Isolation and identification of *Amycolatopsis* sp. strain 1119 with potential to improve cucumber fruit yield and induce plant defense responses in commercial greenhouse

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Abstract

**Background and aims** The application of chemical fungicides is the first strategy to control plant fungal diseases. This approach is highly polluting for the environment and affects human health. The artificial introduction of beneficial rhizobacteria into the soil can be an economical and practical way to control phytopathogenic fungi in commercial greenhouses. Here, we recount the travel of a rare Actinomycete (*Amycolatopsis* strain 1119) from a maize field to a commercial cucumber greenhouse.

**Methods and results** Culturable bacteria from rhizosphere and bulk soils of dicot and monocot crops were isolated and screened. About 20% of the representative colonies showed Actinobacteria appearance. 106 Actinobacteria that had antagonistic activity against *Phytophthora capsici* and were able to produce IAA-like molecules were selected for further analysis. Two *Streptomyces* strains (432 and 615) and 2 *Amycolatopsis* strains (3513 and 1119) that showed a positive effect on plant growth in greenhouse conditions were selected to evaluate the biocontrol potential. Strains 432, 3513, 615 and 1119 controlled incidence of the damping-off by 65%, 42%, 83% and 100% respectively. Application of strain 1119 under commercial greenhouse conditions resulted in an increase in fruit yield (20%) and a decrease in fruit nitrate content (70%). Increased antioxidant enzymes activity and increased LOX and APX transcription and also, increased expression of two genes *PRI-1a* and *GLU* (SAR genes) showed that strain 1119 could...
induce both ISR and SAR in cucumber without pathogen exposure.

**Conclusion**  
Our results demonstrate that the *Amycolatopsis* strain 1119 has a great potential to be used as an active principle for bio-inoculant development because of the ability to improve cucumber fruit yield and induce plant defense responses in a commercial greenhouse.

**Keywords**  
*Amycolatopsis* · Biocontrol · Damping-off · Fruit quality · PGPB · *Phytophthora capsici*

**Introduction**

At present, the application of chemical fungicides is the first strategy to control and manage plant fungal diseases. This approach is highly polluting and has consequences on the development of resistant strains of phytopathogenic fungi (Café-Filho and Ristaino 2008). Disease-suppressive soils are defined as soils in which, despite the presence of virulent pathogens, disease incidence occurs at a low rate (Kinkel et al. 2011). Plant Growth-Promoting Bacteria (PGPB), including some species of Actinobacteria, non-pathogenic *Pseudomonas* and *Bacillus* are consistently associated with disease suppression (Schlatter et al. 2017). The potential of PGPBs to suppress plant root fungal diseases was reported in many studies (Abbasi et al. 2020; Islam et al. 2016; Karimi et al. 2012; Rangarajan et al. 2003). Exudate of the plant root is the main source of the nutrients in the rhizosphere and attracts PGPBs to themselves selectively (Lareen et al. 2016). Microbial communities in the root environment can be deliberately manipulated to increase plant health and yield. The development of manipulation of the rhizobacterial communities to transfer disease suppressiveness to non-suppressive soil is a promising way to reduce chemical fungicides. In this regards, several studies have been done to examine the effect of crop rotation (Niu et al. 2016), application of organic fertilizers (Zaller et al. 2004), biofertilizers containing living PGPBs (Vessey 2003) and bulk soil (Weller et al. 2002) on the suppressiveness properties of soil. Based on the results of these researches, manipulation of the cropping system and artificially introduction of PGPBs into the soil, as biofertilizer or biocontrol agents, are two practical methods to control phytopathogenic fungi in crop fields.

The Actinobacteria is a phylum of Gram-positive bacteria and can be found in bulk and rhizosphere soil (Sharma and Salwan 2018). *Streptomyces* is a well-known genus of Actinobacteria. Diverse species of *Streptomyces* produce more than 70% of the antibacterial and antifungal components are used in medicine, veterinary and agriculture (Landwehr et al. 2016). Also, *Streptomyces* produce many secondary metabolites, including volatile compounds (Cordova et al. 2015) and extracellular hydrolytic enzymes are active in fungal cell wall degradation (Brzezinska et al. 2014; Sadeghi et al. 2017). Cordovez et al. (2015) study showed that *Streptomyces* species were the most dynamic and abundant (70% of the isolates) in a *Rhizoctonia* suppressive soil. Although many studies present control destructive plant pathogens by *Streptomyces* species (Viaene et al. 2016), the biocontrol potential of non-*Streptomyces* Actinobacteria are rarely reported (El-Tarabily et al. 2006; Mingma et al. 2014; Tanvir et al. 2014). Furthermore, little is known about the diversity and distribution of the antagonistic *Streptomyces* or non-*Streptomyces* Actinobacteria among the rhizosphere soil of different crops. Davelos et al. (2004) investigated the diversity of the antagonistic *Streptomyces* in natural habitat (non-agricultural soil). They found that there is a considerable variation in culturable *Streptomyces* communities among different locations of a habitat. Also, it is reported that the density of the antagonistic *Streptomyces* associated with rhizosphere soil significantly differed among species of prairie plants (Bakker et al. 2013). A more detailed study showed that diversity, phylogenetic composition, and pathogen inhibition activity among *Streptomyces* communities of non-agricultural and agricultural soils were similar and only intensity and breadth of inhibitory activities differed among phylogenetic groups (Bakker et al. 2010). Niu et al. (2016) have reported the importance of crop plants as a key factor in determining the function and genetic diversity of the antagonistic PGPBs. Their culture-independent study showed that only tobacco-maize rotation cropping system but not lily or turnip decrease the incidence of tobacco bacterial wilt. It has recently been reported that the rhizosphere soil of a field in which maize is frequently grown has a high diversity in terms of cultivable bacteria that stimulate plant growth (Tchuisseu Tchakounte et al. 2018).
Actinobacteria, especially Actinomycetales, Streptomycetales and Pseudonocardiaceae, are playing fundamental roles in growth promotion and biological control of insect pests and pathogens which cause damage to grain crops such as leguminous and cereals (Gopalakrishnan et al. 2019). *Amycolatopsis* is a genus of Actinomycetales within the family Pseudonocardiaceae which are Gram-positive filamentous bacteria with a high GC content in their genomes (Tang et al. 2010). To the best of our knowledge, the genus *Amycolatopsis* contains 87 recognized species and four subspecies (https://lpsn.dsmz.de/genus/amycolatopsis). Unfortunately, the significance of this genus in agriculture is not well known.

We hypothesized that there are differences between culturable bacteria especially, the Actinobacterial population in the rhizosphere and bulk soil of dicots and monocots and recognizing these differences may be useful for isolating efficient PGPBs. In addition, we assumed that a growth-promoting *Amycolatopsis*, which is effective in research greenhouse, could also increase cucumber yield in a commercial greenhouse.

The objectives of the present research were to (1) select and characterize Actinobacterial isolates from bulk and rhizosphere soil of different monocot and dicot crops fields, with the ability to inhibit the growth of the most important fungal pathogens that cause damping-off and wilt in cucumber (2), detect hydrolytic enzyme activity and PGP (plant growth promotion) traits of the antagonistic isolates, (3) investigate the PGP effect of selected strains in greenhouse condition (4), evaluate the biocontrol of *Phytophthora capsici*, the causal agent of cucumber damping-off by PGP strains under greenhouse conditions and (5), evaluate the effect of the most promising strain (*Amycolatopsis* sp. strain 1119) on yield, fruit quality and molecular and cellular defense mechanisms of cucumber plants grown under commercial greenhouse conditions.

