricot	Stem/ diseased	Epi	Ilkhchi
111	MH717363	218-3	*Erwinia* sp. MJJ-R3 (99.26%)	Almond	Stem/ diseased	Epi	Ilkhchi
112	MH717364	205-2	*Pseudomonas rhizosphaerae* DSM 16,299 (99.57%)	Apricot	Bud/healthy	Epi	Basmenj
113	MH717365	16 – 1	*Pantoea agglomerans* BBPE8284 (99.68%)	Almond	Stem/ diseased	Endo	Ajabshir

A phylogenetic tree using the Bayesian method and evolutionary distances were calculated based on the obtained partial 16 S rRNA gene sequences and sequences of selected bacterial reference strains downloaded from NCBI (Fig. 2). Phylogenetic trees based on the partial sequences of the *cts* and *gyr*B genes, respectively, revealed relationships between the isolated members of the genera *Pseudomonas* spp. and *Pantoea* spp./*Erwinia* spp. and selected reference strains (Figs. 3 and 4). Phylogenetic trees based on *cts* gene sequencing showed that the most of *Pseudomonas* strains (57%) isolated from almond and apricot had the highest similarity to the *P. fluorescens* reference strains, some (34%) to the *P. graminis* reference strain. Phylogenetic trees based on *gyr*B gene sequencing showed that the *Pantoea* spp. and *Erwinia* spp. strains isolated in this study were identified as *P. agglomerans* and *E. billingiae* with the highest similarity to the *P. agglomerans*, and *E. billingiae* reference strains.

Antagonistic activity of strains

Thirty five of the 113 sequenced isolates produced an inhibition zone against the Pss-170 strain in the primary dual culture test. Eighteen of these isolates also produced an inhibition zone against the Pss-170 strain in the complementary dual culture test. Of these 18 isolates, 13 were identified as *P. fluorescens* with the highest similarity to the antagonistic *P. fluorescens* A506 strain based on 16 S rRNA and *cts* genes sequencing (Figs. 2 and 3), four isolates were identified as members of the genus *Lysinibacillus* sp., and one isolate as *Paenibacillus* sp. based on the 16 S rRNA sequence analysis (Fig. 2). *Lysinibacillus* strains showed high similarity to *Lysinibacillus fusiformis* strains based on 16 S rRNA gene sequencing. Isolate 185-2 belongs to the *Paenibacillus* genus showed high 16 S rRNA gene sequence similarity to *Paenibacillus polymyxa* DBB1709.

Isolates showed the highest inhibition when plated at an OD_{600} of 0.1 and when the pathogen was plated at an OD_{600} of 0.01 and 0.1 with inhibition zone diameters ranging from 8.5 to 13.5 mm and 6.5 to 12 mm, respectively. Only the five *P. fluorescens* strains (11 – 1, 190-2, 35 – 1, 136-2, 159-5, and 199-3 isolates) inhibited the Pss-170 when the pathogen was plated at an OD_{600} of 1 with an inhibition zone diameter range from 6.6 to 8.5 mm. Among the *P. fluorescens* strains, 190-2 and 146-k-1 isolates showed the greatest inhibitory effect on the pathogen with an inhibition zone diameter of 13.5 and 12 mm at an OD_{600} of 0.01 and 0.1, respectively (Fig. 5).

In spite of *Pseudomonas* isolate 11 – 1, all other isolates with antagonism against the Pss-170 strain were isolated from apparently infected trees with oozing and canker symptoms, including 11 almond and seven apricot trees from seven geographic areas. Most of the antagonistic isolates, approximately 72%, were endophytes isolated from nine almond and four apricot trees (Table 1).

