Streptomyces Strains Induce Resistance to *Fusarium oxysporum* f. sp. *lycopersici* Race 3 in Tomato Through Different Molecular Mechanisms

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Plant growth promoting rhizobacteria (PGPR) are potential natural alternatives to chemical fungicides in greenhouse production via inducing plant immune system against biotic stresses. In this research, 126 *Streptomyces* isolates were recovered from rhizosphere soils of 13 different commercial vegetable greenhouses in Iran. *Streptomyces* isolates were screened for *in vitro* Plant growth promoting (PGP) traits and ability to antagonize *Fusarium oxysporum* f. sp. *lycopersici* race 3 (*FOL*), the causal agent of Fusarium wilt of tomato (FWT). Six isolates with the highest antagonistic activity and at least three PGP traits were selected and compared with chemical fungicide Carbendazim® in a greenhouse experiment. All bacterial treatments mitigated FWT disease symptoms like chlorosis, stunting and wilting at the same level or better than Carbendazim®. Strains IC10 and Y28 increased shoot length and shoot fresh and dry weight compared to not inoculated control plants. Phenotypic characterization and 16S rRNA gene sequencing showed, strains IC10 and Y28 were closely related to *S. enissocaesilis* and *S. rochei*, respectively. The ability of the superior biocontrol strains to induce antioxidant enzymes activity and systemic resistance (ISR) was investigated. Increased activity of catalase (CAT) in plant treated with both strains as well as an increase in peroxidase (POX) activity in plants treated with Y28 pointed to a strain specific-induced systemic resistance (ss-ISR) in tomato against *FOL*. The differential induced expression of *WRKY70* and *ERF1* (two transcription factors involved in plant defense) and *LOX* and *TPX* by the analyzed *Streptomyces* strains, especially after inoculation with *FOL*, suggests that ss-ISR is triggered at the molecular level.

**Keywords:** Fusarium wilt, induced systemic resistance, Streptomyces, tomato growth promoting, siderophore production

**INTRODUCTION**

In recent decades, overuse of chemical fertilizers and fungicides to proliferate and protect greenhouse vegetables has been a threat to human safety. Using biological agents is the best alternative for chemical treatments (Pilkington et al., 2010). Soil-borne fungus *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder and Hansen (FOL) causing Fusarium wilt of tomato
reduces yield in greenhouses (Fravel et al., 2003; McGovern, 2015). Invading through the vascular tissue and soil-borne feature of the pathogen make it difficult to control this disease. Besides, new races of the mentioned pathogen (e.g., race 3) have emerged that can overcome host resistance (Reis et al., 2005). The symptoms of Fusarium wilt disease caused by race 3 are yel lowing the lower leaves, vascular necrosis, epinasty, defoliation, plant stunting and ultimately plant death (Sanchez-Pena et al., 2010). Biological control of this pathogen has been the subject of many studies (Ben Abdallah et al., 2016). Plant growth promoting rhizobacteria (PGPR) colonize rhizosphere or plant root and improve plant health and growth. Some of the most important plant growth promoting (PGP) activity include diazotrophic nitrogen fixation, siderophore production, solubilization of mineral phosphates and production of hormones such as indole-3-acetic acid (IAA) (Sadeghi et al., 2012; Dahal et al., 2017; Gouda et al., 2018). Plant defense strategies including physical barriers, numerous secondary metabolites and antimicrobial agents, which the effective, aggressive pathogens have to overcome them (reviewed by Bruce and Pickett, 2007). Some plant defense strategies are constitutive while others are inducible and only launch in response to a stimulating pathogen and/or beneficial microbes (Piererse et al., 2012). Plant defense strategies keep damage of specific pests below an economic threshold; however, maintain the beneficial organisms involved in integrated pest management (IPM) (Chellemi et al., 2016). IPM considers available pest control techniques that prevent the development of pest populations and keep pesticides to levels that are economically reasonable and reduces risks to human health and the environment. Developing sustainable biocontrol measures for managing Fusarium wilt disease requires a comprehensive understanding of the molecular basis of plant–pathogen–biocontrol interactions. Hormones, PR proteins, terpenoid synthases, polyketide terpene synthases, peroxidases, lignin synthases, transcription factors, calcium signal transducers, and UDP-glucosyltransferases and ubiquitin-protein ligases are components of the plant defense-related genes (Wang et al., 2015). Generally, the plant responses to microbes are regulated through signaling pathways including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Jasmonic acid pathway is required for defense against necrotrophic pathogens and chewing insects, while SA pathway is involved in a wide range of plant defense responses, which ends to systemic acquired resistance (SAR) and occurs following the exposure to many biotrophic and some necrotrophic pathogens. Induced systemic resistance (ISR) is also an activated resistance process elicited by contacting with non-pathogenic microorganisms. This procedure is independent of SA and is synchronized by JA and ET (Walters and Heil, 2007). Activated induced resistance (via ISR or SAR) is a broad-spectrum and long-term resistance, which usually suppresses a disease up to 20–85% (Walters et al., 2013). Thus, inducing plants by direct interaction with rhizobacteria prior to pathogen infection, so-called priming, decreases disease severity (Beckers and Conrath, 2007). Streptomyces species, are Gram-positive filamentous bacteria, reported as PGP and biocontrol agent of Alternaria alternata (Verma et al., 2011), Rhizoctonia solani (Goudjal et al., 2014), F. oxysporum (Kim et al., 2011), Phytophthora drechsleri (Sadeghi et al., 2017), and Verticillium dahliae (Cao et al., 2016). Little is known about bio-suppression of tomato wilt by Streptomyces in greenhouse conditions.

The aims of this study were to (1) isolate and characterize Streptomyces from vegetable greenhouse soils (2) detect PGP characteristics and antagonistic activity of isolates against FOL race 3 (3) evaluate Fusarium wilt biocontrol in tomato by superior isolates in greenhouse condition (4) establish induced systemic resistance (ISR) of tomato through inducing antioxidant enzymes and defense-related genes by inoculation of plants with biocontrol Streptomyces.

### MATERIALS AND METHODS

#### Microorganisms

Rhizosphere soil samples (500 g soil with pH = 6.2–7 and EC < 2.5 dS/m) were collected from 18 cucumber and tomato commercial greenhouses in Yazd, Isfahan and Kerman provinces of Iran in 2016. There were no symptoms of wilt and damping off diseases developing in these greenhouses. The samples were placed in plastic bags, taken to the laboratory and then air-dried for 7 days. For isolation of Streptomyces, 2 g rhizosphere soil was suspended in 100 mL of sterile saline solution (0.9% NaCl) and shaken for 30 min. Two dilutions (1:100 and 1:1000) were prepared using sterile saline solutions in a final volume of 1 mL. An aliquot of 0.1 mL of each dilution was spread on 1.8% water agar supplemented with 300 mM NaCl. The plates were incubated at 29°C for 7 days. Representative colonies were selected and streaked on plates of “International Streptomyces Project media 2” (ISP2) medium (Kampfer et al., 1991) 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. The plates were incubated at 29°C for 7 days. Then morphologically (color, size, and shape) distinct colonies were stored in 30% glycerol solution at −70°C (Table 1).

