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Evaluation of *Streptomyces* spp. and *Bacillus* spp. for biocontrol of Fusarium wilt in chickpea (*Cicer arietinum* L.)

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ABSTRACT

A study was carried out to test direct and indirect antagonistic effect against Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC), and plant growth-promoting (PGP) traits of bacteria isolated from rhizosphere soils of chickpea (*Cicer arietinum* L.). A total of 40 bacterial isolates were tested for their antagonistic activity against FOC and of which 10 were found to have strong antagonistic potential. These were found to be *Streptomyces* spp. (five isolates) and *Bacillus* spp. (five isolates) in the morphological and biochemical characterisation and 16S rDNA analysis. Under both greenhouse and wilt sick field conditions, the selected *Streptomyces* and *Bacillus* isolates reduced disease incidence and delayed expression of symptoms of disease, over the non-inoculated control. The PGP ability of the isolates such as nodule number, nodule weight, shoot weight, root weight, grain yield and stover yield were also demonstrated under greenhouse and field conditions over the non-inoculated control. Among the ten isolates, *Streptomyces* sp. AC-19 and *Bacillus* sp. BS-20 were found to have more potential for biocontrol of FOC and PGP in chickpea. This investigation indicates that the selected *Streptomyces* and *Bacillus* isolates have the potential to control Fusarium wilt disease and to promote plant growth in chickpea.

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Introduction

Fusarium wilt caused by *F. oxysporum* Schl. emend. Snyd. and Hans. f. sp. *ciceri* (Padwick; *Fusarium oxysporum* f. sp. ciceri (FOC)) is the third most important disease in chickpea (*Cicer arietinum* L.) throughout the world. It is one of the main yield-limiting factors in all chickpea-growing

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areas of the world (Dubey et al. 2007). General symptoms of Fusarium wilt in chickpea include drooping, yellowing, drying of the leaves and discolouration of vascular system. The pathogen infects the roots of susceptible host, colonises the vascular system of plants and produces toxin(s) that kills plants by blocking xylem vessels and restricts water transport (Anjaiah et al. 2003; Gopalakrishnan et al. 2005). FOC can survive saprophytically in the soil or crop debris for more than six years (Trapero-Casas and Jimenez-Diaz 1985). Therefore, it is not possible to control by normal crop rotation. The use of resistant cultivar is the most efficient control measure but the effectiveness of resistance is restricted by the occurrence of eight pathogenic races in FOC (Jimenez-Gasco et al. 2002). Application of fungicides does not always prove economical against soil-borne pathogens and it has also led to environmental pollution, pathogen resistance, increased risk to human and animal health and creates an imbalance in the microbial community in soil (Li et al. 2012; On et al. 2015).

The use of microbial biocontrol agents is an alternative to fungicides for the management of plant diseases because it is one of the most environmentally viable and health-friendly approaches for replacing fungicides (Jiménez-Fernández et al. 2015). Numerous biocontrol agents have been reported to control Fusarium wilt of chickpea, such as *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma* spp. and *Streptomyces* spp. (Gopalakrishnan et al. 2011; Moradi et al. 2012). Different mechanisms have been implicated in the suppression of fungal root diseases by biocontrol agents such as competition for nutrients and production of microbial metabolites such as extracellular antibiotics, siderophores, lytic enzymes and hydrogen cyanide (Das et al. 2008; Naureen et al. 2009; Erdogan and Benlioglu 2010). Such plant growth-promoting (PGP) microbes facilitate plant growth either directly by nitrogen fixation, phosphate solubilisation, iron chelation and phytohormone production (Cakmakci et al. 2006; Vivas et al. 2006; Hanane et al. 2008) or indirectly by inhibiting phytopathogens, and thus promoting plant growth and development. Biocontrol agents are known to be found commonly in forest soil, pasture soil, rhizosphere soil and compost/vermicompost.

Actinobacteria are Gram-positive bacteria with high GC content in their genome and resemble fungi morphologically. Among actinobacteria, *Streptomyces* is the predominant genus followed by *Actinomadura*, *Actinoplanes*, *Frankia*, *Microbispora*, *Micromonospora*, *Nocardia*, *Mycobacterium*, *Nonomurea*, *Saccharopolyspora* and *Verrucosispora* and they found commonly in soil (Gopalakrishnan et al. 2016). Actinobacteria are also known for production of secondary metabolites and of which the genus, *Streptomyces*, is the major producer (39%) of secondary metabolites, including

antibiotics, anticancer agents, antiparasitic drugs, antifungals, antivirals, immune suppressants, insecticides, antioxidants, enzyme inhibitors and herbicides (Berdy 2012). *Bacillus* is another important PGP bacteria that has been found naturally in the rhizosphere soils and reported widely in promoting not only plant growth and yield but also antagonistic against phytopathogens (Sreevidya and Gopalakrishnan 2017). The main objective of the present study was to isolate *Streptomyces* spp. and *Bacillus* spp. from rhizosphere soils of chickpea and to evaluate further for their antagonistic potential against Fusarium wilt disease of chickpea under *in vitro*, greenhouse and field conditions.

Material and methods

Isolation of actinomycetes and *Bacillus spp.*

Rhizosphere soil samples were collected, at the depth of 0—15 cm, with the help of soil core, randomly in the chickpea fields of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Ten grams of rhizosphere soil was added in a conical flask containing 90 mL of physiological saline (0.85% NaCl; in order to provide osmotic protection for bacterial cells in the sample) and kept for shaking on an orbital shaker (at 100 rpm) at 28 ± 2 °C for 1 h. At the end of shaking, the samples were serially diluted up to 10^6 dilutions. Dilutions 10^4—10^6 dilutions were spread plated (0.1 mL) on actinomycetes isolation agar (AIA; HiMedia Laboratories, Mumbai, India) for isolating actinomycetes and *Bacillus* differential agar (BDA; composition: yeast autolysate – 0.2 g, mannitol – 5.0 g, monohydrogen ammonium phosphate – 1.0 g, potassium chloride – 0.2 g, magnesium sulphate – 0.2 g, bromocresol purple – 0.0075 g, agar 15 g, final pH 7.2 ± 0.2) for isolating *Bacillus* spp. The plates were incubated at 28 ± 2 °C for 7 days for actinomycetes and 2 days for *Bacillus* spp. Prominent colonies were isolated, purified (by picking isolated colonies) and stored on AIA or BDA slants at 4 °C for further studies.

In vitro antagonistic activity against FOC

The prominent actinomycetes and *Bacillus* isolates were screened for their antagonistic activity against FOC (acquired from legume pathology, ICRISAT, Patancheru, India) by dual-culture assay on glucose cassamino acid yeast extract (GCY) agar as per the protocols of Gopalakrishnan et al. (2011). In brief, a disc of FOC (6 mm dia.) was placed on one edge (1 cm from the corner) of the GCY agar plate and actinomycetes/*Bacillus* spp. was streaked on the other edge of the plate (1 cm from the corner).

The plates were incubated at 28 ± 2 °C for 5 days. Inhibition of the FOC was recorded as positive and the inhibition zone measured.

Enzymatic activities and secondary metabolite production by the selected actinomycetes and *Bacillus* isolates

The selected isolates were evaluated for their production of siderophore, cellulase, protease, lipase, hydrocyanic acid (HCN), indole acetic acid (IAA) and β-1, 3-glucanase. Siderophore production was estimated as per the protocol of Schwyn and Neilands (1987). Production of cellulase and protease and lipase was detected by using standard protocols of Hendricks et al. (1995) and Bhattacharya et al. (2009), respectively. HCN was qualitatively assessed by the method described by Lorck (1948). Estimation of IAA and β-1,3-glucanase was done as per the protocols of Patten and Glick (1996) and Singh et al. (1999), respectively. The rating scales for cellulase, lipase and protease were as follows: 0 = no halo zone; 1 = halo zone of 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm. For HCN production, the following rating scale was used: 0 = no colour change, 1 = light reddish brown, 2 = medium reddish brown and 3 = dark reddish brown.