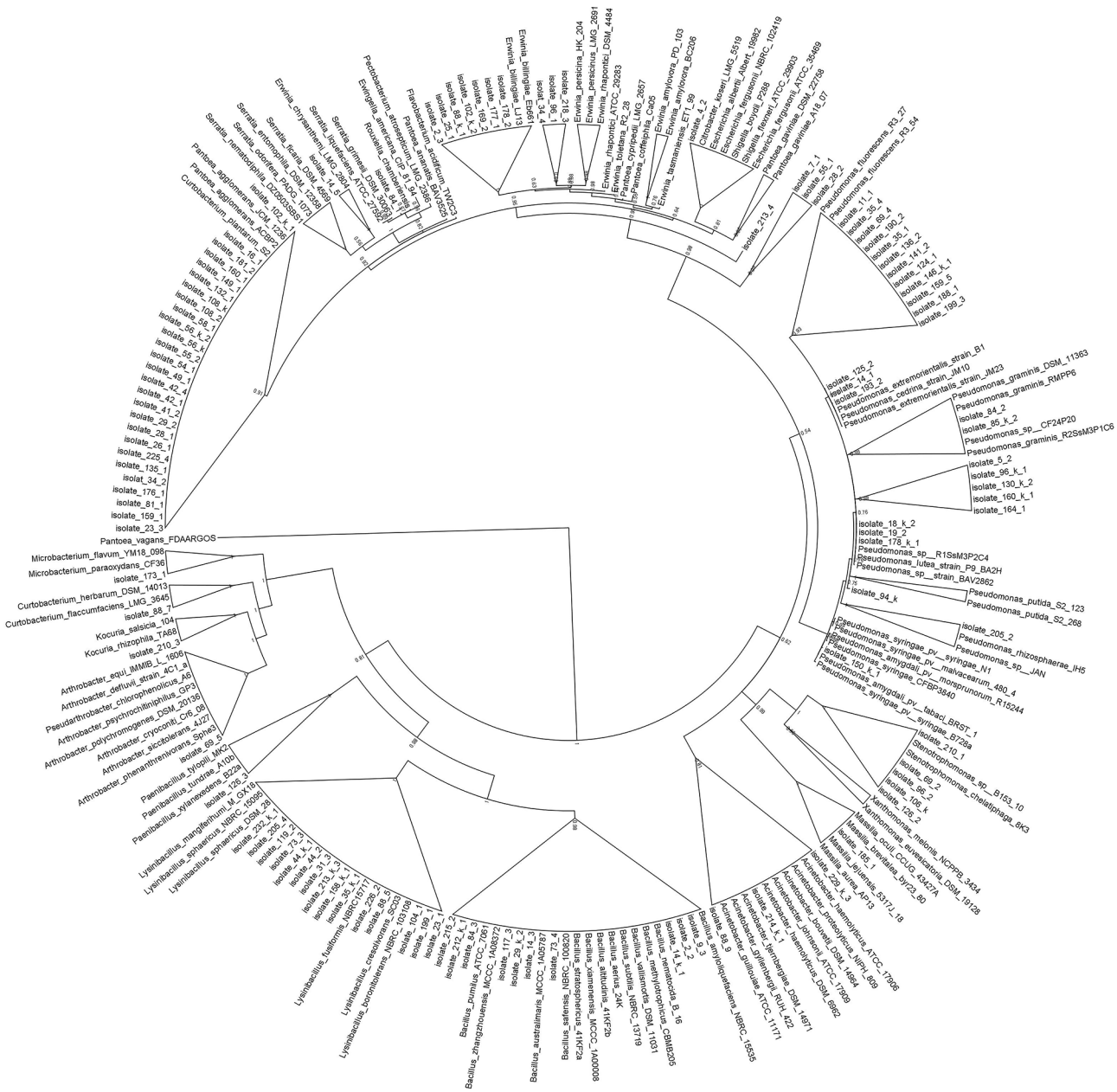

Fig. 2 Phylogenetic tree of partial 16S rRNA gene sequences of bacteria isolated from almond and apricot trees constructed by Bayesian inference using the GTR + I + G model. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes obtained for 100,000,000 replicates

Evaluation of plant growth promoting properties

All 18 isolates with antagonistic activity against the Pss-170 strain were selected for evaluation of their plant growth promoting ability including siderophore, protease, HCN, GA, IAA, and ACC production, and phosphate solubilization.

Results from the quantitative biosynthesis assay of GA showed differences among isolates. While all strains had some GA production, the highest and lowest one was detected in *P. fluorescens* 120-3 with 13.7 µg/ml of GA and *P. fluorescens* 35 − 4 with 1.8 µg/ml of GA, respectively (Fig. 6).

In the qualitative siderophore production assay, colonies of four isolates had positive results developing yellow to orange haloes on CAS agar (Supplementary Fig. 1 and Table 2). Quantitative siderophore production abilities of these bacteria ranged from 21.2 µg/ml by *P. fluorescens*