The fungal pathogen was isolated from tomato plants displaying disease symptoms and pathogenicity test was conducted using root-dipping inoculation method on tomato seedlings. The fungus was re-isolated from the vascular tissue of a symptomatic plant. Fungal DNA extraction was carried out by the methods described by Zhang et al. (2010), Salehi et al. (2018, 2019). Polymerase chain reaction (PCR) was performed with universal pair primers (ITS4-ITS5). Race determination of fungus was done using four specific primer pairs designed by Hirano and Arie (2006) based on the sequence of the exo-polygalacturonase gene (Table 2). ITS4-ITS5 nucleotide sequence obtained by DNA amplification of fungal pathogen was deposited in NCBI GenBank with accession number MG670445. Blast analysis showed that this pathogen had 99 % similarity to F. oxysporum. The result of the race determination using four specific primer pairs showed that the fungal isolate belongs to F. oxysporum f.sp. lycopersici race 3 (Supplementary Figure S1).

#### Growth on Nitrogen-Free Medium

Screening of the free-living (non-symbiotic) diazotroph isolates was carried out according to the procedure described by Xu and Zheng (1986). Each colony was spot-inoculated on nitrogen-free
isolates were spot-inoculated onto the center of Chrome Azurol agar (CAS) medium. To the ratio of the clear zone diameter to colony diameter solidification of the medium, bacterial isolates were spot-inoculated onto the center of the plated plates for 48 h. Then, one fungal isolate was inag medium incubated at 29°C for 14 days, and then growth or lack of growth was compared to ISP2 medium.

### Inorganic Phosphate Solubilization
Pikovskaya’s medium (PVK) was used to measure calcium phosphate \([Ca_3(PO_4)_2]\)-solubilizing activity. Sterilized PVK medium with pH 7.2 was poured into petri plates. After solidification of the medium, bacterial isolates were spot-inoculated onto the center of the plates and incubated at 29°C for 7 days. Solubilization index was evaluated according to the ratio of the clear zone diameter to colony diameter (Soltani et al., 2010).

### Siderophore Production
Siderophore production was evaluated as described by Alexander and Zuberer (1991) on Chrome Azurol agar (CAS) medium. *Streptomyces* isolates were spot-inoculated onto the center of the plate and incubated at 29°C for 7 days. The formation of orange halo around the colonies were considered as siderophore-producing isolates. After 3 days, the ratio of the halo zone diameter to colony diameter was calculated as siderophore production.

### IAA Production
To determine amounts of IAA produced by each isolate, 1 mL of bacterial culture in ISP2 broth was inoculated in Tryptic Soy Broth supplemented with 150 mg/L L-tryptophan. Approximately 1 mL of culture solution was centrifuged at 12000 rpm for 5 min, and 1mL of the supernatant was mixed with 2 mL of Salkowski’s reagent (150 mL concentrated H₂SO₄, 250 mL distilled water, 7.5 mL 0.5 M FeCl₃·6H₂O) and incubated for 20 min in darkness at room temperature (de Oliveira-Longatti et al., 2014). IAA production was qualitatively assayed as pink color development and quantified by measurement of absorbance at 530 nm using a spectrophotometer infinit M200 (Tecan, Switzerland). The calibration plot was constructed using dilutions of a standard IAA (Fluka, Switzerland) solution and the uninoculated medium with the reagent as a standard curve (0, 5, 10, 20, 50, and 100 μg/mL). The quantity of IAA in the culture was expressed as μg/mL.

### Antagonistic Effect of PGPRs
Bacterial suspension of candidate PGPR isolates (Table 1) (20 μL of the 10⁷ cfu/mL sterile saline solution) was inoculated at 29°C linearly at the two opposite sides (1 cm from the plate edge) of potato dextrose agar (PDA) plates for 48 h. Then, one fungal

| Isolate | Host crop | Location | Growth on N free medium | Inorganic P solubilization | Siderophore production | IAA production | Cellulase | Protease | Chitinase |
|---------|-----------|----------|--------------------------|---------------------------|------------------------|----------------|-----------|----------|----------|
| IC6     | Cucumber  | Isfahan  | 1 | 0.1 ± 0.1 | 0.3 ± 0.1 | 9.2 ± 0.0 | 1.0 ± 0.0 | 0.3 ± 0.0 | 1.0 ± 0.0 |
| IC10    | Cucumber  | Isfahan  | 1 | 0.2 ± 0.0 | 0.4 ± 0.0 | 27.1 ± 0.1 | 0.0 ± 0.0 | 1.0 ± 0.0 | 0.9 ± 0.1 |
| IC13    | Cucumber  | Isfahan  | 1 | 0.3 ± 0.0 | 0.4 ± 0.0 | 32.3 ± 2.6 | 0.0 ± 0.0 | 0.6 ± 0.0 | 0.9 ± 0.1 |
| IS8     | Cucumber  | Isfahan  | 1 | 1.9 ± 0.1 | 0.4 ± 0.0 | 21.9 ± 0.9 | 0.0 ± 0.0 | 0.4 ± 0.0 | 1.0 ± 0.0 |
| SS12    | Cucumber  | Isfahan  | 1 | 0.1 ± 0.0 | 0.3 ± 0.0 | 24.1 ± 1.9 | 1.0 ± 0.0 | 0.5 ± 0.0 | 0.8 ± 0.1 |
| CU122   | Cucumber  | Isfahan  | 1 | 0.2 ± 0.0 | 0.1 ± 0.0 | 20.5 ± 0.7 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| IC15    | Cucumber  | Isfahan  | 1 | 0.3 ± 0.0 | 0.3 ± 0.0 | 9.6 ± 1.2 | 0.0 ± 0.0 | 0.3 ± 0.0 | 0.9 ± 0.0 |
| SS14    | Cucumber  | Isfahan  | 1 | 0.0 ± 0.0 | 0.3 ± 0.0 | 24.5 ± 0.7 | 1.0 ± 0.0 | 0.5 ± 0.0 | 0.0 ± 0.0 |
| IT20    | Tomato    | Isfahan  | 1 | 0.2 ± 0.0 | 0.4 ± 0.0 | 24.0 ± 1.3 | 1.0 ± 0.0 | 0.5 ± 0.0 | 0.0 ± 0.0 |
| IT8     | Tomato    | Isfahan  | 1 | 0.0 ± 0.0 | 0.4 ± 0.0 | 25.8 ± 0.5 | 1.0 ± 0.0 | 0.2 ± 0.0 | 0.0 ± 0.0 |
| IT25    | Tomato    | Isfahan  | 1 | 0.2 ± 0.0 | 0.5 ± 0.0 | 25.8 ± 1.8 | 0.0 ± 0.0 | 0.3 ± 0.0 | 0.8 ± 0.2 |
| Y7      | Cucumber  | Yazd     | 1 | 0.5 ± 0.1 | 0.0 ± 0.0 | 7.7 ± 0.9 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Y18     | Cucumber  | Yazd     | 1 | 0.1 ± 0.0 | 0.0 ± 0.0 | 12.0 ± 0.6 | 1.0 ± 0.0 | 0.9 ± 0.1 | 0.8 ± 0.2 |
| Y27     | Tomato    | Yazd     | 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 30.8 ± 1.1 | 1.3 ± 0.4 | 0.3 ± 0.0 | 0.0 ± 0.0 |
| TO612   | Tomato    | Yazd     | 1 | 0.0 ± 0.0 | 0.5 ± 0.0 | 24.7 ± 0.3 | 1.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Y17     | Tomato    | Yazd     | 1 | 0.0 ± 0.0 | 0.4 ± 0.0 | 13.7 ± 2.8 | 1.0 ± 0.0 | 0.4 ± 0.0 | 0.6 ± 0.3 |
| Y28     | Tomato    | Yazd     | 1 | 0.4 ± 0.0 | 0.3 ± 0.0 | 16.8 ± 2.1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Y281    | Tomato    | Yazd     | 1 | 0.3 ± 0.0 | 0.3 ± 0.0 | 10.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Y16     | Tomato    | Yazd     | 1 | 0.0 ± 0.0 | 0.4 ± 0.1 | 11.4 ± 0.5 | 1.4 ± 0.4 | 0.4 ± 0.0 | 0.0 ± 0.0 |
| Y33     | Tomato    | Yazd     | 1 | 0.2 ± 0.0 | 0.0 ± 0.0 | 9.3 ± 0.2 | 1.0 ± 0.0 | 0.3 ± 0.0 | 0.8 ± 0.2 |
| Y34     | Tomato    | Yazd     | 1 | 0.3 ± 0.1 | 0.3 ± 0.0 | 11.8 ± 0.9 | 1.2 ± 0.2 | 0.2 ± 0.0 | 0.7 ± 0.3 |
| KH12    | Cucumber  | Kerman   | 1 | 0.0 ± 0.0 | 0.1 ± 0.0 | 7.5 ± 0.7 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.0 ± 0.0 |
| K40     | Cucumber  | Kerman   | 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 27.5 ± 2.2 | 0.2 ± 0.0 | 0.4 ± 0.0 | 1.0 ± 0.0 |
| K43     | Cucumber  | Kerman   | 1 | 0.0 ± 0.0 | 0.0 ± 0.0 | 22.5 ± 3.5 | 1.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

