

Research Note

Characterization of Antagonistic *Streptomyces* As Potential Biocontrol Agent Against Fungal Pathogens of Chickpea and Sorghum

Gottumukkala Alekhya and Subramaniam Gopalakrishnan*

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

*Author for correspondence; e-mail: s.gopalakrishnan@cgiar.org; Phone: +91-40-30713610; Fax: +914030713074

Twenty-seven actinomycetes isolated from vermicompost and rhizosphere soils were screened for their antagonistic potential against fungal pathogens of chickpea and sorghum. Eight isolates (BCA-657, BCA-671, BCA-679, BCA-687, BCA-690, CAI-67, CAI-70 and CAI-98) showed broad-spectrum antagonistic activity in dual-culture assay and metabolite inhibition assay. The isolates exhibited growth at pH 7–11, up to 10% saline (except BCA-687, CAI-67 and CAI-70) at 20–40 °C (BCA-679 and BCA-690 exhibited growth even at 50 °C) and produced siderophore, chitinase, cellulase, lipase, and protease with some exceptions, including hydrocyanic acid and indole acetic acid. At field application level, all the isolates tolerated the fungicide Bavistin. The sequences of 16S rDNA gene of the isolates matched with *Streptomyces* but with different species in the BLAST analysis. This study indicates that the selected actinomycetes have broad-spectrum biocontrol and plant growth promotion potential.

Key Words: antagonistic actinomycetes, chickpea, plant pathogens, PGP traits, sorghum

Abbreviations: BCA – biocontrol actinomycetes, CAI – compost actinomycete isolate, FOC – *Fusarium oxysporum* f. sp. *ciceri*, HCN – hydrocyanic acid, IAA – indole acetic acid, PDA – potato dextrose agar, PGP – plant growth promotion, SCB – starch casein broth, SRI – system of rice intensification

INTRODUCTION

Chickpea (*Cicer arietinum* L.) and sorghum (*Sorghum bicolor* [L.] Moench) are major food crops grown in the semi-arid tropics of Asia and Africa. Productivity of these crops can be considerably improved if the adverse effects of both biotic and abiotic stresses are addressed. Chickpea is severely affected by *Fusarium* wilt, *Ascochyta* blight, dry root rot, collar rot and botrytis grey mold, resulting in reduced crop yield (Nikam et al. 2007; Akhtar and Siddiqui 2010; Sharma et al. 2010) whereas yield loss in sorghum is mainly due to infection by charcoal rot, rust, leaf blight and grain mold (Shamarao et al. 2001).

In conventional agriculture, chemical fungicides are used to control plant diseases. However, the use of excess chemicals poses a serious problem to the environment by increasing resistance and accumulation of chemicals, the result of which is a growing need for substitutes. Biological control is slow, but it is a harmless method to control pathogens; however, it does not eliminate the pathogen completely but maintains the balance (Ningthoujam et

al. 2009). It is gaining greater attention due to its low cost, ecofriendly application and public concern about the usage of hazardous chemicals (Kumar et al. 2007). It can also be an alternative to the use of chemicals (Sharma and Parihar 2010). The use of *Trichoderma* spp., *Pseudomonas* spp., *Bacillus* spp. and *Streptomyces* spp. for biological control of fungal plant pathogens has been reported widely (Gozale and Hernandez 2009; Gopalakrishnan et al. 2011a, b).

Actinomycetes are gram-positive filamentous bacteria that are known to produce antibiotics effective against fungal plant pathogens and possess plant-growth promoting (PGP) traits (Bressen 2003; Shahidi et al. 2004; Gopalakrishnan et al. 2012a, b). The antagonistic activity of the actinomycetes is attributed to production of extracellular hydrolytic enzymes such as chitinase, lipase, protease, cellulase and β -glucanase (Srividya et al. 2012). Soil actinomycetes, particularly *Streptomyces*, possess not only plant-growth promoting traits (Gopalakrishnan et al. 2013), which enhance soil fertility, but also antagonistic activity against soil-borne plant pathogens (Aghighi et al. 2004). In the present investigation, actinomycetes

isolated from rhizosphere soils and vermicompost were evaluated for their physiological properties, fungicide tolerance, PGP traits and antagonistic properties against wilt, collar rot, dry root rot, gray mold diseases of chickpea and charcoal rot and grain mold diseases of sorghum.