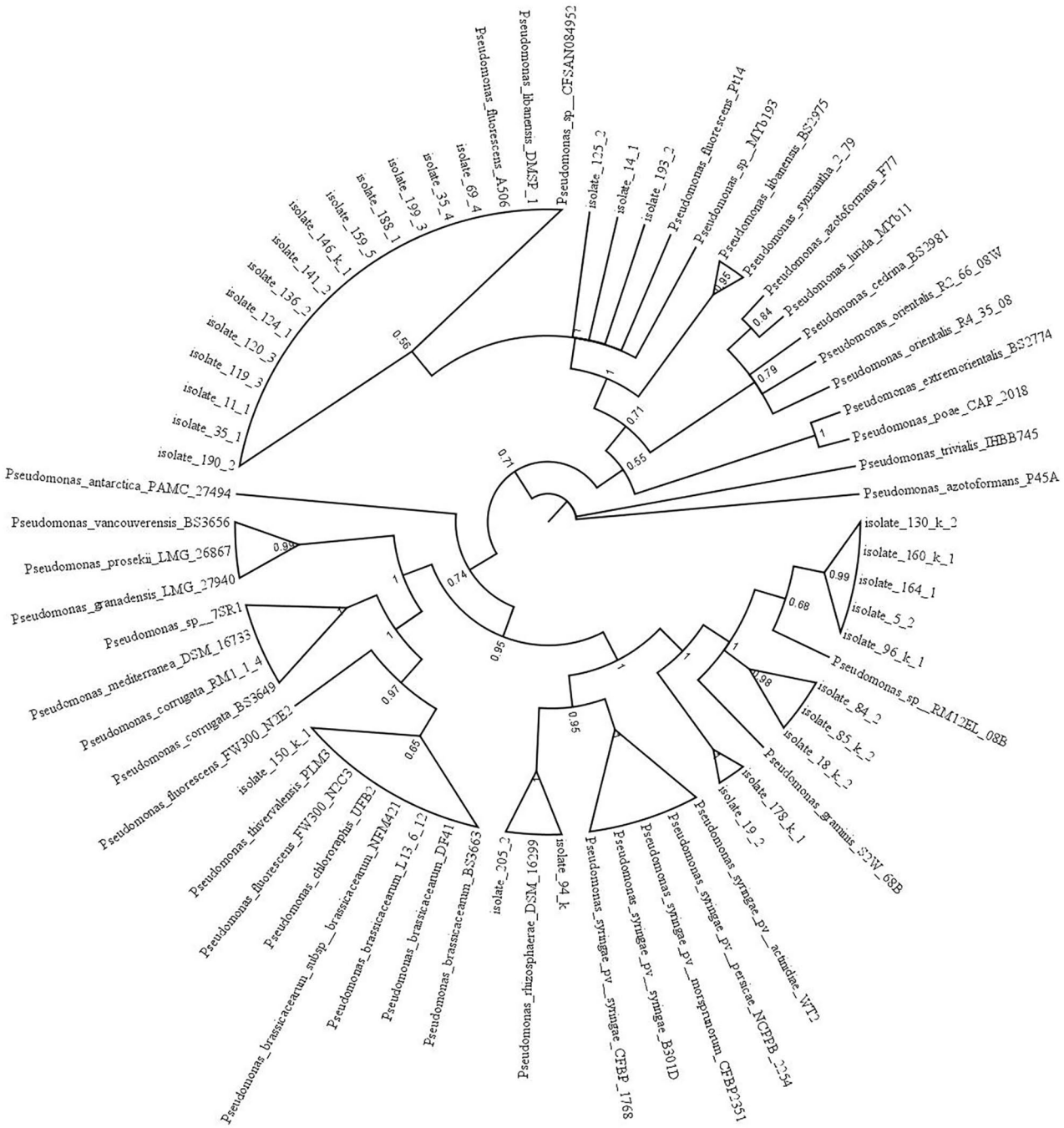

Fig. 3 Phylogenetic tree of partial *cts* gene sequences of *Pseudomnas* spp. isolated from almond and apricot trees constructed by Bayesian inference using the GTR + I + G model. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes obtained for 100,000,000 replicates

11 – 1 to 0.07 µg/ml by *P. fluorescens* 159-5, 136-2, 119-3, and 120-3 (Fig. 7).

In the quantitative IAA assay, the highest production rate was observed by *P. fluorescens* 120-3 with 103 µg/ml of IAA and the lowest production was found in *P. fluorescens* 188-1 and 199-3 with 1.2 µg/ml of IAA (Fig. 8).

Isolates were screened qualitatively and quantitatively for their ability to solubilize phosphate. All isolates were found to be able to solubilize insoluble phosphate by producing phosphatase enzyme based on the formation of a transparent halo around their colonies (Supplementary Fig. 2 and Table 2). Quantitatively phosphate solubilizing abilities of