**Materials and methods**

Isolation, cultivation, and maintenance of Actinobacterial isolates

Wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), tomato (*Solanum lycopersicum* L.) and cucumber (*Cucumis sativus* L.) roots with adhering soil were harvested from healthy plants grown in individual farms around Nazarabad city (35° 55’ 08” N, 50° 36’ 27” E), Alborz province, Iran in 2015. Three farms were considered for each crop and three root samples were randomly collected from each farm. Also, three soil samples without plant residues (assigned as bulk soil) were randomly collected from the margins (5 m away from the last row of plants) of each farm. Excess soils were removed following gentle shaking of the roots, and the soils that remained attached to the roots were considered rhizosphere soils. In total, 36 rhizosphere and 36 bulk soil samples were collected. For isolating Actinobacteria, 2 g soil was diluted in 100 mL of sterile saline solution (0.9% NaCl) and shaken for 30 min. Three serial dilutions (1:100, 1:1000 and 1:10,000) were prepared using sterile saline solutions in a total volume of 1 mL. An aliquot of 0.1 mL of each dilution was plated on water agar (18 g/L pH 7.2). The plates were incubated at 29°C, for seven days. Colonies formed in the second and third dilutions were used to count and calculate the bacterial population. For each dilution, three replicates were prepared and each repetition was a plate with a diameter of 10 cm. Colonies from the second dilution were selected and streaked on new plates of MYA medium (containing 10 g/L malt extract, 4 g/L yeast extract, 4 g/L glucose and 18 g/L agar, adjusted to pH 7.2) at 29°C.

All studied strains including 407, 405, 432, 515, 3637, 3415, 3513, 1118, 1119, 1331, 1317, 614, and 615 were preserved at the microbial collection of the department of microbial biotechnology. Besides, strain 1119 was deposited at the Agricultural Biotechnology Research Institute of Iran Culture collection (ABRIICC) under accession numbers ABRIICC 20716.

Antifungal activity

The dual culture method (Yuan and Crawford 1995) was used to evaluate the antifungal activity of the Actinobacterial isolates against five plant pathogens including, *Phytophthora drechsleri*, *Phytophthora capsici* (ABRIICC 10292) (Abbasi et al. 2020), *Pythium ultimum*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *cucumerinum* (ABRIICC 10294) (Abbasi et al. 2019). The pathogen *P. capsici* strain
SA (GenBank accession number MG670447) and *P. drechsleri* strainAZ94 (GenBank accession number MF138111) were kindly provided by Dr. Azimi, Iranian Research Institute of Plant Protection (IRIPP), Tehran, Iran. *R. solani* AG-2-2 (strain Rh133) and *P. ultimum* (strain MK1248) were kindly provided by Dr. Kakueinezhad, Pathology Research Dept., Sugar Beet Seed Institute (S.B.S.I.), Karaj, Iran. A sterile needle was used to culture one individual bacterial colony count unit (cfu) in the center of the PDA (potato dextrose agar) plate. Plugs from the growing edge of each pathogens fresh culture were placed on the two sides of the PDA plate. Plates were incubated at 23 °C for five days. The growth inhibition percentage was calculated using the formula \( n = \frac{(a - b)}{a} \times 100 \), where 'n' is the growth inhibition percentage, 'a' is the fungal growth radius of a control culture (in cm) and 'b' is the distance of the pathogen growth in the direction of bacteria (in cm).

Cellulase activity

Carboxymethyl cellulase (CMCase) activity was determined by Mandels-Reese medium with carboxymethyl cellulose (CMC) as the sole carbon source (Majidi et al. 2011). The ratio of the clear zone diameter to colony diameter was calculated and recorded as cellulase activity.

Chitinase activity

Chitinase production was determined according to the method of Hsu and Lockwood (1975). Bacterial isolates were grown on chitin agar containing 0.4% colloidal chitin and 1.5% agar adjusted to pH 7.2. The colloidal chitin was prepared according to Berger and Reynolds (1958). Plates were incubated for five days at 29 °C. The ratio of the clear zone diameter to colony diameter was calculated and recorded.

Siderophore production

Siderophore production was evaluated according to Alexander and Zuberer (1991) on the Chrome Azurol agar (CAS) medium. The CAS agar medium was prepared and distributed in Petri dishes, then bacterial isolates were spot-seeded onto the center of the plate and incubated at 29 °C. After three days, the ratio of the orange halo zone diameter to colony diameter was calculated and recorded.

**Presumptive* phosphate-solubilizing activity**

Pikovskaya’s medium (PVK) was used to measure tricalcium phosphate \( [Ca_3(PO_4)_2] \)-solubilizing activity. Bacterial isolates were spot-seeded onto the center of the plate and incubated at 29 °C for seven days. The solubilization index was evaluated according to the ratio of the clear zone diameter to colony diameter (Soltani et al. 2010).

It should be noted that the use of tricalcium phosphate alone is not enough to identify and evaluate phosphate-solubilizing bacteria and this method has restrictions. Metal-P compounds such as Fe-P and Al-P and organic acids also must be used to complete the evaluation of the phosphate-solubilizing activity of PGPRs (Bashan et al. 2013).

Presumptive nitrogen fixation (free-living putative diazotrophs)

The ability of the isolates to grow on a solid nitrogen-free medium (NFM) was evaluated according to Dahal et al. (2017). The bacteria were grown on NFM containing \( K_2HPO_4 \) (0.2 g/L), \( KH_2PO_4 \) (0.5 g/L), \( MgSO_4.7H_2O \) (0.2 g/L), \( FeSO_4.7H_2O \) (0.1 g/L), \( Na_2MoO_4.2H_2O \) (0.005 g/L), \( NaCl \) (0.2 g/L), glucose (10 g/L) and 15.0 g/L agar. After seven days of incubation at 29 °C, the growth of each isolate was evaluated and compared with growth on the MYA medium as a positive control.

Production of indole-3-acetic acid (IAA) like molecules

The production of IAA-like molecules was determined based on the method described by Patten and Glick (2002). Actinobacteria isolates were inoculated in 100 mL flasks containing 25 mL TSB (Tryptic soy broth) medium supplemented with 2 mg/mL L-tryptophan at 29 °C for five days on a rotary shaker (150 rpm). Bacterial cells were then collected by centrifugation at 10,000 g for 15 min. Two mL of Salkowski reagent was added to one mL of the supernatant, and absorbance of the solution was read at 535 nm in a UV–Vis Spectrophotometer (Cary 300).
The IAA-like molecules concentration was determined using a standard IAA (Sigma-Aldrich) calibration curve.

Genotypic characterization

Isolation of DNA was performed according to the method of Tripathi and Rawal (1998). PCR amplification of the 16S rRNA gene was carried out as described by Chun and Goodfellow (1995). The almost-complete 16S rRNA gene sequences (1400 nt) were deposited in the GenBank database under the accession numbers MG995004 (strain 407), MG995003 (strain 405), MN888928 (strain 432), MN888932 (strain 515), MN888935 (strain 3637), MG984768 (strain 3415), MH266469 (strain 3513), MG984616 (strain 1119), MG995001 (strain 1118), MG995005 (strain 1331), MN493628 (strain 1317), MN888939 (strain 614) and MN888940 (strain 615). The sequences aligned manually with corresponding sequences of available Actinobacteria species drawn from the GenBank, EMBL, and DDBJ databases using BLAST (Altschul et al. 1997). The phylogenetic tree was constructed using the MEGA 5.0 software package (Tamura et al. 2011) based on the method of neighbor-joining. Bootstrap analyses were used to evaluate the stability of relationships based on 1000 resampling.