MATERIALS AND METHODS

Evaluation of Actinomycete Isolates for Antifungal Activity

The actinomycetes isolated from vermicompost and rhizosphere soils of rice grown by system of rice intensification (SRI) fields at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were evaluated for antifungal activity against pathogens of chickpea, including *Fusarium oxysporum* f. sp. *ciceri* (FOC; causes Fusarium wilt), *Rhizoctonia bataticola* (causes dry root rot; three strains RB-6, RB-24 and RB-115) and *Botrytis cinerea* (causes Botrytis gray mold), and pathogens of sorghum, including *F. andiyazi* FM-943 and *F. proliferatum* FM-242 (causes grain mold) and *Macrophomina phaseolina* (causes charcoal rot). FOC, *B. cinerea* and the three strains of *R. bataticola* were acquired from the legumes pathology division whereas *F. andiyazi* and *F. proliferatum* were acquired from the cereal pathology division, ICRISAT, Patancheru, India. *M. phaseolina* were acquired from the Directorate of Sorghum Research (DSR), Hyderabad, India. The antagonistic activity of the actinomycetes was evaluated by dual culture assay, based on the protocol of Gopalakrishnan et al. (2011a, b), using Glucose Casamino acid Yeast extract (GCY agar; Anjaiah et al. 1998) plates. The inhibition by the isolates was recorded as follows: – (no inhibition zone) = no inhibition; + (1–3 mm zone of inhibition) = little inhibition; ++ (4–8 mm zone of inhibition) = good inhibition; and +++ (8 mm and above inhibition zone) = excellent inhibition.

Metabolite Inhibition Assay

The actinomycetes were grown in Starch Casein Broth (SCB) for 6 d. The culture filtrate was separated by centrifugation at 10,000g for 15 min and passed through Millipore filter (0.2 mm). The filter-sterilized culture filtrate was evaluated for antagonistic activity against FOC, *R. bataticola* (three strains RB-6, RB-24 and RB-115), *S. rolfisii*, *F. proliferatum* (FM-242), *F. andiyazi* (FM-943) and *M. phaseolina*. A fungal disk (6 mm diameter) was bored and kept in the center of the quarter strength potato dextrose agar (PDA) plate

amended with 10% culture filtrate. Control plates contained no culture filtrates. The plates were incubated at 28 ± 2 °C for 5 d and fungal inhibition was recorded as follows: – = no inhibition; + = little inhibition (1–3 mm zone of inhibition); ++ = good inhibition (4–8 mm zone of inhibition); and +++ = excellent inhibition (8 mm and above zone of inhibition).

Evaluation of Actinomycete Isolates for Physiological Traits

Physiological properties such as pH, temperature and salinity tolerance were studied for all the potential actinomycete isolates. For pH and salinity, the isolates were streaked on Bennet agar, adjusted to different pH (5, 7, 9 and 11), saline concentrations (0–10% at 2% interval) and incubated at 28 °C for 5 d. For temperature, the Bennet agar plates were streaked with the actinomycetes and incubated at different temperatures (20 °C, 30 °C and 40 °C) for 5 d, whereas at 50 °C, the isolates were inoculated in Bennet broth. Responses of the isolates to salinity, pH and temperature were recorded as follows: – = no growth; + = little growth; ++ = moderate growth; and +++ = good growth.

Evaluation of Actinomycetes for PGP and Biocontrol Traits

The actinomycetes were evaluated for their PGP and biocontrol traits, including siderophore, chitinase, cellulase, lipase, protease, hydrocyanic acid (HCN) and indole acetic acid (IAA) production. Siderophore production was estimated based on the protocol of Schwyn and Neilands (1987) and Gopalakrishnan et al. (2011a). Chitin agar plates amended with colloidal chitin suspension and mineral salts were prepared according to standard protocols of Hsu and Lockwood (1975) whereas chitinase estimation was done based on the protocols of Gopalakrishnan et al. (2011a). The standardized protocols of Hendricks et al. (1995) were used to evaluate cellulase production. Protease and lipase production was estimated based on the protocols of Bhattacharya et al. (2009). HCN was qualitatively assessed by the method described by Lorck (1948) and Gopalakrishnan et al. (2011a). Estimation of IAA was done based on the protocols of Patten and Glick (2002) and Gopalakrishnan et al. (2011a).