The means halo zone diameter/ colony diameter of three replications ± standard deviation is represented. 1, growth; 0, no growth. The data indicated for IAA production was in μg/mL.
plug (0.5 cm diameter) was inoculated at the center of PDA plate (Kunova et al., 2016) and then incubated for 4 days. The growth inhibition percentage was calculated using the formula $(a - b)/a \times 100$, where “a” is the fungal growth radius of a control cell and “b” is the distance of the pathogen growth in the direction of bacteria (in cm).

**Chitinase Activity**

Chitinase production was determined according to the method of Hsu and Lockwood (1975). *Streptomyces* isolates were grown on chitin agar containing 0.4% colloidal chitin and 1.5% agar. Plates were incubated for 5 days at 29°C. The ability of chitinase production was shown by a clear halo around the colonies. The ratio of the clear zone diameter to colony diameter was calculated as chitinase activity.

**Cellulase Activity**

Carboxymethyl cellulase (CMCase) activity was determined by Mandels-Reese medium with carboxymethyl cellulose (CMC) as sole carbon source (Majidi et al., 2011). The bacteria were grown on CMC agar containing 0.4 g/L KH$_2$PO$_4$, 0.02 g/L CaCl$_2$, 0.02 g/L NaCl, 0.02 g/L FeSO$_4$ 7H$_2$O, 2.5 g/L CMC, and 15.0 g/L agar. The pH was adjusted to 7.2 with 1 M NaOH. The CMC agar plates were incubated at 29°C for 7 days to allow the secretion of cellulase. To visualize the hydrolysis zone, agar medium was flooded with an aqueous solution of Congo red (1 mg/mL) for 20 min. Congo red solution was then poured off, and the plates were further treated by flooding with 1 M NaCl for 15 min. To indicate cellulase activity, the diameter of the clear zone around each colony was measured. The ratio of the clear zone diameter to colony diameter was calculated as cellulase activity.

**Protease Activity**

Extracellular protease activity of *Streptomyces* isolates was assayed by a modification method of Mehrotra et al. (1999). Each bacterial isolate was streaked on skim milk agar containing 15 g/L skim milk powder, 0.5 g/L yeast extract and 10 g/L agar. After incubation at 29°C for 48 h, the ratio of the clear zone around bacterial colony to colony diameter was measured as protease activity.

**Phenotypic and Molecular Characterization of the Selected Isolates**

The potent PGP and antagonist isolates were further characterized by differential morphological traits on ISP2, ISP3, and ISP4 media (Shirling and Gottlieb, 1966), melanin formation (Suter, 1978), growth in high temperatures (37°C and 42°C), and growth on medium supplemented with 6, 10, and 12% NaCl (Kutzner, 1981).

Molecular characterization of the selected isolates was done using PCR amplification of 16S rRNA gene sequence. DNA was extracted according to the method described by Tripathi and Rawal (1998). PCR amplification was performed using the primers 27F: 5′-AGAGTTTGATCCTGCGTGCAAG-3′ and 1525R: 5′-AAAGAGTGTGATCCAGCACG-3′ as described by Chun and Goodfellow (1995). Almost-complete 16S rRNA gene sequences (1400 nt) were deposited in the GenBank database under the accession numbers of MG722685 (strain IT25), MG786938 (strain TO612), MG786894 (strain Y17), MG786896 (strain Y28), MG654776 (strain IC10), and MG676358 (strain IC13). The sequences were aligned manually with corresponding sequences of available *Streptomyces* species deposited in the GenBank, EMBL and DDBJ databases using BLAST search tool (Altschul et al., 1997). Phylogenetic tree was constructed using the MEGA 6.0 software package (Tamura et al., 2013) based on neighbor-joining method. Bootstrap analysis was used to evaluate the stability of relationships based on 1000 resampling. Strains IC10 and Y28 were preserved and deposited in the Agricultural Biotechnology Research Institute of Iran Culture collection (ABRIICC) under accession numbers of ABRIICC 20108 and ABRIICC 20114, respectively.