Greenhouse experiments

Experiment 1: evaluation of plant growth promotion

Surface-sterilized cucumber seeds (C. sativus L. Soltan cultivar) were pre-germinated at room temperature for 72 h. Thirty-five seedlings were placed into a 35-cell plug tray (25×25×2.5 cm deep), with one seedling occupying each cell. The trays were filled with a sterile mixture of field soil and peat moss (1:2). For bacterial treatments, bacterial spore suspension in sterile saline solution was added to autoclaved sand and well mixed. After air-drying (2 days at room temperature under sterile conditions) the final cfu/g was adjusted to 10^6. A mixture of sand and sterile saline solution was used as untreated control. Bacterial formulation (one-gram sand/seedling) was added to the mixture of soil and peat moss just before filling the trays. The trays were kept in a greenhouse at 27 °C and 16 h/8 h brightness/darkness. Soil irrigated with tap water and drain water from each tray was added to the soil. There were four replicates (4 trays) of each bacterial treatment and untreated control and the experiment was arranged in a randomized complete design (CRD). The seeds were watered every two days. To evaluate the effects of the bacterial treatment on plant growth, dry weight of root and shoot and plant height were measured 30 days after planting.

Experiment 2: evaluation of biocontrol activity

The PGPB isolates were selected based on the results of the first experiment, and the second experiment was repeated as experiment 1 with 8 replications (8 trays) for each bacterial treatment and control. After 14 days of bacterial treatment, trays were divided into two groups inoculated and uninoculated. Cucumber seedlings in the inoculated group were inoculated artificially with the pathogen. An agar plug from a fresh culture of P. capsici on PDA was placed on the surface of each cell (at a distance of 0.5 cm from the crown), and disease signs were monitored daily. The inoculated seedlings were checked daily, and the number of diseased seedlings (with a thin and brown stem or completely death) was recorded. Final disease incidence was determined 16 days after inoculation based on total diseased seedlings percentage (%). At the end of the experiment (thirty days after planting), the plants of the uninoculated group were harvested, and the dry weight of roots and shoots were measured.

Experiment 3: performance evaluation of strain 1119 at the commercial greenhouse conditions

Greenhouse experiments were conducted in spring 2019 in a 1250 square meter soil-based typical solar commercial greenhouse located in Mohammadabad-e Arab (35° 15’ 48” N, 51° 43’ 53” E), a village in Varamin County, Tehran Province, Iran. The experimental design was a randomized complete block (RCB) with two treatments and four replicates. Each replicate was a 20-m-long row containing 30 plants. Treatments consisted of strain 1119 and untreated as a control. Greenhouse bed preparation, use of animal manure, seed (C. sativus L. Soltan cultivar) germination and transplanting in the soil, irrigation, light and temperature management, weed removal, use of fungicide and pest management were carried
out according to the typical methods in the area. The deficiency of minor elements was identified by the specialist during the plant growth and development and was treated with foliar spray. Livestock manures (cow manure (10 ton/ha) and poultry manure (30 ton/ha)), super triple phosphate (200 kg/ha) and potassium phosphate (250 kg/ha) were applied during bed preparation. Sixty days after planting NPK fertilizer (2 g/L NPK, 10–8-40) were injected directly into the irrigation water at three doses weekly until the end of the cultivation period. Ten days after plants transfer to soil, treatment with strain 1119 was done. Fifteen days after treatment, the leaves of four plants from each replicate were harvested and pooled, frozen in liquid nitrogen and kept at -80 °C for more analysis. One hundred days after treatment, 15 times (with a two-day interval), fruits were harvested and their fresh weight was recorded.

**Fruit quality, dry weight percent and content of sugar and nitrate**

To determine the percentage of dry weight, 100 g of the fresh cucumber (4 fruits were selected from each replicate and then 25 g of each fruit was pooled) was cut into 5 mm pieces and dried in an oven at 55 °C. For sensorial evaluation, the samples were presented to 40 untrained panelists (25 males and 15 females in the range of 24–50 years old). Each participant was asked to evaluate fruits by scoring characteristics (bitterness, fragility, aroma, juiciness, appearance, flavor, and overall acceptance) with grades ranged from 1 to 5, where 1 = weakly accepted and 5 = excellent quality.

To estimate total soluble sugar, 200 mg of frozen fruit was added to a centrifuge tube and homogenized with 1.5 mL of 80% ethanol solution in a vortex for 50 s. The sample was centrifuged at 5000×g for 10 min, and the supernatant was put in an oven at 50 °C to evaporate ethanol. Respectively, 10 mL of deionized water, 0.47 mL of 0.3 N BaOH, and 0.5 mL of 5% Zn (SO₄)₂ solution were added to the sample. The tube containing the sample was centrifuged at 5000×g for 10 min. Phenol (5%, 0.5 mL) and sulfuric acid (98%, 2.5 mL) were added to 1 mL of the supernatant. After 45 min, the absorbance of solutions was read at 485 nm using a spectrophotometer (Cary 300, Agilent, USA). Soluble sugar content was calculated using glucose as a standard curve (Dubois et al. 1956).

To evaluate the nitrate content, 100 mg of frozen fruit was added to a centrifuge tube and homogenized with 1 mL of deionized water and placed in Bain-Marie at 45 °C for 60 min. The homogenate was centrifuged at 8000×g for 10 min, and 100 μL of supernatant was mixed to 400 μL deionized water, then 100 μL of the mixture was added to 400 μL salicylic acid (5% salicylic acid in sulfuric acid). After 20 min, 9.5 mL NaOH 2 M was added, and the absorbance of the solution was read at 410 nm using the spectrophotometer. Potassium nitrate (KNO₃) was used to prepare a standard curve (Cataldo et al. 1975).

**Protein, MDA (malondialdehyde) and H₂O₂ content and antioxidant enzymes activity**

Frozen leaves (500 mg fresh weight) were homogenized in Na-Pi buffer containing 10 mg polyvinylpyrrolidone. The homogenate was centrifuged at 8000×g for 30 min at 4 °C and, the supernatant was used as a crude enzyme extract to determine total protein content (Bradford 1976) and activity of ascorbate peroxidase (APX: EC 1.11.1.11), catalase (CAT: EC 1.11.3.6) and peroxidase (POX: EC 1.11.1.7). Catalase (CAT) activity was assayed using the method of Cakmak and Horst, 1991. The APX activity was determined following the method of Cakmak and Marschner (1992). The POX activity was assayed following the colorimetric determination of pyrogallol oxidation, according to Hasan et al. (2011). The specific activity of the enzymes was expressed as U/mg protein. Lyophilized tissue (100 mg) was extracted with 0.1% trichloroacetic acid (TCA) and used to measure MDA content, according to Stewart and Bewley (1980). The MDA concentration was defined by its extinction coefficient of 155 mM⁻¹ cm⁻¹. The H₂O₂ concentration was measured on lyophilized tissue crushed in 5 mL of cold 0.1% (w/v) TCA and then centrifuged for 15 min at 12,000×g in a refrigerated centrifuge. The supernatant (0.5 mL) was added to 100 mM phosphate buffer (pH 7.0) and 1 M of iodate potassium (KI) solution and the absorbance was measured at 390 nm (Loreto and Velikova 2001). The amount of H₂O₂ was calculated using a standard curve prepared with known concentrations of H₂O₂.
qRT-PCR analysis of the defense-related genes