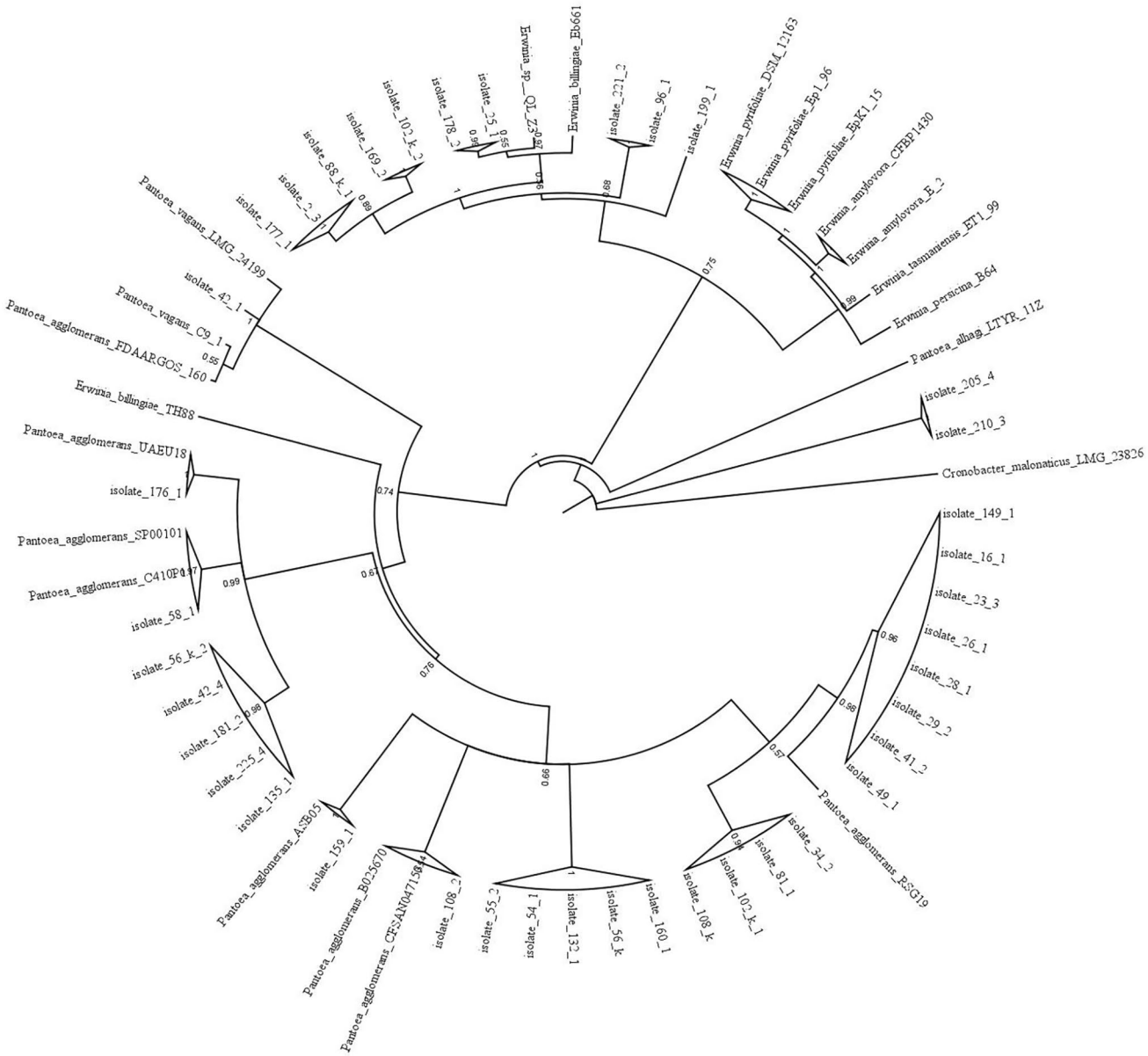

Fig. 4 Phylogenetic tree of partial *gyr*B gene sequences of *Pantoea* spp./*Erwinia* spp. isolated from almond and apricot trees constructed by Bayesian inference using the GTR + I + G model. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes obtained for 100,000,000 replicates

these bacteria ranged between 17 µg/ml by *Lysinibacillus* sp. 44-k-1 to 513 µg/ml by *P. fluorescens* 35 − 1 (Fig. 9).

None of the isolates were able to produce protease and hydrogen cyanide. In vitro ACC production indicative of potential plant growth promoting activity was detected for 11 of the 18 isolates (Table 2).

Discussion

Bacterial canker is one of the most dangerous diseases of cultivated *Prunus* spp. in Iran and the world (Agrios 2005; Ahmadi et al. 2017). One of the causal agents of the disease is the Gram-negative bacterium *Pss.* Disease management strategies for bacterial canker caused by *Pss* are important but laborious because of little available knowledge of host resistance, the endophytic nature of the pathogen during some phases of the disease cycle, and the lack of effective systemic chemical bactericides. Copper compounds are the