**Greenhouse Experiments**

Tomato (*Solanum lycopersicum* L.) cv. Rio Grande susceptible to *FOL* races 2 and 3 (Barker et al., 2005), was used in greenhouse experiments. Seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) and incubated in a growth chamber at 25°C for 7 days. Germinated seedlings were placed into 84-cell plug tray (50 × 30 × 5 cm deep) filled with sterilized soil and peat moss (1:2), with one seedling occupying each cell. Seedlings were watered every day with tap water and kept at 27°C and 16 h brightness/8 h darkness. After 21 days, the seedlings were transferred to pots (15 × 20 cm) filled with a sterile mixture of field soil and peat moss (2:1). For bacterial treatments, *Streptomyces* cell and spore were centrifuged at

| Table 2 | The list of primers used in the q-RT PCR in this study. |
|---------|--------------------------------------------------------|
| Gene Name | Amplicon size (bp) | Sequence |
| UnF | 670 | F-5′-ATACCTTGTGCGCAACTTCA3′ |
| Sp13 | 445 | F-5′-GTCAGTCCATGCTGCTTCT3′ |
| Sp23 | 518 | F-5′-CTCTGTTGCTTTTGCTC3′ |
| Sprl | 947 | F-5′-GATGTTGAGAAGCTGATGCG3′ |
| UDP-G | 122 | F-5′-GATGAAACGCGACCCTTTAG3′ |
| WRKY70 | 131 | F-5′-TGTAAGAGCATAGTGACTCAAC3′ |
| PAL1 | 148 | F-5′-CACTGAGGGAGAAAGAGCAAT3′ |
| LOX E | 104 | F-5′-CTCGGCACTGCTTCTTCT3′ |
| TPX | 133 | F-5′-AGCACATGGAACACAGCTAC3′ |
| PR1 | 136 | F-5′-GTAAGACGAGAGCAGAA3′ |
| ERF1 | 126 | F-5′-GAATGAGGAGGTGAA3′ |
| TUB | 146 | F-5′-CAAGAAGCCTGCTCATTGTG3′ |

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8000 rpm for 15 min and then the pellet re-suspended in 10 mL sterile saline solution. Bacterial suspension was added to autoclaved sand and final cfu/g sand adjusted to 10⁸. Seven gram of sand containing bacteria was added to the surface of each cultivated pot immediately after transferring of plant to the pot. Sterilized sand was used as a negative control. The pots were watered every 2 days. For FOL inoculation, 10 days after bacterial treats, tomato seedlings were uprooted and inoculated with conidial suspension for 30 min (root-dip method). Seedlings submerged in sterile distilled water (mock inoculation) were used for bacterial treatments and negative control. To prepare FOL suspension (pathogen inoculum), fungi was cultivated on PDA for 10 days. Conidia were harvested by scraping in sterile water (10 mL/plate) and final conidia/mL adjusted to 5 × 10⁷ using hemocytometer. The treatments were negative control plants (mock inoculation), positive control (FOL inoculated), Carbendazim® (soil drenched with fungicide in a concentration of 1.5 g/L) and six Streptomyces treatments inoculated or non-inoculated with FOL. The plants were harvested 60 days after transferring to the pots. The growth parameters comprising of shoot length, fresh and dry weight of shoot and root were measured and recorded. Further, disease severity (DS) was assessed on a scale from 0 to 5: 1 = symptoms free = 0%; 2 = slight chlorosis, stunting, or wilting = 25%; 3 = moderate chlorosis, stunting, or wilting = 50%; 4 = severe chlorosis, stunting, or wilting = 75%; 5 = death = 100% (Marlatt et al., 1996). Percentage of control value was calculated using the formula (DC − DT)/DC × 100, where “DC” is disease index of inoculated control (FOL) and “DT” is disease index of inoculated treatment (%) (Song et al., 2004).

A greenhouse experiment was conducted separately for the next two tests. The procedure of this experiment was similar to the first one, but the experiment duration was shorter and the plants were harvested 14 days after transfer to the pots, also 1 cm of the root end of the seedlings was cut before FOL/mock inoculation. Foliar was sprayed with plant hormones (1 mM SA or 10 mM MeJA) 48 h before FOL inoculation. The treatments were negative control plants (mock inoculation), positive control (FOL inoculated), Carbendazim® (soil drenched with fungicide in a concentration of 1.5 g/L) and two Streptomyces treatments (strains IC10 and Y28) inoculated or non-inoculated with FOL. Plants leaves were harvested 2 days after FOL inoculation, frozen in liquid nitrogen and kept at −70°C for the following analysis.

**Antioxidant Enzymes Activity**

Frozen leaf samples (100 mg fresh weight) with 10 mg polyvinyl pyrrolidone (PVP) was transferred to 1.5 mL tube and homogenized in 1 mL Na-Pi buffer (1 mM, pH 7). The homogenate was centrifuged at 15000 × g for 15 min. All operations were performed at 4°C. The supernatant was used as a crude enzyme extract. The activities of antioxidant enzymes including peroxidase (POX: EC 1.11.1.7) and catalase (CAT: EC 1.11.3.6) were measured in a reaction containing 250 µL 0.1 M phosphate buffer (pH 7.0), 250 µL 10 mM guaiacol, 30 µL H₂O₂, and 40 µL crude enzyme extract (Chance and Maehly, 1955). The enzyme activity (U/mL) was measured spectrometrically (Cary 300, United States) by monitoring of the degradation of H₂O₂ by the increase in the absorbance at 470 nm and the decrease in the absorbance at 290 nm over 3 min.

**Quantitative Real-Time PCR Analysis of the Defense-Related Genes**

Total RNA was extracted from shoots using RNEasy plant mini kit (QIAGEN). cDNA was synthesized using 1 µg of each RNA sample after treating with RNase-free DNase I (Invitrogen) using iScript cDNA synthesis kit (BioRad) according to manual description. Quantitative PCR was performed in a 25 µL reaction containing 1 µL of template cDNA, 0.5 µL of 10 pM of each forward and reverse specific primer (Table 2) and iQ SYBR Green Supermix kit (BioRad) on BioRad multicolor real-time PCR detection system. The PCR profile included an initial denaturation step at 95°C for 3 min, followed by 40 cycles of denaturation (95°C/30 s), primer annealing (60°C/30 s) and primer elongation (72°C/30 s), by a final elongation step (72°C/ 5 min) and recording melting curves. Results were expressed as the normalized ratio of mRNA level of target gene to internal control tubulin gene (TUB). Changes was estimated by using the Relative Expression Software Tool (REST 2009) (Pfaffl et al., 2002).

**Statistical Analysis**

Statistical analysis was performed using analysis of variance (ANOVA) by Microsoft Excel (Microsoft Corporation, United States) and SPSS version 16.0 (SPSS Inc. Chicago, IL, United States). All data shown are average value of three (in vitro experiments) biological replicates ± SE. The greenhouse experiments were carried out in randomized blocks design with four blocks and there were four biological replications for each treatment. The significance differences of the treatments were evaluated using multivariate generalized linear model (GLM) with Duncan multiple range test post hoc analysis at level of P < 0.05. The relationship between in vitro and in vivo data was studied using bivariate Pearson test at P ≤ 0.01.

**RESULTS**

**Isolation and Selection of Streptomyces Bacteria**

A total of 126 colonies displaying Streptomyces appearance including compact colored heaped and wrinkled, waxy, chalky, powdery or velvety colony, were isolated from tomato and cucumber rhizosphere soil collected from Isfahan, Yazd and Kerman (Figure 1A). Totally, 106 isolates (84%) were able to grow on solid nitrogen-free medium (Figure 1B) of which thirty-eight isolates were able to solubilize inorganic phosphate. Three isolates comprising of IS8, Y7, and Y28 with the ratio 1.9, 0.5, and 0.4 had the most potential to solubilize tricalcium phosphate, respectively (Figure 1C). Further, sixty-six percent of 106 isolates were able to produce siderophore (Figure 1D). The maximum orange halo due to iron chelation was recorded for isolate TO612 after 7 days of incubation. All 106 isolates produced IAA in a range of 7.0–40.9 µg/mL.
of which twenty percent produced IAA more than 27 µg/mL. Thirty-two isolates from 106 showed proteolytic enzyme activity. The greatest halo zone/colony diameter ratio for protease activity was 1.0 and recorded for isolate IC10 (Table 1). Forty-four isolates were found to produce cellulose. The greatest halo zone/colony diameter ratio for cellulase activity was 1.4 which recorded for Y16. Furthermore, biosynthesis of the chitinolytic enzyme was detectable for 22 isolates. Isolates IC13, K40, SS12, IC6, and Y17 were able to produce all three examined hydrolytic enzymes. Overall, 24 isolates containing at least three PGP traits and hydrolytic enzymes activity were selected for evaluation in the following experiments as shown in Table 1.