Molecular identification of actinomycetes and *Bacillus* isolates

Pure cultures of the selected antagonistic actinomycetes and *Bacillus* isolates were sent to Macrogen Inc. Seoul, Korea for identification based on their 16S rDNA analysis. Macrogen used universal bacterial primers 27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492 R (5′-TACGGYTACCTTGTTACGACTT-3′) for amplification of the 16S rDNA gene (Bazzicalupo and Fani 1995). The obtained sequences were compared with similar sequences retrieved from GenBank (using the BLAST program and aligned using the Clustal W software) and the dendrogram was constructed by the neighbour-joining method (Saitou and Nei 1987; Alschul et al. 1990; Thompson et al. 1997). Bootstrap analysis was performed using the MEGA version 4 program to estimate the statistical stability of the branches in cluster with 1000 replications. The sequences were submitted to NCBI and accession numbers obtained.

Antagonistic and PGP potentials of the selected actinomycetes and *Bacillus* isolates under greenhouse conditions

The five most potential antagonistic actinomycetes and *Bacillus* isolates against FOC, from the *in vitro* studies, were evaluated individually for

their antagonistic potential in pots in the greenhouse. Pot mixture was prepared by mixing Vertisol, sand and farm yard manure at 3:2:1 (w/w) and was filled (1 kg) in 8 in. plastic pots (Hosco Horticultural Supplies Co, Mumbai, India) followed by inoculation with FOC inoculum (20% of pot weight, 200 g pot^{-1}; two weeks before sowing). FOC inoculum was mass-multiplied on chickpea grains (variety JG62; highly susceptible to Fusarium wilt, acquired from the Legumes Pathology Division, ICRISATE) as per the methodology of Gopalakrishnan et al. (2011). Inoculum was thoroughly mixed with the pot mixture and the pots were covered with polythene sheets, in order to maintain the moisture in the soil so that inoculum will be developed. The whole setup was incubated at 26 ± 2 °C for 15 days to have Fusarium wilt disease conditions. Two weeks later, the seeds of chickpea variety JG62 were surface-sterilised (with 2.5% sodium hypochlorite solution in water for 5 min and rinsed 8 times with sterilised double distilled water) and treated with respective actinomycetes/*Bacillus*. Each actinomycetes/*Bacillus* isolates were inoculated by three different methods, namely M1 = inoculation of the seeds by soaking in the respective actinomycetes/*Bacillus* culture for 1 h; M2 = inoculation of the potting mixture with actinomycetes/*Bacillus* culture at the time of sowing (10 ml of well-grown culture [10^8 CFU ml^{-1}] applied on the seed and covered with soil; CFU was obtained by spread plating the actinomycetes/*Bacillus* culture on AIA/BDA plates) and M3 = M1 +M2. Six inoculated seeds were sown (at 2—3 cm depth) in each pots and one week later thinned to retain three seedlings. The experiment had six replications. Plants were irrigated once every two days with 20 ml of sterilised distilled water. Incidence of Fusarium wilt disease (number of plants showing wilt symptoms to the total number of plants in a pot) was recorded on 5, 10, 15, 20 and 29 days after sowing (DAS). Disease incidence was calculated using the method reported by Cao et al. (2011) with the following formula:

$$\textbf{Disease incidence}\ (\%) = \textbf{Number of diseased plants/Total number of plants} \times 100$$

The selected five *Streptomyces* and *Bacillus* isolates were also evaluated for their PGP traits under greenhouse conditions. Soil mixture containing Vertisol, sand and farm yard manure (3:2:1) was prepared and filled in plastic pots (8″). A total of 13 treatments (five *Streptomyces* isolates + their consortia, five *Bacillus* isolates + their consortia and one uninoculated control) with three replications were maintained. The seeds of chickpea variety JG11 were surface-sterilised and treated with respective *Streptomyces*/*Bacillus* isolates as described earlier. Six seeds were sown (at 2—3 cm depth) in each pots and one week later thinned to retain three seedlings. The experiment had six replications. At the seven days

interval, a booster (additional) dose of *Streptomyces/Bacillus* was added. At 30 DAS, PGP traits including plant height, branches number, pod number, nodule number, nodule weight, leaf area, leaf weight, root weight, stem weight, total plant weight, surface area, root length and root volume were recorded. At 45 DAS, observation recorded including plant height, branches number, nodule number, nodule weight, flower number, pod number, root weight, stem weight, leaf weight and total plant weight and at harvesting, pod number, pod weight, shoot weight, seed number and seed weight were recorded.

Field studies

Field trials were performed in 2016 and 2017 post-rainy seasons at ICRISAT, Patancheru (17°30.861′N; 78°16.080′E; altitude = 549 m) in the Telangana State of India. The experimental field soils are classified as 51% clay, 22% silt and 26% sand with an organic carbon content of 0.4 – 0.5% and an alkaline pH of 7.5 – 8.1. The mineral content of the experimental field rhizosphere soil (top 15 cm) includes 24 mg kg^{-1} soil of available nitrogen, 8 mg kg^{-1} soil of available phosphorous and 294 mg kg^{-1} soil of available potassium. The experimental field was kept fallow except for post-rainy season. The maximum and minimum temperatures recorded during the cropping season were 29.01—32.71 and 12.05— 14.43, respectively. The experimental plots were laid out of 4 m × 3 m ridges (rows) arranged in a randomised complete block design with three replications.

Three genotypes of chickpea (JG62 and K850 – susceptible to Fusarium wilt, early and later wilter, respectively; JG11- popular cultivar) were surface-sterilised, treated with respective *Streptomyces*/*Bacillus* isolates, as described earlier, and sown by hand at 5 cm depth. A booster doses of *Streptomyces/Bacillus* peat-based formulation (10^8cfu ml^{-1}) were applied to soil at an interval of 15 DAS until flowering stage. Control plots were maintained without the application of *Streptomyces*/*Bacillus*. Weeding was performed as and when required. No serious insect pest or phytopathogens (except FOC) were observed during the cropping period. During the cropping season, a maximum temperature range of 27.1—31.0 °C and a minimum temperature range of 7.0—19.2 °C were recorded. Incidence of Fusarium wilt (number of plants showing wilt symptoms to total number of plants in a plot) was recorded in JG62 on 18, 22 and 26 DAS and in K850 on 45, 58 and 75 DAS till the susceptible check showed close to 100% mortality.

In JG11 cultivar, at 35 DAS, PGP traits, including plant height, nodule number, nodule weight, leaf area, leaf weight and stem weight were

recorded. At 60 DAS, plant height, nodule number, nodule weight, shoot weight, pod number and pod weight and while at final harvest, pod number, pod weight, seed number, stover weight and grain weight were recorded. The roots of chickpea were tested for colonisation by *Streptomyces* and *Bacillus* spp. by SEM analysis (Gopalakrishnan et al. 2015). The samples were examined with a scanning electron microscope (JOEL-JSM 5600) as per the standardised procedure at RUSKA lab, College of Veterinary Science, Rajendranagar, Hyderabad, India. Observations of the presence of *Streptomyces* and *Bacillus* spores/cell on root surfaces were recorded.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) (GenStat 10.1 version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) to evaluate the efficiency of biocontrol agent's application in both the greenhouse and field studies. Significance of differences between the treatment means was tested at $p = 0.01$ and 0.05.

Results

Selection of antagonistic actinomycete and *Bacillus* isolates against FOC

A total of 20 actinomycete and 20 *Bacillus* isolates, the most prominent ones which were found abundantly and inhibited the adjacent colonies, were isolated from AIA and BDA, respectively, and further screened for their antagonistic potential against FOC by dual-culture assay. The five most potential FOC antagonistic actinomycetes (AC-5, AC-6, AC-10, AC-18 and AC-19) and *Bacillus* (BS-10, BS-15, BS-17, BS-19 and BS-20) isolates were selected for further evaluation. Of the selected 10 FOC antagonistic isolates, BS-20 inhibited the most (inhibition zone 29 mm) followed by AC-5 (27 mm) and AC-10 (25 mm) (Figure 1).