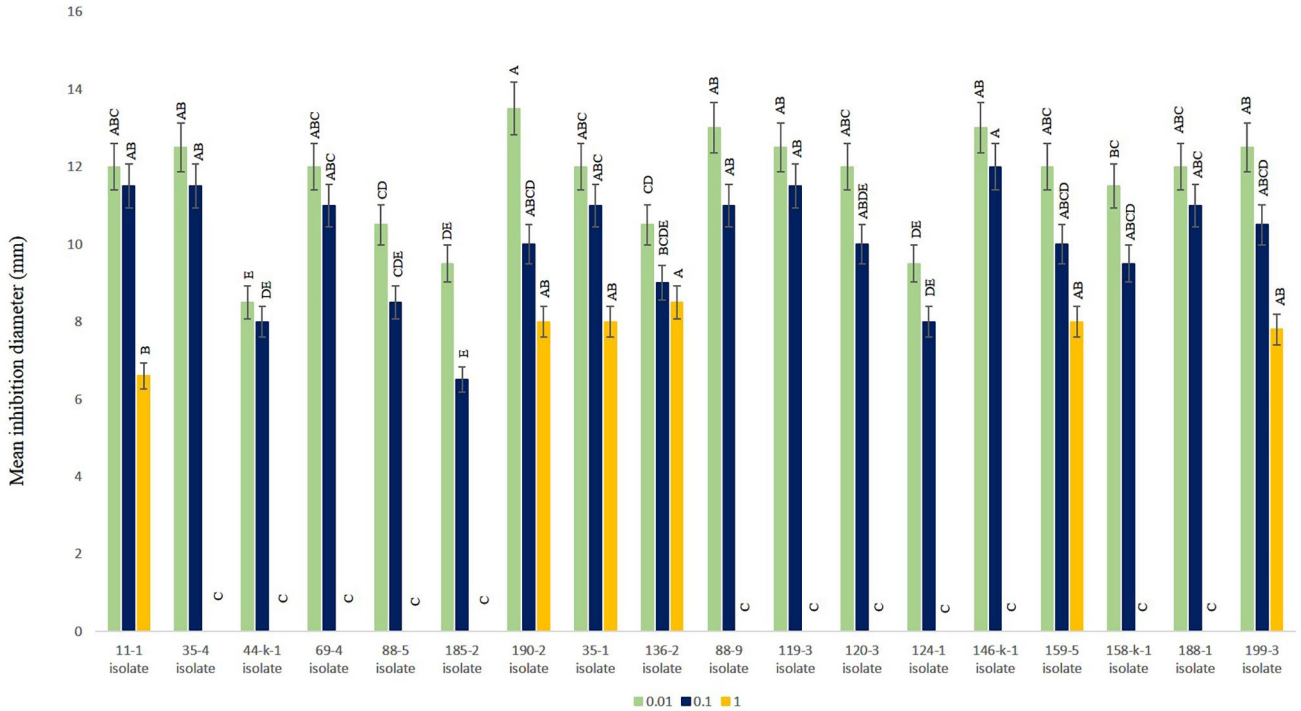

Fig. 5 Antagonistic activity of bacterial isolates against *Pseudomonas syringae* pv. *syringae* at three concentrations (OD_{600} of 0.01, 0.1, and 1) of the pathogen using a dual culture test

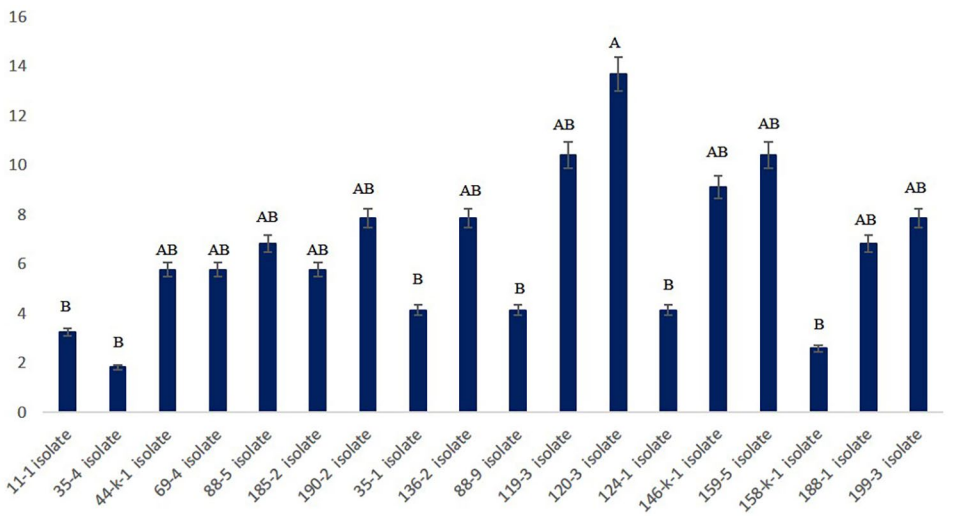

Fig. 6 Phylogenetic tree of partial *cts* gene sequences of *Pseudomonas* spp. isolated from almond and apricot trees constructed by Bayesian inference using the GTR + I + G model. The scale bar represents the average number of substitutions per site, and posterior probability values are shown at the nodes obtained for 100,000,000 replicates

standard bactericides for controlling bacterial canker disease but they are not able to kill the pathogen systemically, they may induce emergence of copper-resistant strains, persist in fruit with harm to consumers, and exhibit phytotoxicity (Kennelly et al. 2007). Therefore, developing alternative control strategies, such as biological control is desirable. Biocontrol using antagonistic bacteria can be an alternative strategy in the management of plant pathogens (Hallmann and Berg 2006). Endophytic bacteria that occupy the internal spaces of plants in vicinity to plant pathogens, are promising biocontrol agents (Berg et al. 2005). Antagonistic bacteria that produce antimicrobial compounds, phytohormones, and siderophores, and that induce systemic resistance can inhibit disease development by plant pathogens (Compant et al. 2010; Zachow et al. 2015).