Total RNA was isolated from fresh shoots using TRIzol® (Invitrogen). One microgram RNA was used for synthesizing cDNA after treating with RNase-free DNase I (BioLabs) using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific). Gene expression was assayed using Roche LightCycler® 96 real-time PCR system and iQ SYBR Green Supermix kit (BioRad), according to the manual description. Transcription of each gene was studied by RT-PCR with 0.5 μL of 10 pM of each forward and reverse specific primer designed in this study (Table S) and 1 μL of template cDNA (100 ng). The following PCR profile was used: 5 min at 95 °C; 40 cycles (30 s at 95 °C, 30 s at 64 °C, 30 s at 72 °C); 10 min at 72 °C and recording melting curve. The transcription of the EF (elongation factor) gene was used as an internal control. The gene expression ratio was calculated using the REST 2009 software (Pfaffl et al. 2002).

Statistical analysis

There were at least three biological replicates for each treatment or control group and all in vitro and in vivo experiments were repeated at least twice. Statistical analysis was carried out by SPSS version 16.0, SPSS, Chicago, IL, USA. First, data were subjected to a variance homogeneity test based on Levene’s test as a diagnostic tool. When population variances were equal according to $p > 0.05$ then the statistical analysis was performed with one-way ANOVA followed by Duncan significant difference test at a level of $p \leq 0.05$ (to compare more than two means obtained from greenhouse experiments 1 & 2) or Student’s t-test (to compare two means obtained from greenhouse experiment 3 and evaluations of the bacterial population).

Results

Isolation of Actinobacteria

In this research, the total number of colonies were isolated from 12 fields was 6596. The abundance of bacteria (cfu/g dry soil) isolated from different fields was not the same and differences in the number of colonies isolated from the rhizosphere and bulk soils of cucumber and tomato fields were significant ($P \leq 0.01$). In contrast, there were no significant differences in the richness of culturable bacteria between the rhizosphere and bulk soils of all three wheat fields. Also, there was not a significant difference

![Fig. 1 The abundance of culturable bacteria isolated from bulk and rhizosphere soil of different fields of tomato, cucumber, maize, and wheat. Data represent the mean values±SE of three samples. The values related to rhizosphere marked with an asterisk are significantly different from the bulk according to Student’s t-test ($p < 0.05$)](image-url)
between wheat fields. The differences between the number of colonies isolated from the rhizosphere and bulk soils of maize fields were not significant too (Fig. 1). Compared to dicots, the ratio of colonies isolated from bulk soils was higher in monocots fields. Also, the distribution of cultivable bacteria in the rhizosphere and bulk soils of maize and wheat was similar. This status was observed for dicots, cucumber, and tomato (Fig. 2). About twenty percent of the representative colonies (1335 of 6596) showed Actinobacteria appearance including compact colored heaped and chalky, waxy, wrinkled, powdery, or velvety after culture on ISP2 medium and possessed an earthy odor or a good scent like vanilla. The share of tomato, cucumber, maize, and wheat from total Actinobacteria isolates were 27%, 35%, 20% and 18% respectively. In total, nine hundred and six (69%) and 405 (31%) Actinobacteria colonies were isolated from rhizosphere and bulk soils, respectively.

Antifungal activity

One hundred and six Actinobacterial isolates (8%) that showed antagonistic activity against \( P. \) capsici were selected for further analysis. In some fields, the antagonistic Actinobacteria were found in both rhizosphere and bulk soils, and in others, antagonistic isolates were observed only in the rhizosphere or bulk soil. Furthermore, lots of the antagonistic Actinobacterial isolates (64%) were isolated from bulk soils (Fig. 3a). The share of monocots and dicots from total antagonistic isolates were 79% and 21%, respectively (Fig. 3b). All of the antagonistic isolates (isolated based on the inhibition of \( P. \) capsici growth) showed antagonistic activity against \( P. \) drechsleri, \( P. \) ultimum (31%), \( R. \) solani (45%) and \( F. \) oxysporum (45%). The number of antagonistic isolates, which is isolated from monocot crops, was higher than dicots, among them, wheat possesses higher antagonistic isolates (Table 1). Twenty out of 106 antagonistic isolates showed an inhibitory effect against all the five phytopathogenic fungi.

Fig. 2 The percentage of culturable bacteria isolated from bulk and rhizosphere soil of different crops. The soil was suspended in the sterile saline solution and diluted in ratios of 1:100, 1:1000, and 1:10,000. An aliquot of 0.1 mL was plated on water agar (18 g/L pH 7.2). After seven days of incubation at 29 °C, colonies formed in the second and third dilutions were recorded and used to calculate the bacterial populations.

Fig. 3 Distribution and percentage of 106 antagonistic Actinobacteria isolated from a bulk and rhizosphere soil of b monocots and dicots crops. The dual culture method (Yuan and Crawford 1995) was used to evaluate the antifungal activity of the Actinobacterial isolates against \( P. \) drechsleri, the causal agent of cucumber damping-off.
Table 1 Isolation site and distribution of characteristics of 106 Actinobacteria isolated in this study

| Host crop | Place of collection | Number of Actinobacteria | Antagonism against | With enzyme activity | With PGP activity |
|-----------|---------------------|--------------------------|--------------------|----------------------|-------------------|
|           |                     |                          | P. capsici | P. drechsleri | P. ultimum | R. solani | F. oxysporum |                          | Cellulases | Chitinases | Growth on N free medium | Tricalcium phosphate-solubilization | IAA-like molecules production | Siderophore production |
| Tomato    | Field 1 (rh)        | 4                        | 4          | 0            | 0          | 0          | 4          | 3          | 2                     | 0             | 0               | 4                     | 2               |
| Tomato    | Field 2 (b)         | 2                        | 2          | 0            | 0          | 0          | 2          | 0          | 0                     | 0             | 0               | 2                     | 2               |
| Tomato    | Field 3 (b)         | 3                        | 3          | 1            | 0          | 0          | 3          | 1          | 1                     | 1             | 1               | 3                     | 1               |
| Cucumber  | Field 1 (rh)        | 1                        | 1          | 1            | 1          | 1          | 1          | 1          | 0                     | 0             | 0               | 1                     | 1               |
| Cucumber  | Field 1 (b)         | 1                        | 1          | 0            | 0          | 0          | 1          | 1          | 0                     | 0             | 0               | 1                     | 0               |
| Cucumber  | Field 2 (rh)        | 1                        | 1          | 1            | 1          | 1          | 1          | 1          | 0                     | 0             | 0               | 1                     | 1               |
| Cucumber  | Field 2 (b)         | 1                        | 1          | 0            | 0          | 0          | 1          | 1          | 0                     | 0             | 0               | 1                     | 1               |
| Cucumber  | Field 3 (rh)        | 3                        | 3          | 1            | 0          | 0          | 3          | 0          | 2                     | 2             | 2               | 3                     | 1               |
| Maize     | Field 1 (rh)        | 5                        | 5          | 0            | 0          | 0          | 4          | 2          | 0                     | 4             | 0               | 5                     | 2               |
| Maize     | Field 1 (b)         | 5                        | 5          | 2            | 2          | 0          | 4          | 2          | 2                     | 2             | 2               | 5                     | 2               |
| Maize     | Field 2 (rh)        | 4                        | 4          | 2            | 0          | 0          | 2          | 2          | 2                     | 4             | 4               | 2                     | 2               |
| Maize     | Field 2 (b)         | 7                        | 7          | 0            | 0          | 1          | 3          | 3          | 1                     | 2             | 2               | 7                     | 2               |
| Maize     | Field 3 (rh)        | 8                        | 8          | 4            | 6          | 1          | 5          | 2          | 7                     | 8             | 8               | 3                     | 3               |
| Maize     | Field 3 (b)         | 1                        | 1          | 1            | 0          | 0          | 1          | 0          | 0                     | 1             | 1               | 1                     | 0               |
| Wheat     | Field 1 (rh)        | 13                       | 13         | 5            | 8          | 8          | 10         | 4          | 0                     | 1             | 1               | 13                    | 5               |
| Wheat     | Field 2 (b)         | 42                       | 42         | 13           | 33         | 35         | 34         | 8          | 0                     | 1             | 1               | 42                    | 15              |
| Wheat     | Field 3 (rh)        | 2                        | 2          | 0            | 0          | 0          | 0          | 0          | 0                     | 0             | 0               | 2                     | 0               |
| Wheat     | Field 3 (b)         | 3                        | 3          | 2            | 0          | 2          | 0          | 0          | 0                     | 0             | 0               | 3                     | 2               |
| Total     |                     | 106                      | 106        | 33           | 48         | 48         | 79         | 30         | 17                    | 26            | 106             | 42                    |
Hydrolytic enzymes activity