Fungicide Tolerance

The fungicide tolerance of the actinomycetes was evaluated at full strength and half strength of field application levels based on the protocols of Gopalakrishnan et al. (2012b). In brief, the

actinomycetes were streaked on actinomycetes isolation agar plates containing different concentrations of fungicides. The fungicides studied include Thiram (dimethylcarbamothioylsulfanyl *N*, *N*-dimethylcarbamodithioate), Bavistin (carbendazim 50%; methyl benzimidazol-2-ylcarbamate), Benlate (benomyl 50%; methyl [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl] carbamate), Captan (captan 50%; *N*-trichloromethylthio-4-cyclohexene-1, 2-dicarboximide), Benomyl (methyl [1-[(butylamino)carbonyl]-1H-benzimidazol-2-yl] carbamate) and Radonil (*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl) alanine methyl ester) at field application levels of 3000, 2500, 4000, 3000, 3000 and 3000 $\mu\text{g mL}^{-1}$ concentrations and half strength of field application levels, respectively. At the end of 5 d incubation, the growth was recorded as follows: – = no growth; + = little growth; ++ = good growth; and +++ = excellent growth.

Molecular Identification of the Antifungal Potential Actinomycetes

The antifungal actinomycetes were identified by 16S rDNA sequencing. Pure cultures of antagonistic actinomycetes were grown in SCB broth until log phase (4 d) and genomic DNA was isolated according to the protocol of Bazzicalupo and Fani (1995). The amplification of 16S rDNA gene was done using universal primers 1492R (5'-TAC GGY TAC CTT GTTACG ACT T-3') and 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') based on the conditions described by Pandey et al. (2005). The polymerase chain reaction (PCR) product was sequenced at MacroGen Inc., Seoul, Korea. The sequences obtained were compared with those from the GenBank using the BLAST program (Alschul et al. 1990) and aligned using the Clustal W software (Thompson et al. 1997); phylogenetic trees were inferred using the neighbor-joining method (Saitou and Nei 1987). Bootstrap analysis using MEGA version 4 programs was done to estimate statistical stability of the branches in the cluster with 1,000 replicates (Tamura et al. 2007).

RESULTS

Of the 27 isolates screened for their antagonistic potential against eight plant pathogens of chickpea and sorghum, eight actinomycetes showed broad spectrum activity. Of the eight isolates studied on chickpea diseases, all showed inhibition on *R. bataticola* strain RB-6, *R. bataticola* strain RB-24 (except BCA-657), *R. bataticola* strain RB-115 (except CAI-70), *B. cinerea* (except BCA-657) and FOC (except CAI-98),

whereas on sorghum diseases, all showed inhibition on *M. phaseolina* (except CAI-70), *F. proliferatum* strain FM-242 (except BCA-657 and BCA-687) and *F. andiyazi* strain FM-943 (only for CAI-671, CAI-679, CAI-67 and CAI-70; Table 1).

The cell-free extracts of the selected isolates were further evaluated for their secondary metabolite production capabilities, among which BCA-690 (except *S. rolfssii*) and CAI-70 (except *R. bataticola* strain RB-115) exhibited antagonistic activity against eight pathogens. Of the remaining six isolates, BCA-657 (except FOC and *S. rolfssii*) and BCA-679 (except *R. bataticola* strain RB-115 and *S. rolfssii*) showed antagonistic activity against seven pathogens whereas the remaining four isolates showed antagonistic activity against only four pathogens (Table 2).

Physiological properties such as pH, salinity and temperature were evaluated for the promising eight isolates of actinomycetes. All eight isolates tolerated a wide range of pH (7–11) whereas maximum sporulation was observed at pH 9 (data not shown) and no growth was observed at pH 5. Among the isolates, CAI-98, BCA-657, BCA-671, BCA-679 and BCA-690 showed salt tolerance up to 10%; however, maximum sporulation was observed at 2% NaCl concentration (data not shown). All the isolates were found to grow at 20–40 °C. However, at 50 °C, BCA-690 was able to grow well while BCA-679 had little growth (Table 3).

The biochemical and PGP traits such as chitinase, cellulase, lipase, protease, HCN, siderophore and IAA were estimated for the promising isolates. All the isolates produced chitinase (except BCA-657, CAI-67 and CAI-70), cellulase, lipase (except CAI-70), protease (except CAI-67, CAI-70 and CAI-98), HCN, siderophore (except CAI-67) and IAA. Of the eight isolates, CAI-98 was found to produce the highest chitinase whereas BCA-690 produced the highest amount of cellulase, lipase (also BCA-671 and BCA-679), protease, HCN, siderophore and IAA (52.9 $\mu\text{g mL}^{-1}$) (Table 4).