Enzymatic activities and secondary metabolite production by the selected actinomycetes and *Bacillus* isolates

All the selected actinomycete and *Bacillus* isolates were found to produce cellulase (except AC-5), protease (except AC-5, AC-10 and BS-10), lipase (except AC-5), HCN (except AC-18 and BS-10), IAA and β-1,3-glucanase. Siderophore was produced only by four isolates (AC-10, AC-18, BS-15 and BS-20). Of the selected 10 FOC antagonistic isolates, only BS-20 was found to produce all the traits (Table 1).

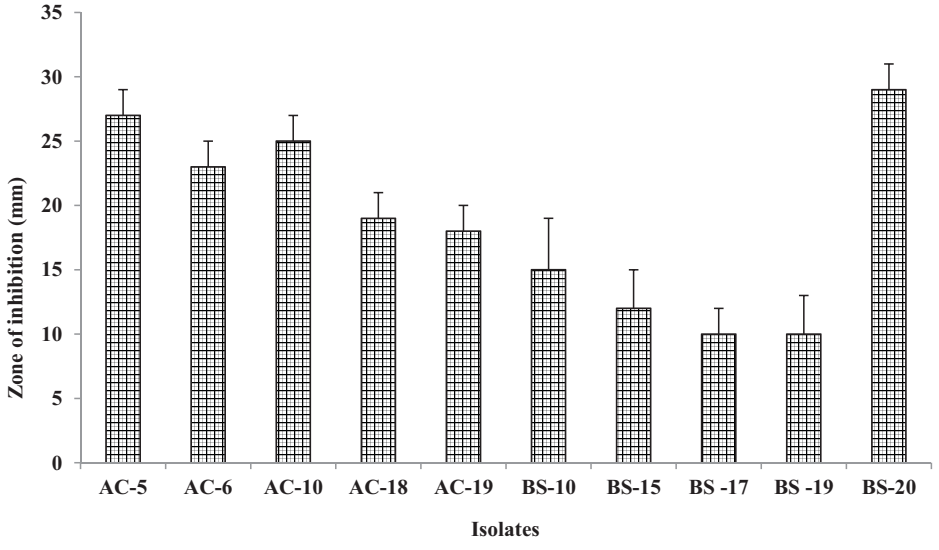


Figure 1. *In vitro* antagonistic activity of actinomycetes and *Bacillus* isolates against FOC.

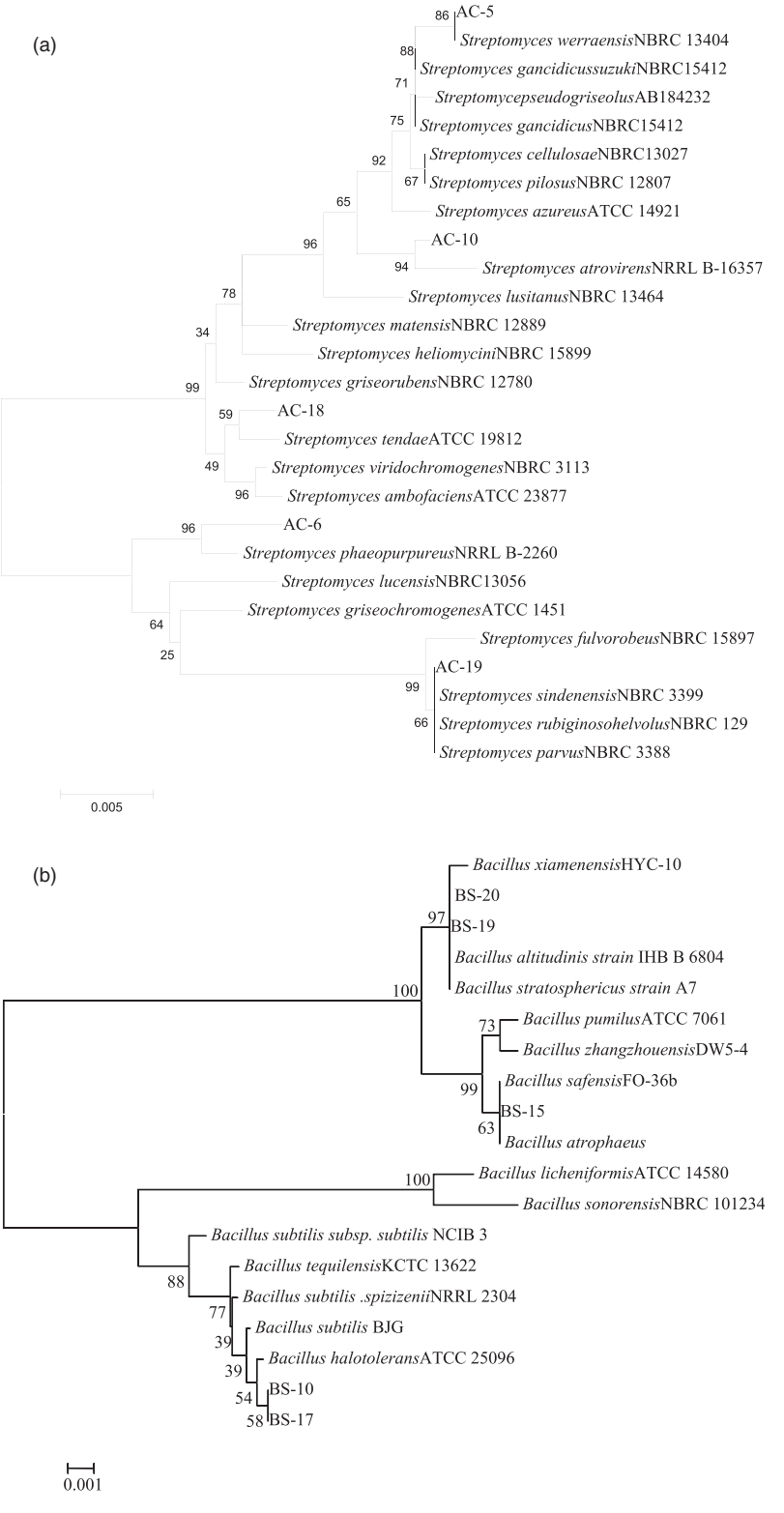
Table 1. *In vitro* evaluation of actinomycetes and *Bacillus* isolates for different PGP traits.

Isolate	Cellulase	Protease	Lipase	Siderophore (% units)	HCN	IAA ($\mu g ml^{-1}$)	β-1,3-glucanase units[a]
AC-5	0	0	0	0.0	1	2.4	0.28
AC-6	1	1	3	0.0	1	10.1	2.08
AC-10	2	0	2	51.5	2	31.6	0.77
AC-18	2	2	3	54.0	0	9.8	2.08
AC-19	2	2	3	0.0	2	12.2	2.15
BS-10	3	0	2	0.0	0	14.1	0.18
BS-15	3	2	2	16.5	1	12.5	1.29
BS-17	3	1	1	0.0	2	10.8	1.01
BS-19	3	1	1	0.0	2	23.0	1.34
BS-20	3	2	3	23.5	2	25.0	1.72
SE±	0.39	0.30	0.27	0.31	0.14	1.57	0.30
LSD (1%)	1.29	0.97	0.89	0.97	0.44	5.11	0.97

[a]One units of β-1,3-glucanase activity was defined as the amount of enzyme that liberated 1 μg ml of glucose $hour^{-1}$ at defined conditions.
HCN: Hydrocyanic acid; IAA: Indole acetic acid.
The rating scales for cellulase, lipase and protease were as follows: 0 = no halo zone; 1 = halo zone of 1–10 mm; 2 = halo zone of 11–20 mm; 3 = halo zone of 21–30 mm; 4 = halo zone of 31–40 mm; and 5 = 41–50 mm. For HCN production, the following rating scale was used: 0 = no colour change, 1 = light reddish brown, 2 = medium reddish brown and 3 = dark reddish brown.