All 16 (100%) antagonistic Actinobacteria were isolated from the dicots fields had cellulase activity (Table 1), while this ratio was only 70% for monocots. Thirty out of 106 (28%) antagonistic isolates had chitinase activity, of which 23% and 77% belonged to dicots and monocots fields, respectively. At a different glance, 44% and 26% of antagonistic Actinobacteria were isolated from the dicots and monocots fields, respectively had chitinase activity. The shares of rhizosphere and bulk soils from isolates with the ability to inhibit P. capsici were 39% and 61%, respectively, which were in accordance with their share in cellulase activity (Table 1).

PGPB traits evaluation at in vitro level

Seventeen, 26, and 42 antagonistic isolates had the ability to grow on N-free medium, solubilize tricalcium phosphate, and produce siderophore, respectively. All 106 isolates were able to produce IAA-like molecules (Table 1). Only three Actinobacteria, one of them isolated from cucumber bulk soil (isolate 3415) and the others from maize rhizosphere soil (strains 1119 and 1331), had all tested PGP traits (Table 2). Compared to bulk soils, most of the PGP Actinomycete were resident in the rhizosphere. The numbers of the isolates had one of the PGP traits were more in the monocots fields, but the percentages of antagonistic isolates that had PGP activity were higher in the dicots fields (Table 1). Thirteen isolates with both antagonistic and PGP traits were selected for further studies (Table 2).

Genotypic characterization of potential PGPB strains and phylogenetic tree of strain 1119

It is apparent from Table 2 that Actinobacteria, which were isolated from dicots and monocot soils, were associated with two genera Streptomyces and Amycolatopsis. The diversity of genus and species of Actinomycete were higher in dicots in comparison with monocots. While all isolates from wheat fields belong to S. monomycini and most of the strains of maize soils belong to different species of Amycolatopsis (some of the results are shown in Table 2). The initial analysis of the 16S rRNA gene sequence showed that strain 1119 is associated with species of the genus Amycolatopsis with 99.22% and 99.15% sequence similarity to A. thailandensis JCM 16380T (NMQT01000085) and A. umgeniensis UM 16T (DQ110876), respectively. Also, the constructed phylogenetic tree from the 16S rRNA gene illustrates that isolate 1119 is a member of the Amycolatopsis genus and is placed in a clade with these two type strains (Fig. 4).

Greenhouse experiments

Experiment 1: evaluation of potential PGPB strains for cucumber growth-promoting activity

There were significant differences between seed germination in the untreated control group and Actinomycete treatments (Table 3). Compared to the control group, seed germination in treatment with strain 1118, 1331, 3415, 407, and 405 was 35%, 60%, 80%, 80%, and 100% lower, respectively. Also, the growth of germinated seeds in the soil treated with these bacteria was less. Strains 3637 and 1317, which did not have a negative effect on seed germination, also reduced seedling growth. On the contrary, the plants grown in soil treated with strains 3513, 1119, 432 or 615, increased shoot length (Fig. 5) and shoot dry weight compared to the control. The increase in dry weight of shoot was in the range of 9–25% (Table 3).

Experiment 2: biocontrol activity of potential PGPB strains

Four strains 615, 1119, 3513 and 432 that caused a significant increase in shoot dry weight were selected for biocontrol activity evaluation. Thirteen days after seedlings inoculation with P. capsici, the damping-off symptom was observed. The highest incidence of the disease was in the untreated control and was about 80%. Strains 432, 3513, 615 and 1119 controlled incidence of the disease by 65%, 42%, 83% and 100% respectively (Fig. 6). The PGP activity of strains 3513 and 1119 was observed for the second time in this experiment as an increase in shoot and root dry weight (Fig. 7).
Table 2  Antagonistic activity against plant phytopathogens, hydrolytic enzyme activity, PGP properties and genotypic characterization of 13 antagonistic isolates selected for greenhouse experiments

| Isolate | Host crop | Antagonistic activity (%) | Hydrolytic enzymes (halo zone diameter/colony diameter) | PGP activity | Closest type strain | Similarity | Accession No |
|---------|-----------|---------------------------|--------------------------------------------------------|--------------|-------------------|------------|--------------|
|         |           |                           | P. capsici | P. drechsléri | P. ultimum | R. solani | F. oxysporum | cellulases | chitinases | siderophore production | Growth on N free medium | Tricalcium phosphate-solubilization |
| 407     | Tomato    | 100 | 33 | − | − | − | 3 | 1.8 | + | + | − | A. lurida DSM 43 134 T (AJ577997) | 98.15 | MG9995004 |
| 405     | Tomato    | 20 | 63 | − | − | − | 4.7 | 1.1 | + | + | − | A. lurida DSM 43 134 T (AJ577997) | 98.08 | MG9995003 |
| 432     | Tomato    | 20 | 63 | − | − | − | 4.7 | − | − | − | − | − | S. bellus ISP 5185 T (AJ599476) | 99.01 | MN888928 |
| 515     | Tomato    | 100 | 50 | 60 | − | − | 2.9 | 1.2 | − | − | − | S. fulvovirideus NBRC 15897 T (AB184711) | 97.75 | MN888932 |
| 3637    | Cucumber  | 100 | 55 | − | − | − | 2.5 | − | − | − | + | S. cyaneus NRRL B-2296 T (AF346475) | 98.39 | MN888935 |
| 3415    | Cucumber  | 20 | 60 | 50 | − | − | 1.3 | − | + | + | + | A. roodepoortensis M29 T (KF771262) | 98.65 | MG984768 |
| 3513    | Cucumber  | 95 | 53 | − | − | − | 4.3 | − | − | − | + | A. roodepoortensis M29 T (KF771262) | 98.16 | MH266469 |
| 1119    | Maize     | 30 | 50 | 85 | − | − | − | − | + | + | + | A. thailandensis JCM 16380 T (NMQT01000085) | 99.22 | MG984616 |
| 1118    | Maize     | 30 | 48 | 86 | − | − | − | − | + | + | + | A. umgeniensis UM16 T (DQ110876) | 98.00 | MG9995001 |
| 1331    | Maize     | 40 | 41 | 43 | 60 | − | − | − | + | + | + | A. lurida DSM 43 134 T (AJ577997) | 98.08 | MG9995005 |
| 1317    | Maize     | 30 | 30 | − | 60 | − | 2 | − | + | + | + | S. monomycini NRRL B-24309 T (JNYL01000005) | 99.51 | MN493628 |
| 614     | Wheat     | 95 | 60 | 100 | 67 | 84 | − | 1.3 | + | + | − | S. monomycini NRRL B-24309 T (JNYL01000005) | 99.86 | MN888939 |
| 615     | Wheat     | 100 | 54 | 100 | 69 | 63 | 1.1 | − | + | + | − | S. monomycini NRRL B-24309 T (JNYL01000005) | 99.65 | MN888940 |