**Growth Inhibition of the Selected Isolates Toward FOL**

Six isolates (25% of the selected isolates) showed more than 30% inhibitory effect against FOL in dual culture test. The highest percentages of growth inhibition were 69, 49, 48, 42, 39, and 38, which were recorded for IC10, IT25, Y17, TO612, Y28, and IC13, respectively (Table 3). The biocontrol potential of these isolates (superior PGP antagonistic isolates) was evaluated in a greenhouse experiment.

**Phenotypic and Genotypic Characterization of the Superior Isolates**

Phenotypical characterization was performed using aerial hyphae and spore chains color of 10-days-old bacterial culture on ISP media. On the medium ISP2, isolates TO612 and Y17 were differentiated from IC10, IC13, IT25, and Y28 according to the color of spore chains. On the medium ISP3, isolates Y28, IT25, and IC10 were distinct from the others based on the color of aerial hyphae. Further, on the medium ISP4, isolate IC13 was differentiated from IC10 by its color of aerial hyphae. Also, these two isolates were recognizable on ISP3 according to the color of their aerial hyphae. Isolates IC10 and Y28 were well distinguished from TO612, Y17 and IC13 using ISP media and from IT25 based on melanin production. Isolates IC10 and Y28 slightly were different from each other on ISP2 medium (Supplementary Figure S2). Physiological tests showed only IC13 had potential...
Biocontrol Potential of the Superior Isolates

Biocontrol efficacy of the superior isolates against FOL causing wilt disease was evaluated in the greenhouse and compared to the chemical fungicide Carbendazim®. The high level of disease severity (4.3) was observed in positive control. In Carbendazim®, treatment, disease severity was 2.1. Disease severity in the plants treated with IC10 and TO612 were 1.6 and 1.9, respectively. Strain IC10 increased control value by 12.5% compared to the chemical fungicide (Figure 3). Minimum shoot length, fresh and dry weight were related to positive control and minimum fresh and dry root weight were related to Carbendazim®. All superior isolates significantly increased shoot length, fresh and dry weight of shoot, compared to positive control (Table 6). Strains IC13 increased tomato total dry weight by 30 and 51% compared to control and Carbendazim® respectively. Strains Y28 and IC10 with the highest PGP and biocontrol activity were selected to determine their role in the induction of tomato-systemic resistance through antioxidant enzymes and defense-related genes.

Antioxidant Enzymes Activity

 Peroxidase activity significantly increased 24 h after inoculation of plants with FOL. Further, MeJA individually increased the enzyme activity at the same time interval. Plants inoculated with FOL in SA or Y28 treatment increased POX activity after 24 h like positive control (FOL). In SA treatment, POX activity increased 48 h after exogenous application. The level of POX activity remained constant in plant treated with FOL and MeJA and also SA and Y28 inoculated with FOL. In soil treated with strain IC10 and inoculated or non-inoculated with the pathogen, POX activity did not increase during the experiment time (48 h) (Figure 4). CAT activity of tomato plants increase 48 h after FOL inoculation. Treatments including bacterial strains and plant hormones increased CAT activity after 24 h. Pathogen inoculation slightly suppressed CAT activity in the SA treatment (Figure 5).

Quantitative Real-Time PCR Analysis of the Defense-Related Genes

Plant inoculation with FOL and MeJA increased UDP-glycosyltransferase (UDP) transcripts by 103- and 98-fold,

### Table 3 | In vitro antagonistic activity against Fusarium oxysporum f.sp. lycopersici by selected isolates.

| Isolates | Growth inhibition (%) | Isolates | Growth inhibition (%) | Isolates | Growth inhibition (%) |
|----------|-----------------------|----------|-----------------------|----------|-----------------------|
| IC6      | 4.0 ± 0.11a           | IT25     | 49.1 ± 0.80p          | IC34     | 1.7 ± 1.5p            |
| IC10     | 68.5 ± 1.00a          | Y7       | 0.6 ± 1.11l           | KI12     | 30.0 ± 0.00d          |
| IC13     | 38.2 ± 0.90f          | Y18      | 1.3 ± 1.11l           | K40      | 4.0 ± 0.00f           |
| IS8      | 10.6 ± 1.11h          | Y27      | 0.6 ± 1.11l           | K43      | 10.0 ± 2.00n          |
| SS112    | 0.6 ± 1.11l           | TO612    | 41.5 ± 1.80c          |          |                       |
| CU122    | 0.0 ± 0.00f           | Y17      | 47.5 ± 2.56p          |          |                       |
| IC15     | 17.9 ± 1.49g          | Y28      | 38.7 ± 1.1c           |          |                       |
| SS14     | 1.0 ± 1.11l           | Y281     | 2.3 ± 2.5l            |          |                       |
| IT20     | 19.3 ± 0.85f          | Y16      | 2.6 ± 2.5l            |          |                       |
| IT8      | 9.6 ± 0.40p           | Y33      | 1.6 ± 2.8l            |          |                       |

Values are the means (averaged from three replicates) ± SE. *Same letters within each column represent non-significant difference according to Duncan’s Multiple Range Test (P < 0.05).

to grow at 42°C (Table 4). All six isolates were able to grow on NaCl 6 % while only isolates Y28 and Y17 were able to grow on medium containing NaCl 10 % (Table 4). The preliminary phylogenetic analysis of the 16S rRNA gene sequences showed IC10 and Y28 were closely related to species of the genus Streptomyces. The phylogenetic tree constructed from 16S rRNA sequences that isolates IC10 and Y28 are two members of Streptomyces genus with more than 99.5% sequence similarity to S. enissocaesilis NRRL B-16365T and S. rochei NBRC 12908T, respectively (Figure 2).