Molecular identification of the selected actinomycete and *Bacillus* isolates

When the sequences of the selected actinomycetes (AC-5, AC-6, AC-10, AC-18 and AC-19) and *Bacillus* (BS-10, BS-15, BS-17, BS-19 and BS-20) isolates were analysed, the results revealed that all actinomycetes matched (100%) with *Streptomyces* but different species (such as *S. warrensis*, *S. phaeopurpureus*, *S. atrovirens*, *S. griseorubens* and *S. parvus*, respectively) while the *Bacillus* isolates matched (100%) with *Bacillus* but different species (such as


(a)
AC-5
Streptomyces werraensisNBRC 13404
Streptomyces gancidicussuzukiNBRC15412
Streptomycepseudogriseolus AB184232
Streptomyces gancidicusNBRC15412
Streptomyces cellulosaeNBRC13027
Streptomyces pilosusNBRC 12807
Streptomyces azureusATCC 14921
AC-10
Streptomyces atrovirensNRRL B-16357
Streptomyces lusitanusNBRC 13464
Streptomyces matensisNBRC 12889
Streptomyces heliomycinNBRC 15899
Streptomyces griseorubensNBRC 12780
AC-18
Streptomyces tendaeATCC 19812
Streptomyces viridochromogenesNBRC 3113
Streptomyces ambofaciensATCC 23877
AC-6
Streptomyces phaeopurpureusNRRL B-2260
Streptomyces lucensisNBRC13056
Streptomyces griseochromogenesATCC 1451
Streptomyces fulvorobeusNBRC 15897
AC-19
Streptomyces sindenensisNBRC 3399
Streptomyces rubiginosohelvolusNBRC 129
Streptomyces parvusNBRC 3388
0.005
(b)
Bacillus xiamenensisHYC-10
BS-20
BS-19
Bacillus altitudinis strain IHB B 6804
Bacillus stratosphericus strain A7
Bacillus pumilusATCC 7061
Bacillus zhangzhouensisDW5-4
Bacillus safensisFO-36b
BS-15
Bacillus atrophaeus
Bacillus licheniformisATCC 14580
Bacillus sonorensisNBRC 101234
Bacillus subtilis subsp. subtilis NCIB 3
Bacillus tequilensisKCTC 13622
Bacillus subtilis .spizizeniiNRRL 2304
Bacillus subtilis BJG
Bacillus halotoleransATCC 25096
BS-10
BS-17
0.001

B. xiamenensis, *B. safensis*, *B. subtilis*, *B. altitudinis* and *B. altitudinis*, respectively) (Figure 2(a,b)). The sequences of 16S rDNA of AC-5 (1467 bp), AC-6 (1476 bp), AC-10 (1008 bp), AC-18 (1483 bp), AC-19 (1481 bp), BS-10 (1502 bp), BS-15 (1502 bp), BS-17 (1509 bp), BS-19 (1499 bp) and BS-20 (1488 bp) were submitted to GenBank and accession numbers, MF361862, MF359563, MF359746, MF359734, MF359745, MF359733, MF359735, MF359737, MF370070 and MF370069, respectively, were obtained.

Antagonistic and PGP potentials of the selected *Streptomyces* and *Bacillus* isolates under greenhouse conditions

Antagonistic potentials

Under greenhouse conditions, when the selected *Streptomyces* and *Bacillus* isolates were evaluated for their antagonistic potential against FOC, up to 78% and 89% reduction of Fusarium wilt disease incidence, respectively, was observed at 30 DAS over the positive (FOC inoculated) control. In the positive control, 100% disease incidence was noticed within 20 DAS itself. Of the three method of inoculations, such as seed treatment, soil application and seed treatment + soil application, reduction in Fusarium wilt incidence of up to 44%, 78% and 56%, respectively, for *Streptomyces* and up to 88%, 89% and 84%, respectively, for *Bacillus* isolates were observed. Of the five selected *Streptomyces* isolates, reduction of disease incidence was found maximum in AC-19 followed by AC-5 and AC-10 whereas for *Bacillus* isolates, BS-20 followed by BS-19 and BS-15 over the positive control. Both *Streptomyces* and *Bacillus* consortia also showed reduction of disease incidence, 11—44% and 40—55%, respectively, over the positive control (Figure 3(a,b)).

PGP potentials

Under greenhouse conditions, at 30 DAS, the selected five *Streptomyces* and five *Bacillus* isolates, significantly enhanced plant height (up to 34% and 27%, respectively), branches number (47% and 47%, respectively), pod number (up to 100% and 100%, respectively), nodule number (64% and 32%, respectively), nodule weight (71% and 81%, respectively), leaf

Figure 2. (a) Phylogenetic relationship between the five FOC antagonistic *Streptomyces* isolates and representative species based on full length 16S rDNA sequences constructed using the neighbour-joining method. The number at each branch is the percentages of times the group of strains in that branch occurred, based on 1000 cycles in bootstrap analysis. (b) Phylogenetic relationship between the five FOC antagonistic *Bacillus* isolates and representative species based on full length 16S rDNA sequences constructed using the neighbour-joining method. The number at each branch is the percentages of times the group of strains in that branch occurred, based on 1000 cycles in bootstrap analysis.

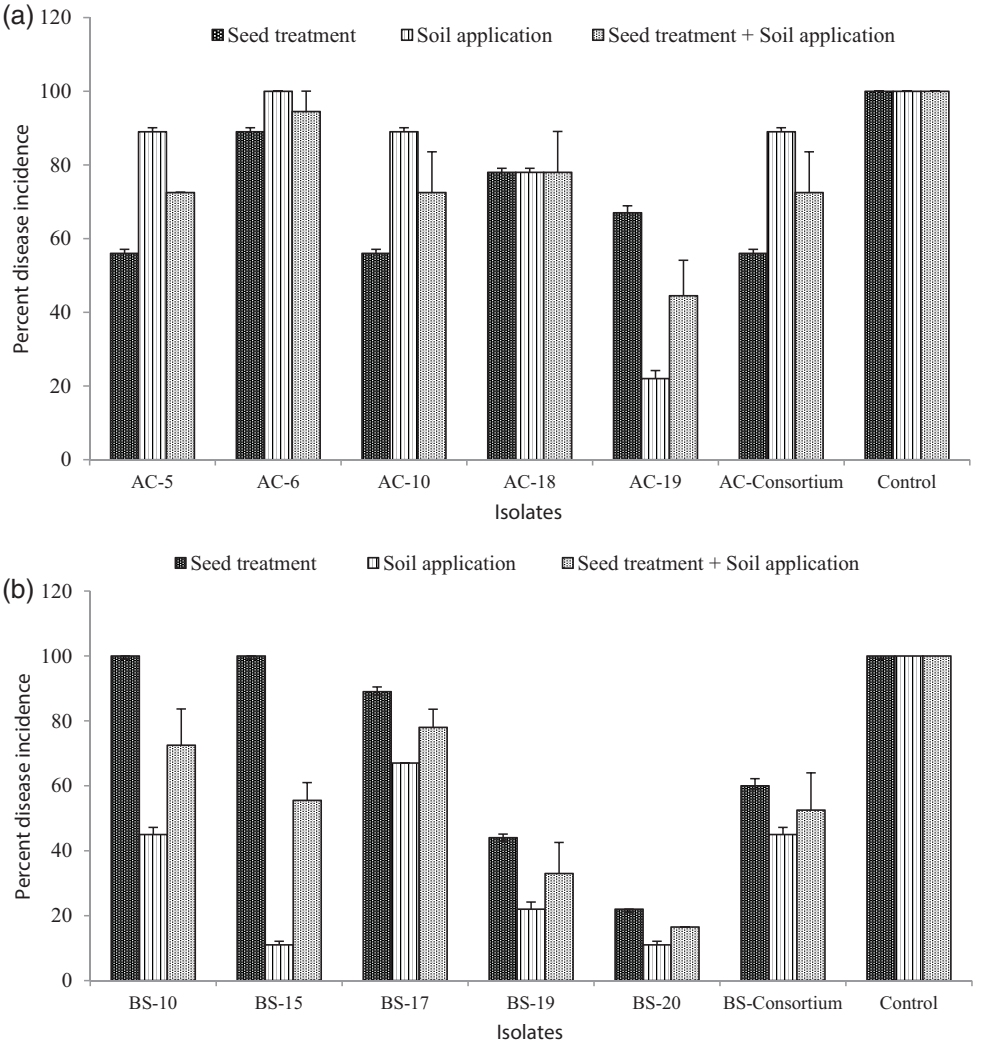


Figure 3. (a) Evaluation of *Streptomyces* isolates for their antagonistic activity against FOC in wilt sick pots in the greenhouse conditions – at 30 days after sowing. (b) Evaluation of *Bacillus* isolates for their antagonistic activity against FOC in wilt sick pots in the greenhouse conditions – at 30 days after sowing.