Fungicide tolerance test of the isolates was conducted at the concentrations at full and half of field application levels. All the isolates showed excellent growth at field level application of Bavistin and good growth at field level application of Thiram (except BCA-679 and BCA-687). None of the isolates tolerated Captan, Benlet, Radonil and Benomyl; however, at half the field level application, the isolates CAI-70 and BCA-679 were able to tolerate Benlate, BCA-690 on Radonil and BCA-657, BCA-679 and BCA-690 on Benomyl (Table 5).

The antagonistic actinomycetes were identified by 16s rDNA analysis. Neighbor-joining dendrogram was

Table 1. Biocontrol potential of eight actinomycetes on the fungal pathogens of chickpea and sorghum.

Isolate	Inhibition on							
	FOC	<i>M. phaseolina</i>	RB-6	RB-24	RB-115	<i>B. cinerea</i>	FM-242	FM-943
BCA-657	+	+++	++	-	+++	-	-	-
BCA-671	++	+++	+++	+++	++	+++	+	+
BCA-679	++	++	+++	+++	+++	+++	+	+
BCA-687	+	++	++	+	++	+++	-	-
BCA-690	++	++	+++	+++	+++	+++	+++	-
CAI-67	+	+	+++	+++	++	+++	++	+
CAI-70	+++	-	+++	+++	-	+++	++	++
CAI-98	-	+++	+++	+++	+	++	++	-
Control	-	-	-	-	-	-	-	-

FOC = *Fusarium oxysporum* f. sp. *ciceri*, RB = *Rhizoctonia bataticola* strains (RB-6, RB-24 and RB-115), *B. cinerea* = *Botrytis cinerea*, FM-242 = *Fusarium proliferatum*, FM-943 = *Fusarium andiyazi*, *M. phaseolina* = *Macrophomina phaseolina*, +++ = excellent inhibition, ++ = good inhibition, + = little inhibition, - = no inhibition.

Table 2. Metabolite activity of eight potential antagonistic actinomycetes on the fungal pathogens of chickpea and sorghum.

Isolate	Inhibition on								
	FOC	RB-6	RB-24	RB-115	<i>S. rolfsii</i>	FM-242	FM-943	<i>M. phaseolina</i>	<i>B. cinerea</i>
BCA-657	-	+++	+	++	-	+	++	+++	+
BCA-671	-	+++	+	-	-	-	-	+++	+++
BCA-679	++	+++	+++	-	-	+++	++	+++	+++
BCA-687	-	-	-	+++	-	-	+	+	++
BCA-690	++	+++	+++	+++	-	+++	+++	+++	+++
CAI-67	-	+++	+++	-	-	-	-	+++	++
CAI-70	+++	+++	+	-	+++	+++	+++	++	++
CAI-98	-	-	-	+++	-	++	-	+	+++
Control	-	-	-	-	-	-	-	-	-

FOC = *Fusarium oxysporum* f. sp. *ciceri*, RB = *Rhizoctonia bataticola* strains (RB-6, RB-24 and RB-115), *S. rolfsii* = *Sclerotium rolfsii*, FM-242 = *Fusarium proliferatum*, FM-943 = *Fusarium andiyazi*, MP = *Macrophomina phaseolina*, *B. cinerea* = *Botrytis cinerea*, +++ = excellent inhibition, ++ = good inhibition, + = little inhibition, - = no inhibition.

Table 3. Physiological properties of eight potential actinomycete isolates.

Isolate	pH				Salinity						Temperature			
	5	7	9	11	0%	2%	4%	6%	8%	10%	20°C	30°C	40°C	50°C
BCA-657	-	+++	+++	++	++	+++	+++	+++	++	+	+++	+++	+++	-
BCA-671	-	+++	+++	++	+++	+++	+++	+++	++	++	+++	+++	+++	-
BCA-679	-	+++	+++	++	+++	+++	+++	+++	++	+	+++	+++	+++	+
BCA-687	-	+++	++	+	++	+++	+++	+	-	-	++	+++	++	-
BCA-690	-	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
CAI-67	-	+++	+++	++	++	+++	+++	++	++	-	++	++	++	-
CAI-70	-	+++	+++	++	++	+++	+	-	-	-	++	++	++	-
CAI-98	-	+++	+++	+++	+++	+++	++	+++	+++	+	++	++	++	-

+++ = Excellent growth, ++ = good growth, + = little growth, - = no growth

Table 4. Enzymatic activities and plant-growth-promoting traits of eight antagonistic actinomycetes.