In the present study, a total of 2992 bacterial strains were isolated from aerial parts of almond and apricot trees of which 113 were identified based on 16 S rRNA gene sequencing. The sequenced strains belonged to 15 bacterial genera including *Pseudomonas*, *Pantoea*, *Erwinia*,

Table 2 Qualitative plant growth-promoting properties of selected bacterial antagonists isolated from almond and apricot trees

Number of strains	Strain code	Protease production	HCN production	ACC production	Phosphate solubilization	Siderophore production
1	*P. fluorescens* 11 – 1	-	-	-	+	+
2	*P. fluorescens* 35 – 4	-	-	-	+	+
3	*Lysinibacillus* sp. 44-k-1	-	-	+	+	+
4	*P. fluorescens* 69 – 4	-	-	+	+	+
5	*Lysinibacillus* sp. 88 – 5	-	-	+	+	+
6	*Paenibacillus* sp. 185-2	-	-	+	+	+
7	*P. fluorescens* 190-2	-	-	+	+	+
8	*P. fluorescens* 35 – 1	-	-	-	+	+
9	*bP. fluorescens* 136-2	-	-	+	+	-
10	*Lysinibacillus* sp. 88 – 9	-	-	-	+	+
11	*P. fluorescens* 119-3	-	-	-	+	-
12	*P. fluorescens* 120-3	-	-	+	+	-
13	*P. fluorescens* 124-1	-	-	+	+	+
14	*P. fluorescens* 146-k-1	-	-	-	+	+
15	*P. fluorescens* 159-5	-	-	+	+	-
16	*Lysinibacillus* sp. 158-k-1	-	-	-	+	+
17	*P. fluorescens* 188-1	-	-	+	+	+
18	*P. fluorescens* 199-3	-	-	+	+	+

+: Positive reaction; -: Negative reaction

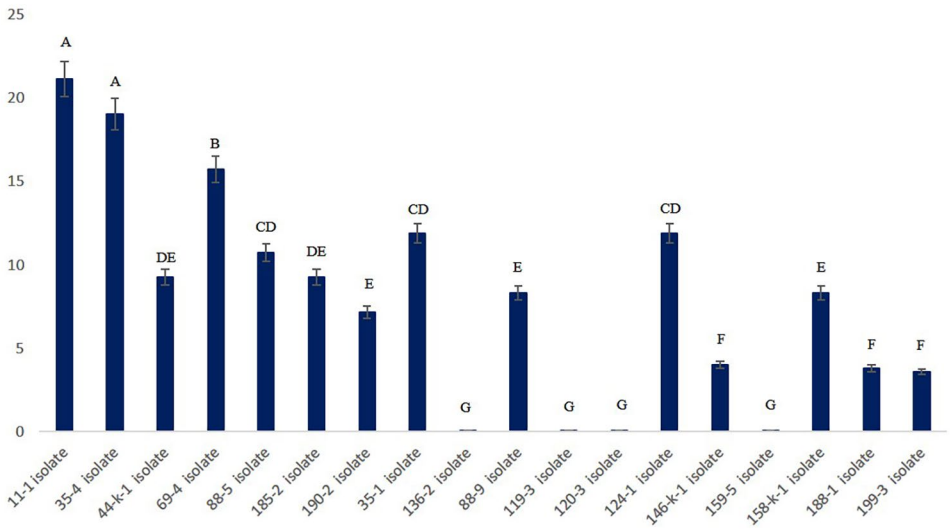

Fig. 7 Quantitative siderophore production (µg/ml) by bacterial isolates. Data represent the mean of three replicates. Means with the same letter are not significantly different

Stenotrophomonas, *Acinetobacter*, *Rouxiella*, *Escherichia*, *Massilia*, *Bacillus*, *Lysinibacillus*, *Paenibacillus*, *Curtobacterium*, *Microbacterium*, *Kocuria*, and *Arthrobacter*. In many studies, some species in these genera were identified as endophytic bacteria of different plants (Rosenblueth and Martínez-Romero 2006). Two Gram negative genera, *Pseudomonas* and *Pantoea*, and two Gram positive genera, *Lysinibacillus* and *Bacillus* were the most abundant genera cultured from aerial tissues of almond and apricot trees. According to previous studies, *Bacillus*, *Microbacterium*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas* have been reported as the most commonly isolated bacterial genera, where *Bacillus* and *Pseudomonas* are the predominant genera (Chaturvedi et al. 2016).

Both, epiphytic and endophytic strains were isolated for all identified genera with exception in the genera of *Rouxiella*, *Escherichia*, and *Curtobacterium*, for which only endophytes were isolated, and in the genera of *Massilia*, *Paenibacillus*, *Microbacterium*, *Kocuria*, and *Arthrobacter*, for which only epiphytes were isolated.

All purified strains were investigated for their antagonistic activity against Pss-170, a strain of the causal agent of apricot canker disease in East Azerbaijan, Iran. Eighteen strains showed antagonistic activity against the pathogen.