area (41% and 60%, respectively), leaf weight (58% and 60%, respectively), root weight (56% and 69%, respectively), stem weight (37% and 46%, respectively), total plant weight (50% and 54%, respectively), surface area (48% and 52%, respectively), root length (42% and 48%, respectively) and root volume (54% and 56%, respectively) over the un-inoculated control. The selected *Streptomyces* and *Bacillus* isolates, at 45 DAS, significantly enhanced plant height (23% and 23%, respectively), branches number (42% and 53%, respectively), nodule number (50% and 47%, respectively), nodule weight (47% and 54%, respectively), flower

number (100%, 100%, respectively), pod number (100% and 100%, respectively), root weight (36% and 33%, respectively), stem weight (51% and 46%, respectively), leaf weight (30% and 38%, respectively) and total plant weight (31% and 36%) and while at final harvest, pod number (29% and 100%, respectively), shoot weight (37% and 100%, respectively), plant dry weight (27% and 100%, respectively) seed number (29% and 100%, respectively) and seed weight (33% and 100%, respectively) over the un-inoculated control. Of the five selected *Streptomyces* isolates, enhanced PGP traits were found maximum in AC-19 followed by AC-5 and AC-10 whereas for *Bacillus* isolates, BS-20 followed by BS-19 and BS-15 over the un-inoculated control. Both *Streptomyces* and *Bacillus* consortium also significantly enhanced all the observed PGP traits at 30 DAS, 45 DAS and final harvest (Tables 2—4).

Antagonistic and PGP potentials of selected *Streptomyces* and *Bacillus* isolates under field conditions

Antagonistic potentials

Under wilt sick field conditions, when the chickpea cultivar JG62 (early wilter) was used, a reduction of wilt incidence of up to 32% and 31% (for *Streptomyces* and *Bacillus* isolates, respectively) was observed at 22 DAS over the control (*Streptomyces*/*Bacillus* un-inoculated control). When the chickpea cultivar K850 (later wilter) was used, a reduction of wilt incidence of up to 45% and 32% (for *Streptomyces* and *Bacillus* isolates, respectively) was observed at 45 DAS over the control (*Streptomyces*/*Bacillus* un-inoculated control). Reduction of disease incidence was found maximum with AC-19 followed by AC-5 and AC-10 for *Streptomyces* and BS-19 followed by BS-20 and BS-15 for *Bacillus* isolates while the other four isolates (AC-6, AC-18, BS-10 and BS-17) showed lower levels of reduction of wilt disease incidence in both JG62 and K850 cultivars over the control (Figure 4(a–d)).

PGP potentials

Under field conditions, when JG11 was used, at 35 DAS, the selected five *Streptomyces* and five *Bacillus* isolates, significantly enhanced plant height (up to 16% and 4%, respectively), nodule number (53% and 54%, respectively), nodule weight (43% and 63%, respectively), leaf area (40% and 37%, respectively), leaf weight (52% and 51%, respectively) and stem weight (48% and 37%, respectively), over the un-inoculated control. The selected *Streptomyces* and *Bacillus* isolates, at 60 DAS, significantly enhanced plant height (18% and 15%, respectively), nodule number (36% and 33%, respectively), nodule weight (61% and 38%, respectively), shoot weight (32% and 22%, respectively), pod number (49% and 24%,

Table 2. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under greenhouse conditions – at 30 days after sowing (DAS).

Isolate	Plant height (cm)	Branch number (plant^{-1})	Pod number (plant^{-1})	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Leaf area (cm^2 plant^{-1})	Leaf weight (g plant^{-1})	Root weight (g plant^{-1})	Stem weight (g plant^{-1})	Total plant weight (g)	Surface area (cm^2 plant^{-1})	Root length (cm plant^{-1})	Root volume (cm^3 plant^{-1})
AC-5	27.2	5.7	0.8	56	35	41.5	0.58	0.36	0.37	0.96	483	2855	6.51
AC-6	24.2	4.5	0.0	80	78	36.5	0.56	0.28	0.38	0.94	493	2805	6.91
AC-10	28.2	4.0	0.7	56	102	40.9	0.62	0.32	0.43	1.05	476	2605	6.94
AC-18	32.2	4.7	0.7	47	93	52.9	0.75	0.37	0.50	1.25	581	2856	9.43
AC-19	33.8	4.7	1.2	89	85	46.0	0.48	0.28	0.44	0.92	353	2214	4.48
AC-consor@	28.7	5.0	0.0	59	125	33.8	0.45	0.30	0.44	0.89	373	2266	4.88
BS-10	25.2	3.7	0.0	47	18	44.6	0.55	0.42	0.34	0.89	626	3181	9.85
BS-15	30.3	4.7	1.7	41	158	78.6	0.80	0.39	0.58	1.38	495	2161	9.37
BS-17	27.8	5.7	2.0	35	50	45.4	0.56	0.32	0.42	0.97	373	2340	4.76
BS-19	28.2	4.5	0.0	34	58	35.7	0.44	0.28	0.41	0.84	326	2132	4.64
BS-20	26.3	3.5	0.0	36	57	36.2	0.45	0.52	0.36	0.81	501	2726	7.36
BS-consor[a]	31.7	5.2	0.2	53	83	65.6	0.65	0.43	0.59	1.24	483	2507	7.45
Control	22.2	3.0	0.0	32	30	31.4	0.32	0.16	0.31	0.63	300	1645	4.35
Mean	28.1	4.5	0.6	51	75	45.3	0.56	0.34	0.43	0.98	451	2484	6.69
SE±	1.66**	0.36***	0.42*	10.2**	15.3***	6.45***	0.056***	0.044***	0.058*	0.112**	39.7***	166.7***	0.907***
LSD (5%)	4.8	1.05	1.23	29.6	44.6	18.8	0.163	0.127	0.168	0.326	115.8	486.7	2.647
CV%	10	14	133	32	35	25	17	22	23	20	15	12	24

[a]Consortium.
*Statistically significant at 0.05.
**Statistically significant at 0.01.
***Statistically significant at 0.001.

Table 3. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under greenhouse conditions – at 45 days after sowing (DAS).

Isolate	Plant height (cm)	Branch number (plant^{-1})	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Flower number (plant^{-1})	Pod number (plant^{-1})	Root weight (g plant^{-1})	Stem weight (g plant^{-1})	Leaf weight (g plant^{-1})	Total plant weight (g)
AC-5	33.8	4.7	98	377	1.3	0.8	0.67	0.51	1.01	1.52
AC-6	35.5	6.0	97	428	0.0	0.0	0.84	0.89	0.93	1.81
AC-10	34.3	4.8	77	363	0.8	0.7	0.69	0.59	1.18	1.77
AC-18	37.2	5.0	87	330	2.5	0.7	0.63	0.50	1.04	1.53
AC-19	35.3	6.0	101	513	2.3	1.2	0.56	0.84	0.92	1.75
AC-consor[a]	35.8	5.3	55	477	0.8	0.0	1.02	0.65	0.86	1.50
BS-10	30.8	5.3	57	588	0.0	0.0	0.81	0.67	1.33	2.00
BS-15	32.0	6.5	96	423	2.2	1.7	0.65	0.81	1.13	1.94
BS-17	31.5	6.8	61	298	0.8	1.0	0.67	0.51	1.17	1.68
BS-19	32.8	5.8	55	275	2.5	1.0	0.59	0.65	1.09	1.74
BS-20	35.7	7.5	73	368	2.0	0.0	0.55	0.77	0.97	1.75
BS-consor[a]	35.7	7.2	55	300	3.3	0.2	0.60	1.01	1.11	2.12
Control	27.3	3.5	51	270	0.0	0.0	0.54	0.43	0.82	1.25
Mean	33.7	5.7	74	385	1.4	0.6	0.68	0.68	1.04	1.72
SE±	1.60**	0.56**	6.9***	39.7***	1.32^{NS}	0.59^{NS}	0.087*	0.103**	0.096*	0.118**
LSD (5%)	4.7	1.63	20.2	115.8	3.86	1.75	0.255	0.301	0.282	0.345
CV%	8	17	16	18	159	188	22	26	16	12

[a]Consortium.
*Statistically significant at 0.05.
**Statistically significant at 0.01.
***Statistically significant at 0.001.
NS = Not significant.