“+” producing or growth, “−” non-producing or no growth
Experiment 3: performance evaluation of strain 1119 at the commercial greenhouse conditions

In the control group, fruit yield was 3.9 ton/1000 m² in a period of one month. Application of strain 1119 significantly increased fruit yield by 20% (4.7 ton/1000 m²) compared to untreated control (Table 4). Sensory evaluation showed that cucumber fruit did not differ significantly from control in the bacterial treatment. However, the fruit score in the bacterial group in terms of juiciness and sweetness was 4% higher and 3% lower than the control, respectively. These results were consistent with the results of the total soluble sugar content and percentage of the dry weight of the fruit. As is shown in Table 4, soil treatment with strain 1119 significantly reduced fruit dry weight percentage (31%) and total soluble sugar content (14%) compared to control. Decreased fruit nitrate content was another effect of bacteria on fruit quality. The content of cucumber nitrate in the bacterial treatment was 9.58 mg/100 g fresh fruit weight less than one-third of the amount of nitrate in the control group (Table 4).

Bacterial treatment increased the level of antioxidant enzymes without significantly altering the amount of leaf protein. The highest increases were in CAT (150%), followed by APX (54%) and POX (25%). Increased activity of these enzymes was associated with a reduction in H₂O₂ level by 50% compared with control. Following the reduction of destructive agent H₂O₂ in the bacterial treatment, the level of MDA, which indicates cell destruction, was 37% lower than the control (Table 4). Here, the results of the gene expression study using RT-PCR showed that the relative frequency of LOX transcription in bacterial treatment was significantly higher than the control. The relative increase in APX transcription and the subsequent increase in APX activity also showed that strain 1119 stimulated plant-induced resistance at the transcription level without pathogen exposure. Systemic acquired resistance (SAR) is associated with the expression of SAR genes that lead to the accumulation of defense proteins. PRI-1a and GLU are two SAR genes that were induced by strain 1119 (Fig. 8).

Discussion

Many studies have shown the rhizosphere effect on bacterial communities. These reports state that the rhizosphere effect is different between plant species due to plant growth stage and root physiology, structure, and exudation (Edwards et al. 2015; Liu et al. 2019; Samad et al. 2017; Sasse et al. 2018). Turner et al. (2013) studied the soil microbiome and revealed a higher rhizosphere effect in pea (dicot) compared to oat and wheat (monocots). As we expected, the number of culturable bacteria in the rhizosphere of monocot crops was not higher than in the bulk soil. Although the cause is not exactly known, three points can be made. First, the roots of wheat and maize do not secrete substances that are attractive or usable to bacteria. Various traits give a bacterium the ability to colonize the rhizosphere. It is illustrated that cheA gene is essential for chemotaxis towards root exudates in bacteria and Pseudomonas fluorescens (Muriel et al. 2015), and Bacillus subtilis (Allard-Massicotte et al. 2016), and cheA mutants are not able to colonize the roots of alfalfa and Arabidopsis thaliana, respectively. de Weert et al. (2002) experiments showed that some organic acids and almost all amino acids have found in tomato root exudate are chemo-attractants for P. fluorescens. However, this chemotactic response was not detected toward sugars in the exudate. Studies on root exudates of dicots plants, including tomato, cucumber, and sweet pepper have shown that the total amount of organic acid was much higher than that of total sugars (Kamilova et al. 2006), while in grasses (monocot plants), these two groups of compounds have an equal share (Kuiper et al. 2002). Second, wheat and barley roots produce metabolites that induce the antagonistic activities of some bacteria that are resident in the rhizosphere. P. fluorescens strains produce the secondary metabolite 2,4-diacetylphloroglucinol (2,4-DAPG), with antibiotic properties. Notz et al. (2001) showed that expression of phlA (a gene that is involved in DAPG biosynthesis) was increased four-fold in the rhizosphere of maize and wheat (two monocots) compared to the rhizosphere of bean and cucumber (two dicots). Third, studied monocots may have attracted endophytic bacteria and have them inside the root tissues. According to studies by Mercado-Blanco et al. (2016), P. fluorescens PICF7 selectively
develops an endophytic lifestyle in two monocots (wheat and barley) roots but not in a dicots plant (*A. thaliana*). The presence of Actinobacteria in the rhizosphere of both monocot and dicot plants has been reported (reviewed in Kumar and Dubey 2020). The ability of Actinomycete strains to colonize the rhizosphere of both monocots and dicots plants has also been demonstrated (Gopalakrishnan et al. 2013). Based on our results, the chance of finding an Actinomycete in the cucumber rhizosphere is higher than in other areas we have studied. Although, to the best of our knowledge, no study has been conducted on comparing the abundance of Actinobacteria in the soil of monocots and dicots fields.

Potential antagonistic Actinomycete strains have been isolated from rhizosphere soil of vegetable crops, especially cucumber and tomato (Chaurasia et al. 2018). However, it is not clear whether the Actinomycete strains isolated from the rhizosphere of cucumber and tomato are *P. capsici* antagonists. Because *P. capsici* often causes disease in pepper, most of the Actinobacteria that inhibit the growth of this pathogen have been isolated from the rhizosphere of kinds of pepper (Balaraju et al. 2016; Joo 2005).

Cellulose is one of the most important components of the cell wall of the phytopathogen *Phytophthora* and is sensitive to the enzyme cellulase. The production of cellulase by antagonistic bacteria in the vicinity of plant roots is one of the ways to prevent the spread of phytopathogenic fungi and disease. Loliam et al. (2012) and Sadeghi et al. (2017) reported antagonistic activity of cellulase-producing Actinobacteria against *Phytophthora*. The correlation between the two traits of cellulose production and inhibition of *Phytophthora* growth shows that searching among cellulase-producing bacteria shortens the path of selection of *Phytophthora* antagonist bacteria. Interestingly, such an association is not observed between chitinase production and inhibition of the growth of...
fungi such as *Rhizoctonia* and *Fusarium* that have chitin in their cell walls.