Isolate	Production Score for						
	Siderophore	Chitinase	Cellulase	Lipase	Protease	HCN	IAA (µg mL ⁻¹)
BCA-657	4.0	0.0	3.0	2.7	1.3	2.0	13.47
BCA-671	4.0	2.0	4.0	5.0	2.0	2.0	37.77
BCA-679	4.3	2.0	4.0	5.0	2.0	1.3	50.05
BCA-687	4.0	2.0	2.7	3.0	2.0	1.3	13.34
BCA-690	5.0	2.0	4.3	5.0	3.0	3.0	52.09
CAI-67	0.0	0.0	1.7	2.0	0.0	1.0	46.27
CAI-70	3.3	0.0	3.0	0.0	0.0	2.7	21.02
CAI-98	3.7	3.0	3.7	4.0	0.0	1.0	31.02

HCN = hydrocyanic acid, IAA = indole acetic acid

The rating scales for siderophore, chitinase, cellulase, lipase and protease were as follows: 0 = no halo zone; 1 = halo zone of <1 mm; 2 = halo zone of 2-3 mm; 3 = halo zone of 4-6 mm, 4 = halo zone of 7-9 mm and 5 = 10 mm and above.

For HCN production, the rating scale used was: 0 = no color change; 1 = light reddish brown; 2 = medium reddish brown; 3 = dark reddish brown.

Table 5. Fungicide tolerance by eight antagonistic actinomycetes.

Isolate	Bavistin		Thiram		Benlate		Captan		Radonil		Benomyl	
	2500	1250	3000	1500	4000	2000	3000	1500	3000	1500	3000	1500
BCA-657	+++	+++	++	++	-	-	-	-	-	-	-	+
BCA-671	+++	+++	++	++	-	-	-	-	-	-	-	-
BCA-679	+++	+++	-	+	-	+	-	-	-	-	-	+
BCA-687	+++	+++	-	-	-	-	-	-	-	-	-	-
BCA-690	+++	+++	+	+	-	-	-	-	-	+	+	+
CAI-67	+++	+++	++	++	-	-	-	-	-	-	-	-
CAI-70	+++	+++	+	++	-	+	-	-	-	-	-	-
CAI-98	++	+++	++	++	-	-	-	-	-	-	-	-

+++ = Excellent growth, ++ = Good growth, + = little growth, - = no growth

generated using the sequences of eight actinomycete isolates and sequences from the database. Based on the maximum similarity, all the actinomycete isolates were identified to be *Streptomyces* species, among which CAI-98 was identified as *Streptomyces olivaceus*, CAI-67 as *Streptomyces caelestis*, BCA-690 as *Streptomyces flavofungini*, BCA-671 as *Streptomyces fungicidicus*, BCA-687 as *Streptomyces badius*, BCA-657 as *Streptomyces pseudogriseolus* ([http://www.ncbi.nlm.nih.gov/nucleotide/90960337?report=genbank&log\\$=nucltop&blast_rank=34&RID=ZTZ1NNKJ01S](http://www.ncbi.nlm.nih.gov/nucleotide/90960337?report=genbank&log$=nucltop&blast_rank=34&RID=ZTZ1NNKJ01S)), CAI-70 as *Streptomyces antibioticus* and BCA-679 as *Streptomyces somaliensis* (Fig. 1).