Table 4. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under greenhouse conditions – at crop maturity.

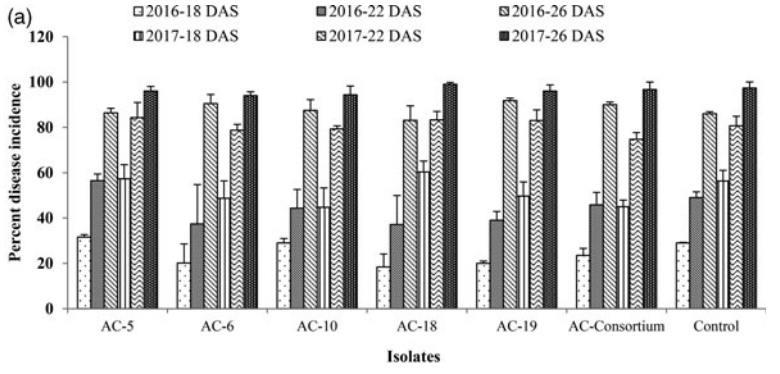
Isolate	Pod number (plant^{-1})	Shoot weight (g plant^{-1})	Pod dry weight (g plant^{-1})	Seed number (plant^{-1})	Seed weight (g plant^{-1})
AC-5	19.7	5.25	4.81	20	3.60
AC-6	18.0	5.24	4.56	19	3.91
AC-10	20.7	4.97	5.17	21	4.06
AC-18	16.0	4.55	4.20	17	3.20
AC-19	19.3	4.57	4.76	20	3.81
AC Consortium	18.3	3.88	4.76	18	3.75
BS-10	19.7	5.02	5.87	21	4.68
BS-15	18.7	7.13	5.25	19	3.60
BS-17	19.7	6.22	5.16	20	3.66
BS-19	18.3	6.09	5.26	18	3.89
BS-20	16.7	6.77	4.97	17	3.74
BS Consortium	26.0	4.44	5.35	26	4.45
Control	14.7	3.29	3.77	15	2.74
Mean	18.9	5.19	4.91	19	3.77
SE±	1.83*	0.459***	0.323*	1.8*	0.299*
LSD (5%)	5.4	1.34	0.942	5.2	0.873
CV%	17	15	11	16	14

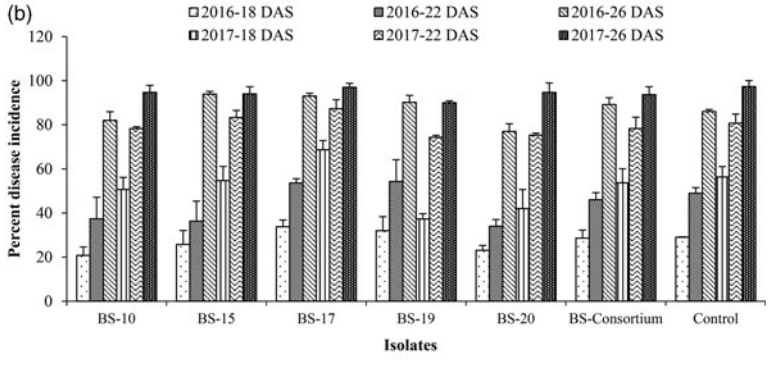
*Statistically significant at 0.05.
***Statistically significant at 0.001.

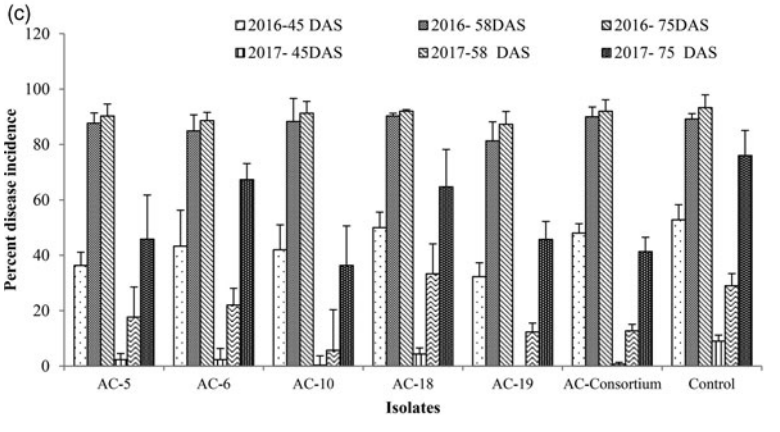
respectively) and pod weight (64% and 45%, respectively) and while at final harvest, pod number (41% and 36%, respectively), pod weight (42% and 34%, respectively), seed number (41% and 34%, respectively), stover weight (37% and 29%, respectively) and grain yield (34% and 28%, respectively) over the un-inoculated control. Of the five selected *Streptomyces* isolates, enhanced PGP traits were found maximum in AC-19 followed by AC-5 and AC-10 whereas for *Bacillus* isolates, BS-19 followed by BS-20 and BS-15 over the un-inoculated control. Both *Streptomyces* and *Bacillus* consortium also significantly enhanced all the observed PGP traits at 35 DAS, 60 DAS and final harvest (Tables 5—7). The colonisation of *Streptomyces* and *Bacillus* isolates on the roots of chickpea was demonstrated by SEM. Extensive colonisation was observed on the roots of chickpea by all the isolates (Figure 5).

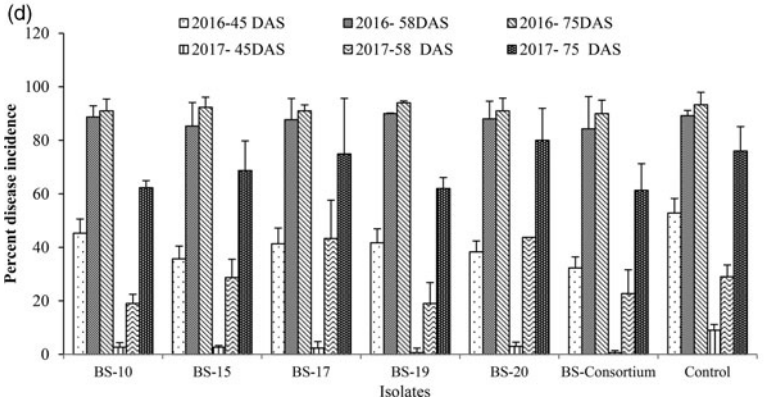
Discussion

Actinobacteria, *Streptomyces* in particular, and *Bacillus* are reported widely for their antagonistic and PGP properties in various crops (Moradi et al. 2012). However, we did not have good biocontrol agents for controlling Fusarium wilt of chickpea. Hence, the present investigation was aimed to isolate these two biocontrol agents from rhizosphere soils of chickpea and to evaluate for their antagonistic potential against FOC and PGP potentials in chickpea. Of the 40 actinomycete and *Bacillus* isolates tested for their antagonistic potential against FOC by dual-culture assay, only 10 of them (25%; 5 each of actinomycetes and


(a)
2016-18 DAS
2016-22 DAS
2016-26 DAS
2017-18 DAS
2017-22 DAS
2017-26 DAS
Percent disease incidence
120
100
80
60
40
20
0
AC-5
AC-6
AC-10
AC-18
AC-19
AC-Consortium
Control
Isolates


(b)
2016-18 DAS
2016-22 DAS
2016-26 DAS
2017-18 DAS
2017-22 DAS
2017-26 DAS
Percent disease incidence
120
100
80
60
40
20
0
BS-10
BS-15
BS-17
BS-19
BS-20
BS-Consortium
Control
Isolates


(c)
2016-45 DAS
2016- 58DAS
2016- 75DAS
2017- 45DAS
2017-58 DAS
2017- 75 DAS
Percent disease incidence
120
100
80
60
40
20
0
AC-5
AC-6
AC-10
AC-18
AC-19
AC-Consortium
Control
Isolates


(d)
2016-45 DAS
2016- 58DAS
2016- 75DAS
2017- 45DAS
2017-58 DAS
2017- 75 DAS
Percent disease incidence
120
100
80
60
40
20
0
BS-10
BS-15
BS-17
BS-19
BS-20
BS-Consortium
Control
Isolates

Bacillus) were found to have good antagonistic potential against FOC, of which BS 20 and AC-5 were found to inhibit FOC the most.