Abbasi et al. (2019) reported that growth ability on a nitrogen-free medium and production of auxin are common traits of *Streptomyces* isolates isolated from the rhizosphere of tomato and cucumber, and only 30% of the isolates have the potential to solubilize tricalcium phosphate. Although bacteria that stimulate plant growth often have several PGP traits, it is possible that bacteria with these traits may not necessarily stimulate plant growth or have different effects on different plants. For example, *Streptomyces rimosus* strain C-2012, which has PGP traits auxin and siderophore production and tricalcium phosphate solubilization (Sadeghi et al. 2012), increased the growth of cucumber (Sadeghi et al. 2017) and peppermint (Esmaeil Zade et al. 2019), although its positive effect on wheat was cultivar-dependent (Akbari et al. 2020). Unfortunately, despite the many studies that have been done, there is still no trait that its presence in Actinobacteria assures us of its positive effect on the growth and yield of all plants.

*Amycolatopsis* are isolated from the rhizosphere of various plants such as rice (Thawai et al. 2018), canola (Lay et al. 2018), birch (Ostash et al. 2014), *Panax notoginseng* (Peng et al. 2019), and eucalyptus (Himaman et al. 2016).

Strain 1119, which belongs to the genus *Amycolatopsis*, raised dry shoot weight up to 17%. Strain 3415 antagonizes three plant pathogens *P. capsici*,

| Isolate | Germination | Plant height | Shoot dry weight |
|---------|-------------|--------------|-----------------|
| 3513    | 0*          | 19           | 17              |
| 1119    | 0           | 28           | 25              |
| 1118    | −35         | −29          | −28             |
| 3415    | −80         | −20          | −15             |
| 1331    | −60         | −51          | −34             |
| 407     | −80         | −66          | −65             |
| 405     | −100        | −75          | −75             |
| 3637    | 0           | −6           | −10             |
| 1317    | 0           | −1           | −8              |
| 432     | 0           | 12           | 9               |
| 614     | 0           | 9            | 0               |
| 515     | 0           | 0            | 0               |
| 615     | 0           | 15           | 20              |

*Positive numbers indicate a positive effect and negative numbers indicate a negative effect of bacteria on the studied parameters. Zero (0) indicates that there is no significant difference (p ≥ 0.05) with control.*
**P. drechsleri**, and **P. ultimum**, and was positive for all four studied PGP traits suppressed seed germination and plant growth. In contrast, strain 1119, which was similar to strain 3415 in terms of studied traits, increased plant growth. As Huang et al. (2017) showed, increased plant dry weight can be an acceptable feature for selecting PGPBs. Strain 1119 and 3415 have the most similarity to **A. thailandensis** (99.22%) and **A. roodepoortensis** (98.65%), respectively. More interestingly, strains 614 and 615, both are very similar (≥ 99.65%) to **S. monomycini** and are quite similar in terms of PGP traits and phytopathogens antagonism, had different effects on the cucumber growth. The same is true for two strains of **A. roodepoortensis**, 3415 and 3513. The effect of strains 407, 405, and 1331, all three of which are most similar to **A. lurida**, on germination and growth of cucumber was negative. Based on these results, the observed negative effect is related to this species and is not related to the ability to dissolve tricalcium phosphate (strain 1331) or lack this ability (407 and 405). Despite all of these, the positive effect of strain 1119

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**Fig. 6** Biocontrol of *P. capsici* damping-off of cucumber seedlings by potential PGPB strains (432, 3513, 615 and 1119). Fourteen days after planting the seeds in treated soil with bacteria (10⁶ cfu/seeding) or untreated soil (control), artificially inoculation with *P. capsici* was performed. Final disease incidence was determined 16 days after inoculation based on total diseased seedlings (with a thin and brown stem or completely death) percentage (%). Data represent the mean values ± SE of 4 replicates. Same letters represent non-significant difference according to Duncan’s test (*p*<0.05)

**Fig. 7** The effect of potential PGPB strains (432, 3513, 615 and 1119) on the growth parameters of cucumber seedlings 30 days after planting under greenhouse conditions (27 °C and 16 h/8 h brightness/darkness). Germinated seeds were planted into trays filled with a sterile mixture of field soil and peat moss (1:2) and one-gram sand containing 10⁶ cfu of bacteria. Sterilized sand was used as control (experiment 2). a Root dry weight; b shoot dry weight. Data represent the mean values ± SE of 4 replicates. Same letters represent non-significant difference according to Duncan’s test (*p*<0.05)
on cucumber growth, which is positive for the ability to grow on N free medium, siderophore production and, tricalcium phosphate solubilization, compared to the lack of effect of strain 432, which is negative for these traits, tempt us to use PGP traits for screening and selecting effective PGPs in subsequent applications too. Although, phosphate-solubilizing bacteria (PSBs) that are commonly selected using tricalcium phosphate, may not be able to dissolve phosphate and stimulate plant growth via improvement of plant phosphate nutrition. In this regard, Bashan et al. (2013) have suggested that metal-P compounds such as Fe-P, Al-P and, phytic acid as well as tricalcium phosphate must be used to identify true phosphate-solubilizing bacteria. Recently, Joe et al. (2018) used a new solid and liquid media named soil extract calcium phosphate (SECP) can be amended with calcium phosphate, rock phosphate, and aluminum phosphate for the isolation and screening of a larger population of phosphate-solubilizing bacteria.

It seems that a closer look at the molecular mechanisms involved in the plant-bacteria interaction to find selectable markers more effective than PGP traits is necessary. Alekhya and Gopalakrishnan (2016) have shown for the first time that one strain of *Amycolatopsis* sp. BCA-696, isolated from the rhizosphere soil of chickpea, is able to promote chickpea and sorghum growth. PGP activity of this strain is related to the production of IAA and siderophore and its good hydrolytic enzyme activity. *Amycolatopsis* sp. strain CRJ2-11 with high similarity (99%) to *A. keratiniphila* was able to produce IAA and siderophore as well as to solubilize inorganic phosphate (Ningthoujam et al. 2016). In contrast, there are strains of this bacterium such as *A. pretoriensis* strain SA3 that do not have any of the PGP traits (Borah and Thakur 2020). Apart from the study on chickpeas and sorghum (Alekhya and Gopalakrishnan 2016), the effect of *Amycolatopsis* species on plants is still unknown. To our knowledge, this is the first report of PGP traits of strains similar to *A. thailandensis* and *A. roodepoortensis* and their positive effects on cucumber growth.

Strain 1119, which thoroughly controlled the disease of seedling damping-off and increased shoot and root dry weight in two separate experiments 1 and 2, was selected for evaluation in commercial greenhouse conditions. Because Actinobacteria have always been considered sources of antibiotic production.

| Treatment  | Fruit Yield (ton/1000 m²) | Leaf DW (%) | TSS g/100 g FW | Proline NC mg/100 g FW | Overall Acceptance  | Protein mg/g FW | CAT U/mg protein | POX U/mg protein | APX U/mg protein | MDA µM/g FW | H₂O₂ µM/g FW |
|------------|---------------------------|-------------|----------------|------------------------|---------------------|----------------|----------------|----------------|----------------|-------------|---------------|
| Control    | 3.9                       | 6.72        | 1.76           | 30.48                  | NS                  | 2.26           | 1.29           | 3.86           | 1.57           | 5.00        | 2.24          |
| Strain 1119| 4.7*                     | 4.6*        | 1.51           | 9.58*                  | NS                  | 2.24           | 3.28*          | 4.94*          | 2.42*          | 2.24*       | 0.65*         |
most reports of these bacteria are about their antagonistic and biocontrol properties. The effect of Actinobacteria on increasing growth and controlling cucumber *Pythium* (El-Tarabily et al. 2009), *Fusarium* (Li et al. 2014), and *Phytophthora* (Sadeghi et al. 2017) diseases have been previously reported. The bacteria reported in most similar studies belong to the largest genus of Actinomycete, *Streptomyces*. The valuable potential of the rare Actinobacteria to increase crops yield and control their diseases is not well known. There are few but promising reports about these rare bacteria. Successful biocontrol of sorghum charcoal rot disease caused by *Macrophomina phaseolina* has been reported by a PGP strain of *Amycolatopsis* (Gopalakrishnan et al. 2019). Complete control of cucumber seedling fluctuations by 1119 pressure observed in this study could be another promising report.