### Table 4 | Cultural characteristics of superior PGP antagonistic isolates.

| Isolate | Color of aerial hyphae – spore chains on ISP media | Melanin production | Growth (in/on) | GeneBank acc. number |
|---------|---------------------------------------------------|--------------------|---------------|---------------------|
|         | ISP2 | ISP3 | ISP4                        | Tyrosine/no tyrosine media | 42°C | NaCl 6% | NaCl 10% | NaCl 13% | |
| IT25    | Yellow – Light purple | Colorless – Light purple | Yellow – purple | −/− | − | ++ | − | − | MG722685 |
| TO612   | Yellow – Light greenish | Yellow – Yellow | Yellow – White | ++/− | − | + | − | − | MG786938 |
| Y17     | Yellow – Greenish | Yellow – Olive | Dark yellow – White | ++/− | − | ++ | + | − | MG788894 |
| Y28     | Yellow – light purple | Colorless – Dark purple | Dark yellow – Purple | −/− | − | ++ | + | − | MG788896 |
| IC10    | Purple – Yellow | Colorless – Purple | Yellow – Purple | −/− | − | + | − | − | MG654776 |
| IC13    | Yellow – Light purple | Dark yellow – Purple | Yellow – Dark gray | −/− | + | + | − | − | MG676358 |
respectively, compared to control. Although *Streptomyces* strains induced UDP transcription, they significantly suppressed gene expression in the plants inoculated with FOL (Figure 6A). Treatments including *Streptomyces* strains and plant hormones and not FOL significantly increased phenylalanine ammonia-lyase (PAL) transcripts compared to control. Salicylic acid and strain IC10 also retained their induction effect in plants inoculated with FOL (Figure 6B). The results revealed that plants treated with SA, IC10, Y28, SA-FOL, and IC10-FOL significantly stimulated (up-regulated) WRKY70 expression. Treatments of FOL and MeJA did not increase relative expression of WRKY70 in tomato plants (Figure 6C). Tomato peroxidase (TPX1) transcripts was significantly up-regulated in all treatments including *Streptomyces* strains and plant hormones. Salicylic
acid and strain Y28 also induced TPX1 gene expression in plants inoculated with FOL (Figure 6D). The highest lipoxygenase (LOX) gene expression level (38-fold) was detected in Y28. There was a significant down-regulation of LOX gene in FOL and IC10 treatments. On the contrary, MeJA and Y28 stimulated LOX expression. Induced LOX expression was also observed in plants treated with bacteria and inoculated with FOL (Figure 6E). In a similar pattern, SA and Y28 significantly increased pathogenesis related protein 1 (PR1) and ethylene response factor 1 (ERF1) transcripts. Strains IC10 and Y28 also

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**TABLE 5** Effect of superior PGP antagonistic isolates (IT25, TO612, Y17, Y28, IC10, and IC13) on tomato growth parameters in greenhouse conditions.

| Treatment          | Shoot length (cm) | Shoot fresh weight (g)/plant | Shoot dry weight (g)/plant | Root fresh weight (g)/plant | Root dry weight (g)/plant |
|--------------------|-------------------|------------------------------|----------------------------|----------------------------|----------------------------|
| Negative control   | 24.18 ± 0.55      | 11.36 ± 0.60                 | 1.79 ± 0.14                | 3.61 ± 0.14                | 1.72 ± 0.10                |
| IT25               | 24.87 ± 0.60      | 10.19 ± 0.35                 | 1.39 ± 0.16                | 2.36 ± 0.35                | 0.90 ± 0.19                |
| TO612              | 29.37 ± 0.32      | 11.63 ± 0.23                 | 1.87 ± 0.19                | 2.78 ± 0.17                | 0.81 ± 0.16                |
| Y17                | 26.50 ± 0.20      | 15.47 ± 0.30                 | 2.18 ± 0.10                | 3.61 ± 0.19                | 1.51 ± 0.25                |
| Y28                | 29.00 ± 0.56      | 14.97 ± 0.49                 | 2.16 ± 0.15                | 2.88 ± 0.12                | 1.23 ± 0.18                |
| IC10               | 28.90 ± 0.25      | 15.37 ± 0.38                 | 2.31 ± 0.39                | 1.64 ± 0.32                | 0.92 ± 0.12                |
| IC13               | 28.62 ± 0.29      | 14.70 ± 0.65                 | 2.45 ± 0.22                | 1.78 ± 0.13                | 0.80 ± 0.24                |

Data recorded 60 days after bacterial treatment. *Same letters within each column represent non-significant difference at 5%, according to Duncan’s tests using the GLM procedure (df = 9, F = 7.16, and p < 0.05).

**TABLE 6** Biocontrol of tomato wilt and stunt caused by *F. oxysporum* f.sp. *lycopersici* race 3 (FOL) using superior PGP antagonistic isolates (IT25, TO612, Y17, Y28, IC10, and IC13) in greenhouse conditions.

| Treatments                        | Shoot length (cm) | Shoot fresh weight (g)/plant | Shoot dry weight (g)/plant | Root fresh weight (g)/plant | Root dry weight (g)/plant | Disease severity | Control value (%) |
|-----------------------------------|-------------------|------------------------------|----------------------------|----------------------------|----------------------------|------------------|------------------|
| Negative control                  | 23.62 ± 0.25      | 11.20 ± 0.63                 | 1.35 ± 0.35                | 3.31 ± 0.10                | 1.41 ± 0.33                | 1.00             | –                |
| Positive control (FOL)            | 14.00 ± 0.28      | 5.56 ± 0.34                  | 0.78 ± 0.18                | 1.66 ± 0.33                | 0.37 ± 0.29                | 4.30             | –                |
| Carbendazim® + FOL                | 15.25 ± 0.22      | 13.00 ± 0.25                 | 2.20 ± 0.16                | 0.23 ± 0.38                | 0.18 ± 0.14                | 2.10             | 71.4a            |
| IT25 + FOL                        | 20.20 ± 0.43      | 10.23 ± 0.27                 | 1.77 ± 0.55                | 2.48 ± 0.49                | 1.00 ± 0.37                | 2.30             | 68.6b            |
| TO612 + FOL                       | 19.00 ± 0.20      | 12.77 ± 0.32                 | 2.40 ± 0.38                | 2.90 ± 0.20                | 1.40 ± 0.38                | 1.90             | 78.1a            |
| Y17 + FOL                         | 19.50 ± 0.35      | 13.38 ± 0.26                 | 2.09 ± 0.58                | 2.76 ± 0.37                | 0.94 ± 0.20                | 2.00             | 75.0b            |
| Y28 + FOL                         | 26.70 ± 0.15      | 11.88 ± 0.75                 | 2.02 ± 0.60                | 2.94 ± 0.28                | 0.59 ± 0.33                | 2.00             | 75.0b            |
| IC10 + FOL                        | 26.60 ± 0.35      | 12.83 ± 0.10                 | 2.07 ± 0.38                | 1.83 ± 0.18                | 0.96 ± 0.24                | 1.60             | 84.4b            |
| IC13 + FOL                        | 21.33 ± 0.39      | 15.00 ± 0.29                 | 2.45 ± 0.41                | 2.54 ± 0.30                | 1.15 ± 0.31                | 2.10             | 71.9a            |

Data recorded 60 days after bacterial treatment. Disease severity was assessed on a scale of 1 to 5 (1, symptoms free; 5, severe symptoms). *Same letters within each column represent non-significant difference at 5%, according to Duncan’s tests using the GLM procedure (df = 11, F = 3.63, and p < 0.05).
were able to stimulate the PRI expression in the presence of FOL (Figures 6F,G).

**Correlation Analysis**

There was a significant relationship between siderophore and IAA production ($r = 0.36$). Besides, a correlation was found between growth inhibition and protease activity ($r = 0.31$). Moreover, growth inhibition of FOL in dual culture assay was significantly correlated to siderophore production ($r = 0.64$) (Table 7). A significant negative correlation ($-0.63$) was observed between growth inhibition (in vitro assay) and disease severity (in vivo assay).