In the present study, the selected 10 actinomycete and *Bacillus* isolates were found to produce IAA, β-1,3-glucanase, cellulase (except AC-5), protease (except AC-5, AC-10 and BS-10), lipase (except AC-5), HCN (except AC-18 and BS-10) and siderophore (only by four isolates; AC-10, AC-18, BS-15 and BS-20). Of the 10 FOC antagonistic isolates studied, only BS-20 was found to produce all the PGP and biocontrol traits. Microbes producing IAA are known to stimulate seed germination, root formation and increase root surface area and length, thereby providing the host plant greater access to water and soil nutrients, fruit formation and abscission control (Khamna et al. 2009). The ability of microbes to produce extra cellular enzymes such as β-1,3-glucanase, cellulase, protease and lipase helps in controlling the plant pathogens by acting on their cell walls, thereby indirectly functions as PGP (Chet and Inbar 1994; Lima et al. 1998; Ellis et al. 2000; Lynd et al. 2002; Haas and Defago 2005). HCN production by microbes is reported widely to play a role in disease suppression (Haas et al. 1991; Wei et al. 1991; Siddiqui 2006). Siderophores function as solubilising agents for iron from minerals under conditions of iron limitation and helps to inhibit the growth of plant pathogens (Tokala et al. 2002). Siderophores producing microbes bind Fe^{3+} from the environment and make it available for its own growth as well as make it available for plants (Wang et al. 2014). In the present study, the selected actinomycetes and *Bacillus* isolates produced one or more extra cellular enzymes and growth-promoting hormones and hence it can be concluded that these have good biocontrol and PGP potentials (Table 1).

Phylogenetic analysis of 16S rDNA sequences of the selected five FOC antagonistic actinomycetes showed that all isolates matched 100% with *Streptomyces* but different species while the selected five *Bacillus* isolates matched 100% with *Bacillus* but different species (Figure 2(a,b)). The sequences of the 10 FOC antagonistic bacteria were submitted to GenBank and the cultures to ICRISAT microbial collection bank.

In the present investigation, the five *Streptomyces* and *Bacillus* isolates were demonstrated for their antagonistic potentials against FOC under both greenhouse as well as wilt sick field conditions. The germination

Figure 4. (a) Evaluation of *Streptomyces* isolates for their antagonistic activity against FOC in wilt sick field conditions – on chickpea cultivar JG62. (b) Evaluation of *Bacillus* isolates for their antagonistic activity against FOC in wilt sick field conditions – on chickpea cultivar JG62. (c) Evaluation of *Streptomyces* isolates for their antagonistic activity against FOC in wilt sick field conditions – on chickpea cultivar K850. (d) Evaluation of *Bacillus* isolates for their antagonistic activity against FOC in wilt sick field conditions – on chickpea cultivar K850.

Table 5. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under field conditions – at 35 days after sowing (DAS).

	Year 2016/2017						Year 2017/2018					
Isolate	Plant height (cm)	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Leaf area (cm^{-2} plant^{-1})	Leaf weight (g plant^{-1})	Stem weight (g plant^{-1})	Plant height (cm)	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Leaf area (cm^{-2} plant^{-1})	Leaf weight (g plant^{-1})	Stem weight (g plant^{-1})
AC-5	24.8	35	104	96	863	480	22.5	24	45	38	589	296
AC-6	20.6	31	89	99	677	469	25.9	26	49	56	853	486
AC-10	25.1	31	73	101	879	533	26.1	40	74	55	785	391
AC-18	21.1	25	68	102	708	493	22.7	22	46	37	602	290
AC-19	21.4	30	64	82	566	479	24.8	22	45	39	560	262
AC-consor[a]	23.5	31	82	98	742	508	20.8	28	45	37	659	311
BS-10	21.8	30	159	93	633	495	24.9	31	71	52	630	446
BS-15	20.1	26	65	86	563	470	24.7	28	80	53	757	402
BS-17	21.1	23	69	98	676	467	21.8	21	47	36	483	268
BS-19	20.7	31	70	83	781	445	22.8	22	44	36	539	256
BS-20	22.3	45	104	106	748	499	24.9	28	56	50	827	384
BS-consor[a]	22.2	35	99	80	614	595	22.9	26	44	39	420	254
Control	21.1	21	59	80	533	441	22.5	19	43	34	409	252
Mean	22.0	30	85	93	691	490	23.6	26	53	43	624	331
SE±	0.99*	3.3**	15.2**	13.8^{NS}	133.0^{NS}	25.7*	0.78***	3.1**	7.1**	6.5^{NS}	65.9***	25.2***
LSD (5%)	2.90	9.7	44.3	40.2	388.3	74.9	2.28	9.1	20.8	18.9	192.4	73.7
CV%	8	19	31	26	33	9	6	21	23	26	18	13

[a]Consortium.
*Statistically significant at 0.05.
**Statistically significant at 0.01.
***Statistically significant at 0.001.
NS = Not significant.

Table 6. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under field conditions – at 60 days after sowing (DAS).

	Year 2016/2017						Year 2017/2018					
Isolate	Plant height (cm)	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Shoot weight (g plant^{-1})	Pod number (plant^{-1})	Pod weight (g plant^{-1})	Plant height (cm)	Nodule number (plant^{-1})	Nodule weight (mg plant^{-1})	Shoot weight (g plant^{-1})	Pod number (plant^{-1})	Pod weight (g plant^{-1})
AC-5	38.3	15	80	11.75	19.3	641	36.0	13	50	5.17	25.0	1.283
AC-6	31.0	14	66	11.50	14.7	587	38.3	20	100	7.49	46.0	2.113
AC-10	37.0	15	88	11.15	9.3	228	40.0	17	64	6.54	28.3	1.653
AC-18	35.0	16	82	13.26	13.7	352	36.7	13	52	5.40	21.7	1.035
AC-19	35.0	17	80	10.00	9.7	214	35.3	14	45	6.46	31.3	2.061
AC-consor[a]	36.3	17	94	9.97	15.3	395	34.0	14	58	5.85	22.7	0.655
BS-10	37.0	16	65	10.22	11.7	307	36.0	15	52	5.35	21.0	1.243
BS-15	33.3	14	73	10.11	12.7	252	35.0	14	41	5.59	26.3	1.581
BS-17	38.7	16	67	11.43	16.3	531	35.3	15	56	5.84	29.7	1.976
BS-19	36.3	19	104	12.66	12.3	312	34.3	15	41	5.56	36.0	1.650
BS-20	36.0	17	102	11.75	16.7	301	31.3	14	43	5.90	23.7	1.108
BS-consor[a]	42.0	19	72	14.53	15.7	532	34.3	13	40	6.73	22.3	1.093
Control	38.7	13	64	9.92	12.7	365	33.7	13	39	5.12	23.7	1.231
Mean	36.5	16	80	11.40	13.9	386	35.4	15	52	5.92	27.5	1.437
SE±	1.83*	1.9^{NS}	20.9^{NS}	0.84**	3.65^{NS}	178.5^{NS}	1.35*	1.3*	9.1**	0.445*	2.72***	0.266*
LSD (5%)	5.34	5.8	61.0	2.45	10.65	520.9	3.94	3.8	26.5	1.300	7.95	0.777
CV%	9	22	45	13	46	80	7	15	30	13	17	32

[a]Consortium.
*Statistically significant at 0.05.
**Statistically significant at 0.01.
***Statistically significant at 0.001.
NS = Not significant.

Table 7. Effect of *Streptomyces* and *Bacillus* isolates on PGP traits of chickpea under field conditions – at crop maturity.