Application of strain 1119 under commercial greenhouse conditions resulted in an increase in fruit yield (20%) and juice and a decrease in fruit nitrate content (70%). Nitrate in the human digestive system reduces to nitrite, a harmful product. Therefore, there are restrictions on the consumption of foods containing nitrate (Santamaria 2006). Cucumber juice is rich in a variety of vitamins, fiber, minerals, and antioxidants beneficial to healthy and slimming diets (Henning et al. 2017). Bacterial treatment by reducing the nitrate content can reduce the limitations of its use.

There are many articles on the positive effect of antagonistic PGPB on plant growth and yield and control of their diseases in conditions of research greenhouse. The results of these studies pave the way to produce commercially available formulations (Tokala et al. 2002; Yuan and Crawford 1995). Although the number of articles describing the effect of selected bacteria in commercial greenhouse and farm conditions is much smaller, fortunately, the products on the market show that their impact is positive and acceptable. Actinovate and Mycostop are two Actinomycete-based formulations commercially available as biofungicide that introduced to control soil-borne fungal diseases caused by *Fusarium, Rhizoctonia, Pythium*, and *Phytophthora* (Vurukonda et al. 2018; www.novozymes.com). However, there are articles on other crops we did not find a report that shows the positive effect of these products on cucumber yield and quality and control of fungal diseases under commercial field conditions. On the other hand, El-Tarabily et al. (2010) reported an increase in cucumber yield and a decrease in *Pythium* disease (seedling damping-off

![Fig. 8](expression_ratio.png)

**Fig. 8** Expression ratio of four defense-related genes including *APX*, *GLU*, *PR* and *LOX* versus internal control (elongation factor, *EF*) in leaves of cucumber (*C. sativus* L. Soltan cultivar) 15 days after treatment with *Amycolatopsis* sp. strain 1119. Plants were grown in commercial greenhouse conditions. Expression ratios were calculated using the relative expression software tool REST 2009 (Pfaffl et al. 2002). Error bars show the standard deviation of the mean values of four biological replicates. Each replicate was a 20-m-long row containing 30 plants. The leaves of four plants from each replicate pooled and considered as one sample. The values marked with one (*p < 0.05*) or two (*p < 0.01*) asterisks represent genes significantly overexpressed relative to control (untreated plants grown in the same conditions).
and root and crown rots) after the use of *Streptomyces spiralis* in commercial farm conditions. Such studies together with the present study demonstrated the effectiveness of biocontrol agents for use in cucumber commercial greenhouses. Though it is not possible to artificially infect plant with pathogens in a commercial greenhouse, there are good markers such as known enzymes and genes involved in stimulating the plant defense system that indicate the plant is ready to deal with possible pathogens. Here, bacterial treatment increased the level of antioxidant enzymes and decreased the content of H$_2$O$_2$ and MDA without significantly altering the amount of leaf protein.

The ability of growth-promoting *Trichoderma longibrachiatum* T6 to increase wheat growth under normal and saline conditions was associated with increased activity of antioxidant enzymes and decreased the content of H$_2$O$_2$ and MDA (Zhang et al. 2019). Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are two forms of plant-defense induction resistance that reduce disease damages if started before the plant is exposed to pathogens (Choudhary et al. 2007). It is reported that the induction of ISR by PGPBs is associated with an increase in LOX activity (Akram et al. 2008). Increased antioxidant enzymes activity and increased LOX and APX transcription showed that strain 1119 induced plant ISR without pathogen exposure. We have already shown that biocontrol *Streptomyces* strains have different functions in ISR induction. *S. rochei* strain Y28 induced LOX expression in non-pathogen inoculated tomato, but *S. enissocaesilis* strain IC10, a strain that phylogenetically is close to that, decreased the expression of the gene. Interestingly, these two strains significantly increased LOX expression in the plant after inoculation with the pathogen (Abbasi et al. 2019). ISR is regulated by jasmonic acid (JA) and ethylene. Lipoxigenase (LOX), encoded by the LOX gene, is the first enzyme in the biosynthesis pathway of JA, so LOX appears to play a pivotal role in activating the plant’s defense responses. Induced expression of LOX in cucumber by non-pathogenic biocontrol *F. oxysporum* (Pu et al. 2014), *Trichoderma asperellum* (Shoresh et al. 2005), and *Pseudomonas putida* (Alipour Kafi et al. 2021) has been reported previously.

SAR is associated with the expression of SAR genes that lead to the accumulation of defense proteins and enzymes (Conrath 2006). *PR1-1a* and *GLU* are two SAR genes that were induced by strain 1119. Zhao et al. (2012) showed that metabolites of *Streptomyces bikiniensis* HD-087 can effectively suppress *F. oxysporum* and trigger induced resistance in cucumber. Plants treated with the fermentation broth of the strain highly increased b-1,3-glucanase activity in cucumber leaves. Their results also showed that the activity of this enzyme, unlike peroxidase, increased regularly during the experiment (from the first to the sixth day after treatment). Considering the increased expression of the *GLU* gene after treatment with strain 1119 in commercial greenhouse conditions (presence of many influential factors), it may be concluded that the activity of this enzyme and the expression of its encoding gene are good indicators for the induction of SAR in cucumber.

Our result suggests that this PGP bacterium could induce both ISR and SAR in cucumber. Stimulation of both types of systemic resistance by *Streptomyces* in tomato has been reported by Abbasi et al. (2019).

**Conclusion**

The results showed that in the study area (Nazarabad, Alborz province), the culturable Actinobacteria population in the rhizosphere of dicots is higher than monocots. However, the number of antagonist Actinobacteria in the rhizosphere of dicots is less and more antagonist Actinomycete bacteria were obtained from bulk soil of monocots fields. These results are important because the studied pathogens are related to dicotyledonous plants. Therefore, when our resources are limited, it may be best to screen the bulk soil of monocot plants first. Although the diversity of Actinobacteria in these regions was less than that of the rhizosphere of dicots, the antagonistic activity of the strains was higher. Another noteworthy point that can be mentioned is the lack of a direct relationship between the plant growth-promoting effect of an antagonistic strain and its PGP traits. Therefore, instead of studying these traits in the screening phase, it might be better to do it in the mechanism finding phase after observing the positive effect of bacteria on plant growth. Here, for the first time, we showed the different effects of four species of *Amycolatopsis* on cucumber. Our results demonstrated
that the Amycolatopsis strain 1119, has a great potential to be used as an active principle for bio-inoculant development because it can improve cucumber fruit yield and induce plant defense responses in a commercial greenhouse. It seems like there are still many beneficial bacteria in the soil that have not been identified. Overall, this study supports the theory that the artificial introduction of PGPBs into the soil can stimulate the plant immune system, control disease, and increase its growth.

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Declarations

Conflict of interest Authors declare that there exists no conflict of interest among them.

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