**DISCUSSION**

Almost half of tomato and cucumber greenhouses in Iran (more than 7000 hectares) is located in Yazd, Isfahan, and Kerman.
The relative level of gene expression (fold) determined by qRT-PCR of seven target genes including UDP (A), PAL (B), WRKY70 (C), TPX (D), LOX (E), PR1 (F), and ERF1 (G) versus reference control (Tubulin gene) in tomato “Rio grande” treated with chemical (MeJA and SA) or biological agents (S. enissocaesilis strain IC10 and S. rochei strain Y28) 48 h after inoculation with FOL/distilled water. Untreated and non-inoculated plants were considered as control (C) and as reference sample. Standard error represents for three biological replicates. Positive values of fold change indicate up-regulation while negative values indicate down-regulation. The values marked with one and two asterisk are significantly different from control at P < 0.05 and P < 0.01, respectively.

provinces. Amount and type of organic fertilizer and soil used to prepare the greenhouse growth bed are different in these provinces. Farmers in Yazd and Kerman use sand from the bed of the nearest rivers to their greenhouse and farmers in Isfahan use soil of the greenhouse floor as a raw material to prepare culture bed (private conversation). Our results showed distribution of the bacterial isolates with the Streptomyces appearance in the three provinces is slightly different. Among the isolates
that showed a PGP trait, a relatively stable share (42–46%) belonged to Isfahan. The contribution of the *Streptomyces* isolated from Yazd or Kerman in each PGP trait was not always constant showing *Streptomyces* with several PGP traits in the field soil is greater than that of rivers sand. A previous study on *Streptomyces* showed that bacterial diversity was more affected by the habitat than the population (Davelos et al., 2004). The restriction of some nutrients in soil may be a major contributor to the change in the microbial diversity due to natural selection.

Growth ability on a nitrogen-free medium and IAA and siderophore production were three dominant characteristics of *Streptomyces* isolated from vegetable rhizosphere soils. A lower percentage (30%) of the isolates had the potential for solubilizing phosphate. Besides, the location of isolation was also effective on this ratio. There was a significant positive correlation between IAA and siderophore production, which may remind a complementary role of the siderophore in plant growth promotion activity (i.e., in addition to its role in antagonistic activity). Such correlation was not observed between other traits involved in antagonist activity and IAA production.

The ability of FOL growth inhibition was very different between *Streptomyces* isolates showing PGP properties. Isolate CU122 with four PGP traits, was not able to inhibit FOL growth. Failure to inhibit the pathogen growth was correlated to the inability of isolate CU122 to produce chitinase and protease enzymes. On the contrary, isolate IC10 with same in vitro PGP features as well as enzymes activity inhibited FOL growth very well. The correlation of protease and chitinase activity with inhibition of FOL growth was positive and respectively, quantitative and non-quantitative. Only two exceptions did not adhere to this relationship. Isolates TO612 and KH12, which did not show enzyme activity, inhibited FOL growth in an acceptable range (equal to or greater than 30%).

This finding revealed that although chitinolytic activity of *Streptomyces* and digestion of the fungal cell wall is an effective mechanism of growth inhibition (Gherbawy et al., 2012), other mechanisms such as antibiotics and even an enzyme like protease (Xu et al., 2016) that is less studied are also involved in antagonism. Interestingly, there was a significant correlation between IAA and siderophore productions and FOL growth inhibition, showing relationship between PGP features and antagonistic activities in *Streptomyces*. IAA production is a common characteristic among antagonistic species of *Streptomyces* (Sreevidya et al., 2016). It seems that IAA production alone does not cause plant growth, and other traits, such as siderophore production, also has an important role in increasing plant growth. In this regard, Kunova et al. (2016) showed that the antagonistic isolates producing IAA did not increase growth of different vegetable species. These isolates were not able to produce siderophore. The significant negative correlation that was observed between FOL growth inhibition (in vitro assay) and disease severity (in vivo assay) confirmed a need for in vitro tests to select the best biocontrol strains. Recently, Wu et al. (2019) reported that a decreased percentage disease index of rice sheath blight caused by *Rhizoctonia solani* was associated with an increase in *Streptomyces* derived antifungal agent concentration. They concluded that the antifungal agent reduces sheath blight symptoms via exerting a strong antagonistic activity against *R. solani* both in vivo and in vitro conditions. S. rochei ACTA1551 from Greek (Kanini et al., 2013), *S. miharaensis* KPE62302H (Kim et al., 2012), and *S. psammoticus* KP1404 (Kim et al., 2011) from Korea, *S. plicatus* from Egypt (Abd-Allah, 2001) and *S. griseorubens* E44G from Saudi Arabia (Rashad et al., 2017) were reported as successful biocontrol agents to control tomato *Fusarium* wilt. In the previous reports, the pathogen race has not been determined and to our knowledge, this is the first report of biocontrol of *Fusarium* wilt caused by *F. oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) race 3 in tomato with *Streptomyces* strains.

Strain Y28 and IC10 were close to *S. rochei* and *S. enissoacaesilis*, respectively and are grouped in a clade on the phylogenetic tree. These PGP strains were isolated from the rhizosphere soils of two greenhouses in two different locations with a distance of 300 kilometers. The high similarity in PGP, and biocontrol activity of these two strains revealed that evolutionary process can keep PGP traits and biological control activities together.

All plants treated with SA, MeJA and Y28 or inoculated with FOL increased POX activity during 48 h after FOL inoculation. The POX activity in hormone or PGPR treated plants was not affected by FOL inoculation. Despite the similar biocontrol activity, isolates IC10 and Y28 had a different effect on the induction of plant POX activity. None of the plants treated with IC10, inoculated or non-inoculated with FOL, increased POX activity. Peroxidase is considered an important pathogen-related protein (PR-protein) or defense protein involved in many physiological responses of plants to biotic stresses. Contribution to biosynthesis of lignin (Chittoor et al., 1997) and antimicrobial compounds such as phytoalexins and quinones (Mayer, 2006) are two well-known POX roles associated with ISR. Increased peroxidase activity of cucumber was reported in plants showing *Fusarium* wilt (Zhao et al., 2012) and also plant treated with *Streptomyces* as biocontrol agent (Salla et al., 2016).

Peroxidase, as well as CAT, are involved in plant antioxidant defense system and reduce the harmful effects of stresses by scavenging of ROS (Das and Roychoudhury, 2014). In this study, the effects of FOL, plant hormones and PGP strains

### Table 7: Pearson correlation coefficients (r) between PGP traits (in vitro assays) and biocontrol activity (in vivo assay).

| Variables                  | In vitro assays | In vivo assay |
|----------------------------|-----------------|---------------|
|                            | IAA production | Fungal growth inhibition (%) | Disease severity |
| Protease activity          | 0.44**          | 0.31*         | −0.47**          |
| Siderophore production     | 0.36**          | 0.64**        | 0.24*            |
| IAA production             | 1               | 0.47*         | −0.01**          |
| Fungal growth inhibition   | −0.63*          |               |                 |

*ns, non-significant; *P < 0.05; and **P < 0.01.*
to induce CAT activity were the same. Induction of systemic resistance through defense-related enzymes POX and CAT in tomato plants treated with salicylic acid or *Pseudomonas fluorescens* was reported by Nikoo et al. (2014). They showed that pathogen inoculation of plants treated with bacterial or hormonal elicitors increased both POX and CAT activity in higher levels compared to non-inoculated treated plants or plants only inoculated with pathogen. This association was not observed in our experiment.