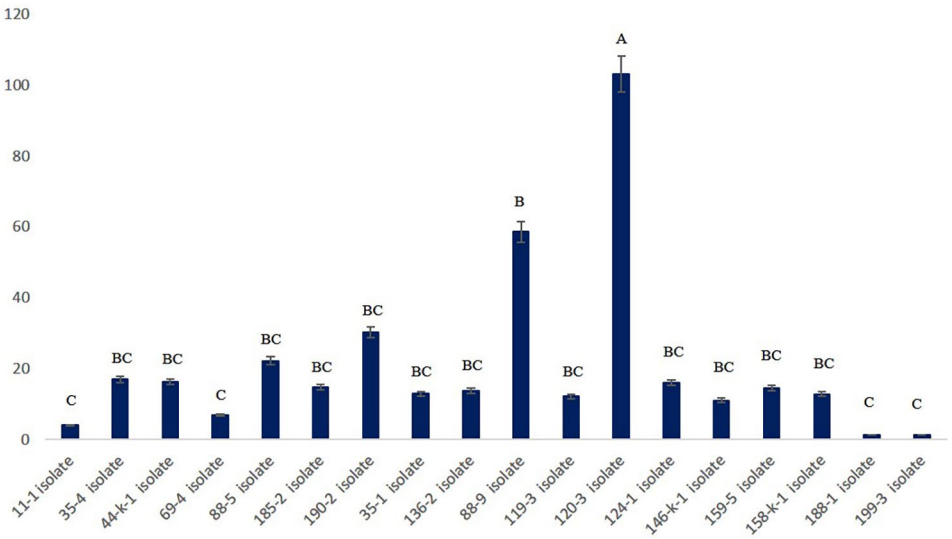

Fig. 8 Quantitative Indole acetic acid production (µg/ml) by bacterial isolates. Data represent the mean of three replicates. Means with the same letter are not significantly different

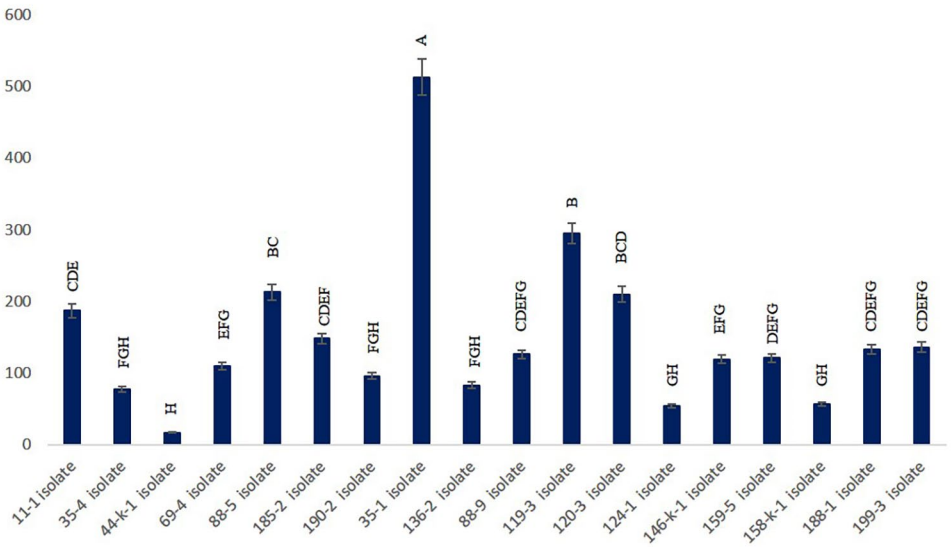

Fig. 9 Quantitative phosphate solubilization (µg/ml) by bacterial isolates. Data represent the mean of three replicates. Means with the same letter are not significantly different

Antagonistic strains belonged to the genera including *Pseudomonas*, *Lysinibacillus*, and *Paenibacillus* based on 16 S rRNA gene sequencing and were isolated both epiphytically and endophytically. These strains were investigated for their plant growth-promoting characteristics such as IAA, GA, and siderophore production, and phosphate solubilization. They were also tested for their biocontrol potential properties such as protease production and HCN production. Almost 100%, 94%, 78%, and 61% of antagonistic strains had the ability to produce GA, solubilize phosphate, produce siderophore, and ACC, respectively, while none of the strains were able to produce protease and HCN.

Synthesis of plant growth regulators, such as indole acetic acid and gibberellic acid, by some bacteria that live in association with plants have beneficial effects for plants by increasing nutrient availability and promoting plant growth under stressful environments (Duca et al. 2014). IAA and GA are phytohormones known to be produced by plant growth promoting bacteria such as *Pseudomonas* and *Bacillus* (Ali et al. 2009; Hussain and Hasnain 2011). Siderophores are low molecular weight bio-molecules secreted by some microorganisms in response to iron starvation. In the present study, both the epiphytic and endophytic antagonistic strains were able to produce siderophores. Siderophore-producing epiphytic and endophytic bacteria are able to compete with phytopathogens for ferrous iron in the rhizosphere as well as inside the host plants and function as a biocontrol agent (van der Lelie et al. 2009). Phosphorus is one of the most important nutrients for plant growth but is usually present in its insoluble form. Many endophytic bacteria with phosphate solubilization activity can enhance phosphorus uptake by plants (Oteino et al. 2015). In agriculture, application of

phosphate solubilizing microorganism was reported to facilitate plant growth (Sahu et al. 2016).