	Year 2016/2017					Year 2017/2018				
Isolate	Pod number (plant^{-1})	Pod weight (g plant^{-1})	Seed number (plant^{-1})	Stover weight (t ha^{-1})	Grain weight (t ha^{-1})	Pod number (plant^{-1})	Pod weight (g plant^{-1})	Seed number (plant^{-1})	Stover weight (t ha^{-1})	Grain weight (t ha^{-1})
AC-5	36	9.2	37	1.36	1.29	27	6.68	28	1.72	1.00
AC-6	43	10.8	44	1.34	1.43	35	8.59	37	2.10	1.24
AC-10	38	9.5	40	1.32	1.29	45	11.12	46	2.76	1.42
AC-18	35	9.4	37	1.36	1.30	28	7.08	28	1.79	0.96
AC-19	38	10.2	40	1.56	1.31	33	8.04	33	2.17	1.09
AC-consor[a]	39	9.5	40	1.53	1.29	30	7.47	31	2.04	1.07
BS-10	43	12.2	43	1.40	1.52	38	9.54	39	2.06	1.31
BS-15	41	11.5	42	1.44	1.41	41	9.57	41	2.30	1.20
BS-17	38	9.4	39	1.35	1.32	37	9.74	37	2.33	1.19
BS-19	38	9.3	39	1.48	1.34	26	7.65	27	2.00	1.08
BS-20	41	11.2	43	1.44	1.52	27	9.98	27	2.47	1.06
BS-consor[a]	44	12.0	45	1.58	1.66	44	9.29	45	2.38	1.24
Control	34	9.0	36	1.32	1.26	26	6.46	27	1.74	0.94
Mean	39	10.3	40	1.42	1.38	34	8.55	34	2.14	1.14
SE±	1.9*	1.58^{NS}	2.0*	0.059*	0.077*	3.2**	1.454^{NS}	3.3**	0.150**	0.068**
LSD (5%)	5.5	4.62	5.8	0.171	0.224	10.0	4.479	10.1	0.462	0.209
CV%	8	27	9	7	10	14	24	14	10	8

[a]Consortium.
*Statistically significant at 0.05.
**Statistically significant at 0.01.
NS = Not significant.

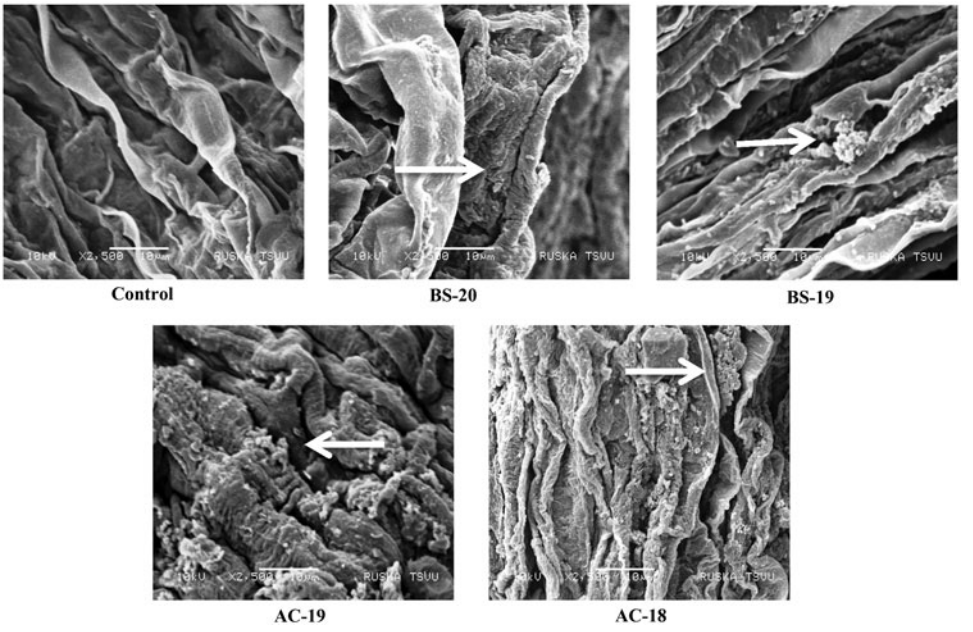


Figure 5. Scanning electron microscopy images of chickpea roots colonisation of *Streptomyces* and *Bacillus* isolates. Note: Arrows indicate chickpea roots colonised by BS-20, BS-19, AC-19 and AC-18.

percentage of the seeds under both greenhouse and field studies were more than 90%, 80% (mean of both JG-62 and K850), respectively, in both test organisms as well as non-inoculated control treatments. Hence, it can be concluded that the test organisms have no negative impact on the germination and growth of chickpea. Of the five selected *Streptomyces* isolates, reduction of disease incidence was found maximum in AC-19 followed by AC-5 and AC-10 whereas for *Bacillus* isolates, BS-19 followed by BS-20 and BS-15 over the positive control in both greenhouse as well as field conditions (Figure 3(a–d)). *Streptomyces* spp. have been reported widely to protect crop plants against plant pathogens such as *Rhizoctonia solani* (causes pea root rot) and *F. oxysporum* f. sp. *cubense* (causes wilt in banana) (Rothrock and Gottlieb 1984; Liu et al. 1996; Getha et al. 2005). *Streptomyces* spp. were also reported to control FOC in chickpea (Bashar and Rai 1994; Nonoh et al. 2010). *Bacillus* spp. have also been reported widely to protect crop plants against phytopathogens (Boulter et al. 2002; Johri et al. 2003; Saharan and Nehra 2011). The mechanisms involved in biocontrol of plant pathogens by antagonistic bacteria include either by competition (Elad and Chet 1987) or by metabolite production such as siderophore, HCN, antibiotics or extracellular enzymes such as cellulase, chitinase, protease, lipase and β-1,3-glucanase (Sang et al. 2006; El Hassni et al. 2007; Idris et al. 2007). In the

present investigation, the selected FOC antagonistic *Streptomyces* and *Bacillus* isolates produced many of these extracellular enzymes and HCN, which could have played a role in suppression of FOC.

In the present study, the selected FOC antagonistic *Streptomyces* and *Bacillus* isolates were found to significantly enhance PGP traits including nodule number, nodule weight, shoot weight, root weight, pod number, pod weight, seed number, seed weight, gain yield and stover yield under both greenhouse as well as field conditions (Tables 2—7). Bacteria are reported to chemo-attract towards the root exudates, colonise in rhizosphere and played an important role in reducing pathogen population and enhancing plant nutrition (Bulluck et al. 2002; Lugtenberg and Kamilova 2009). Such bacteria, including *Streptomyces* and *Bacillus*, are reported widely to enhance not only nodulation and nitrogen fixation but also grain and stover yield in crops, including tomato, wheat, rice, sorghum, bean, pigeonpea and chickpea (Tokala et al. 2002; El-Tarabily et al. 2009; Choudhary and Johri 2009; Sadeghi et al. 2012; Gopalakrishnan et al. 2014, 2015, 2016; Sreevidya and Gopalakrishnan 2017). In the present study, the enhancement of yield and other PGP traits were probably contributed either by IAA or by other hydrolytic enzymes, produced by *Streptomyces* and *Bacillus* isolates.

In the present study, under both greenhouse and field conditions, *Streptomyces* sp. AC-19 and *Bacillus* sp. BS-20 significantly inhibited FOC and enhanced morphological and yield parameters of chickpea such as plant height, leaf area and weight, stem weight, root length, volume and weight, nodule number, nodule weight, stove yield and grain yield over the un-inoculated control. The mechanism by which the AC-19 and BS-20 inhibited FOC and enhanced the plant growth and yield parameters of chickpea could be attributed to their siderophore, IAA, lipase, protease, β-1,3-glucanase, HCN producing capabilities and/or to their ability to survive under harsh environments. Such broad spectrum PGP and biocontrol agents may offer potentially effective novel strategies not only for controlling multiple pathogens and insect pests but also help in conservation of the rapidly eroding agricultural lands. There is a need to do additional comprehensive research for identifying mode of action of AC-19 and BS-20 in controlling Fusarium wilt in chickpea and conduct multi-location trials.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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