Relative expression of several candidate genes encoding transcription factors (*WRKY70* and *ERF1*), *PRI*, *TPX1*, *UDP*, *LOX*, and *PAL* was evaluated in this study. The plant-specific transcription factor *WRKY70* is an important factor in *Arabidopsis* signaling pathways and its expression is activated by SA and repressed by JA. Ren et al. (2008) showed that overexpression of *WRKY70* reduced JA responses and mutually JA treatment inhibited *WRKY70* expression. On the contrary, *WRKY70* is known as a positive regulator of SA-mediated defense because increases *PRI* which is often studied as a marker gene for SA-dependent defense signaling (Li et al., 2004). It is reported that PGP *Bacillus cereus* AR156 stimulated the transcription of *WRKY70* in *Arabidopsis* leaves. According to their study, *WRKY70* modulated B. cereus-triggered ISR through activating SA signaling pathway (Wang et al., 2018). Our results are in accordance with the reported studies and showed induction of *WRKY70* transcription upon treatment with SA, and not with MeJA, in *FOL* inoculated and non-inoculated plants. IC10 and Y28 also significantly induced transcription of *WRKY70* and IC10 continued to induce gene expression after *FOL* inoculation. Following increased *WRKY70* transcripts, expression of *PRI* increased in SA and Y28 treatments. Great increase in *PRI* transcript abundance by Y28 and not IC10 indicates that these two PGPRs stimulate different pathways to elicit defense priming in tomato plant.

UDP-glucose dependent hydroquinone: O-glucosyltransferase (arbutin synthase) is a member of glycosyltransferases catalyze production of arbutin in higher plants (Arend et al., 2000a). Arbutin is a phenolic glycoside that has antimicrobial and antifungal activity (Kundakovic et al., 2014). Arbutin synthase is a multifunctional enzyme converting various natural products, xenobiotics and toxins (Arend et al., 2000b). There is no report about induction of UDP in response to *F. oxysporum* or *Streptomyces* PGPRs in tomato plant. A previous report revealed that induction of members of UDP family, *UGT73B3* and *UGT73B5* is necessary during the hypersensitive response of *Arabidopsis* to plant pathogen *Pseudomonas syringae* (Langlois-Meurinne et al., 2005). Also treatments of *Arabidopsis* with SA and MeJA induced the expression of another gene family member *UGT73C5* involved in mycotoxin detoxification (Poppenberger et al., 2003). Increased UDP transcript in *FOL* inoculated tomato can be attributed to the role of arbutin in detoxification of fungal toxin or its antimicrobial effect (Kuzniak et al., 2015). The increased expression of this gene in treatment of MeJA and not SA represents its role in ISR. Compared to *FOL* inoculation, a significant lower level of UDP expression was observed in plants treated with IC10 and Y28 highlighting the potential role of these PGP strains in tomato defense priming.

**FOL**, SA, MeJA and PGPRs induced expression of *TPX1*. *TPX1*, peroxidase encoding gene, is involved in the synthesis of lignin and suberin (Quiroga et al., 2000). Here, *TPX1* expression induction by Y28 and IC10 revealed their role in ISR. *TPX1* expression at the lower level was observed 48 h after *FOL* inoculation. Different POX activities in plants treated with PGPRs could be related to the *TPX1* transcript abundance.

Phenylalanine ammonia lyase encoded by PAL is involved in the biosynthesis of salicylic acid and defense compounds including flavonoids, phenylpropanoids and lignin. Induction of PAL activity in response to various stimuli as such tissue plant pathogens and hormones was reported (Edwards et al., 1985). In this study, induced expression of PAL in bacterial and hormonal treatments was observed and there was no difference between the induction effects of bacterial strains. The induction of PAL enzymatic activity and PAL gene expression in tomato plants upon pre-treatment with *Bacillus thuringiensis* (Akram et al., 2013) and the mycorrhizal fungus *Funneliformis mosseae* (Song et al., 2015) were also reported.

Members of the ERF protein family are transcription factors involved in ET/JA or SA signaling pathways and cause moderate disease resistance responses in various plant species (Liang et al., 2008). Induced expression of *ERF1* in *Arabidopsis* plants treated with a PGP biocontrol strain of *Paraburkholderia phytofirmans* in response to a model plant pathogen *P. syringae* pv. tomato DC3000 was reported recently (Timmermann et al., 2017). Likewise, induction of *ERF1* by treatment of Y28 tomato was observed in the present study too. On the contrary, plants treated with IC10 decreased *ERF1* expression. Our data supports the hypothesis that studied PGP strains regulate tomato defense responses through different molecular pathways possibly acting at transcriptional level.

**LOX** is a signaling molecule involved in pathogen plant resistance. Strain Y28 slightly induced LOX expression in non-pathogen inoculated plants. *FOL* inoculation and IC10 treatment caused a significant decreased in transcript level of LOX compared to the control plants. *FOL* inoculation of both bacterial treatments caused an induction of gene expression. Our finding is consistent with Mariutto et al. (2011) that showed tomato plants treated with PGP *Pseudomonas putida* did not induce LOX transcription before inoculation with the pathogen *B. cinerea*. Although transcripts of LOX for both strains were higher in *FOL* inoculated plants, gene expression levels in non-inoculated bacterial treatments were different significantly.

**CONCLUSION**

The superior PGP antagonistic *Streptomyces* strains, show biocontrol activities against Fusarium wilt of tomato caused by *F. oxysporum* Schlcht. *F. sp. lycopersici* (Sacc.) race 3. Significant positive correlation between siderophore and IAA production and siderophore accumulation and growth inhibition showed a relationship between PGP and antagonistic traits. A significant
negative correlation between growth inhibition (in vitro assay) and disease severity (in vivo assay) confirmed a need for in vitro tests to select the best biocontrol strains. The correlation of PGP traits with biocontrol activity in phylogenetically close species isolated from distant habitats refers to the role of the natural selection in preserving traits that give superiority to bacteria in the rhizosphere. Here we showed biocontrol Streptomyces stimulate plant defense system through different molecular pathways at the transcriptional level.

**AUTHOR CONTRIBUTIONS**

NS and AS designed and directed the research. MS gave advice during experiments. SA wrote the manuscript with help from NS and AS. All authors read and contributed to the interpretation of the results. SA wrote the manuscript. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2019.01505/full#supplementary-material

**FIGURE S1** | Identification of *Fusarium oxysporum* f. sp. *lycopersici* (FOL) physiological race 3 by selective primers. Polymerase chain reactions carried out using unif, sp13, sp23, and sprl primer sets. 1 kb: 1 kb ladder; C, negative control.

**FIGURE S2** | Bacterial colonies, *S. enusicaesi* strain I010 and *S. roche* strain Y28, on ISP2 medium 10 days after cultivation.
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