One of the key bacterial traits in promoting plant growth and improving plant biomass is the production of the enzyme ACC-deaminase by lowering ethylene accumulation in plants even under stressful conditions such as saline and drought conditions (Gupta and Pandey 2019; Onofre-Lemus et al. 2009). The antagonistic *Paenibacillus* sp. 185-2 strain was able to produce GA, ACC, and siderophore and was weak in the production of IAA and phosphatase activity. Eastman et al. (2014) reported the presence of genes responsible for plant hormone synthesis, and production of antimicrobials in the *P. polymyxa* CR1 genome. *Paenibacillus* species have been isolated from various ecological habitats including soil, air, and rhizosphere. Several studies have shown the antagonistic properties of *Paenibacillus* species against phytopathogenic bacteria and fungi such as *Ralstonia*, *Agrobacterium*, and *Fusarium* and their ability in plant growth promotion and enhancing yield (Algam et al. 2010; Bosmans et al. 2017; Sato et al. 2014; Yadav 2019). Strains of *P. polymyxa* have been reported to possess inhibitory activity against plant pathogenic bacterium *P. syringae* (Eastman et al. 2014; Kwon et al. 2016) with ability in production of HCN, siderophores, phytohormone, and enzymatic activities such as protease and phosphatase production (Gómez-Lama Cabanás et al. 2018).

Four isolates, 44-k-1, 88 – 5, 88 – 9, and 158-k-1, were identified as members of the genus *Lysinibacillus* with high similarity to *L. fusiformis* reference strains. These isolates showed the highest inhibition effects against the Pss-170 strain when used at an optical density of 0.01 and when the pathogen was inoculated at an optical density of 0.1. *Lysinibacillus* is a Gram-positive bacterium that can form dormant endospores under stress conditions which are resistant to heat, chemicals, and ultraviolet light. There are several reports indicating the potential of *Lysinibacillus* spp. for biocontrol activities against phytopathogens and plant growth promotion like phosphate solubilization and nitrogen fixation and production of higher quantity of IAA, phytohormone, siderophore, HCN, and ACC-deaminase (Naureen et al. 2017; Sahu et al. 2018; Sgroy et al. 2009; Verma et al. 2014; Yadav et al. 2016).

The *P. fluorescens* (11 – 1, 35 – 4, 69 – 4, 190-2, 35 – 1, 136-2, 119-3, 120-3, 124-1, 146-k-1, 159-5, 188-1, and 199-3 isolates) were found to be the most abundant antagonistic strains. Among all the *P. fluorescens* antagonistic strains, the 11 – 1, 190-2, 35 – 1, 136-2, 195-5, and 199-3 isolates showed significant inhibition of the Pss-170 strain at all three concentrations of the Pss-170 in the dual-culture assay. All isolated *P. fluorescens* strains showed high similarity to the *P. fluorescens* A506 antagonist strain based on molecular identification. This strain was isolated from pear in California and has the ability to reduce the incidence of fire blight in orchards by 50 to 80% (Stockwell et al. 2010). In the present study, the *P. fluorescens* strains 69 – 4, 190-2, 35 – 1, 124-1, 188-1, and 199-3 showed four properties related to plant growth promotion, including production of ACC and siderophore and phosphate solubilization. The *P. fluorescens* 120-3 strain showed the highest production of the phytohormones IAA and GA compared to the other strains. The *P. fluorescens* strains 11 – 1 and 35 – 4 showed the highest ability in production of siderophore. In many studies, strains of *P. fluorescens* were shown to enhance plant growth promotion and reduce severity of various diseases caused by a range of fungal and bacterial plant pathogens (Gómez-Lama Cabanás et al. 2017; Pujol et al. 2005). This effect is the result of the production of a number of secondary metabolites including antibiotics, siderophores, 2,4-diacetylphloroglucinol, IAA, and hydrogen cyanide as well as ability to solubilize phosphate (Bensidhoum et al. 2016; Couillerot et al. 2009; Duffy and Défago 1999; O'Sullivan and O'Gara 1992; Golanowska et al. 2012) identified the *P. fluorescens* T660 and T777 strains as antagonistic bacteria against the causal agents of stone fruit canker disease caused by *Pss* and *P. syringae* pv. *morsprunorum*.

In conclusion, this study reported the presence and diversity of culturable epiphytic and endophytic bacteria in almond and apricot trees. Based on our information, this is the first reported study in elucidating the epiphytic and endophytic bacterial diversity associated with aerial parts of almond and apricot trees with plant growth promoting and biocontrol potential based on in vitro assays. The existence of such microorganisms with the ability to promote plant growth and control plant disease suggests that they could be utilized as biocontrol agents in future applications, however, further studies based on in vivo and field conditions are required.

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Declarations

Conflict of interest The authors confirm that there is no known conflict of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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