DISCUSSION

Twenty-seven actinomycetes were studied for their antagonistic potential against eight plant pathogens causing diseases of chickpea and sorghum. Among the isolates, eight were found to have broad spectrum antagonistic potential. They were further studied for metabolite production, physiological and biochemical properties, fungicide tolerance and further identified by 16S rDNA analysis. Results of the dual culture assay as well as the cell-free metabolite assay revealed that all the isolates have broad spectrum antagonistic potential against a minimum of four to a maximum of eight pathogens (CAI-70 and BCA-690). In the dual culture assay, inhibition of the pathogens may be attributed to the production of active secondary metabolites in the media which inhibited the fungal mycelial growth, whereas in the metabolite inhibition assay, inhibition of the pathogen was due to the components of cell-free extract amended in the media. There are several reports of actinomycetes used in biocontrol of plant diseases, for instance, control of *Fusarium* spp. in *Pinus taeda* (de Vasconcellos and Cardoso 2009), *Fusarium* wilt in chickpea (Gopalakrishnan et al. 2011a), charcoal rot in sorghum (Gopalakrishnan et al. 2011b), common scab of potato (Kobayashi et al. 2012), *Fusarium* diseases in wheat

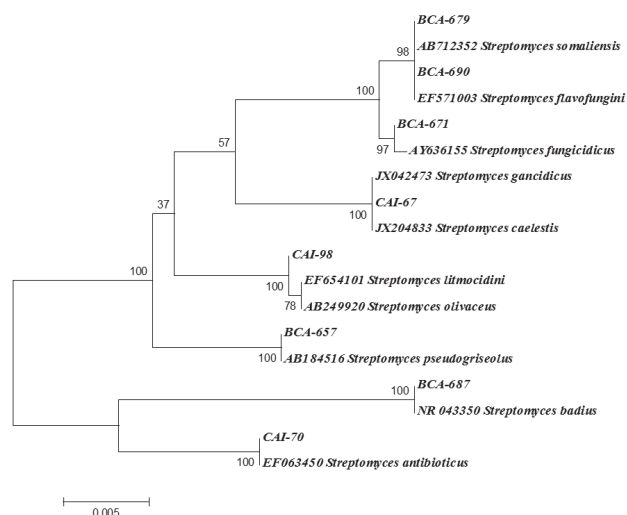


Fig 1. Dendrogram for all the eight promising strains of actinomycete isolates (analyzed by 16S RNA sequencing) using Neighbor-Joining method.

(Sahli and Abdulkhair 2012) and root rot in pepper (Nguyen et al. 2012). CAI-70 and BCA-690 can therefore be exploited for their antagonistic potential against the pathogens of chickpea and sorghum.

Results of tests of tolerance to different levels of pH, salinity and temperature showed growth of the isolates at pH 7–11, temperature 20–40 °C and up to 10% saline (except BCA-687, CAI-67 and CAI-70). Based on these results, the isolates had wide a range of tolerance to pH, temperature and saline conditions and, hence, can be used in harsh environments such as saline and acidic to alkaline pH soils.

The results of biochemical studies indicated that the actinomycetes also had a broad range of PGP and biocontrol traits including HCN, IAA, siderophore, chitinase, cellulase, lipase and protease. HCN was reportedly one of the anti-fungal secondary metabolites produced by biocontrol plant-growth-promoting actinomycetes. In the present study, although all the isolates produced HCN, BCA-690 produced the highest, which established proof of its

antagonistic potential against eight pathogens of chickpea and sorghum. IAA is the common product of L-tryptophan metabolism produced by several microorganisms including plant-growth-promoting rhizobacteria (Lynch 1985; Frankenberger and Brunner 1983). IAA-producing microorganisms are known to promote root elongation and plant growth. In the present investigation, all the eight isolates produced IAA, suggesting that these isolates could be used for plant growth promotion.

Siderophores are usually produced by various soil microbes including actinomycetes to bind Fe^{3+} from the environment and make it available for its own growth; plants also utilize these as an iron source (Wang et al. 1993). Siderophores were also known to facilitate uptake of other heavy metals and their mobilization under certain growth conditions (Chen et al. 1994; Mench and Fargues 1994). In the present investigation, seven of the eight isolates (except CAI-67) were found to be positive for siderophore production. Actinomycetes (*Streptomyces* spp.) isolated from rhizosphere soil have been reported to produce siderophores and inhibit the growth of phytopathogens (Tokala et al. 2002). Actinomycetes found in the rhizosphere need to compete with other rhizosphere plant pathogens for iron, hence, competition for iron is also a possible mechanism to control the phytopathogens.

Chitin, a linear polymer of beta-1, 4-linked N-acetylglucosamine, is synthesized by all the major groups of organisms (Nopakaran et al. 2002) and is a major structural element of the fungal cell wall. Several chitinolytic enzymes have been identified in various *Streptomyces* species (Gupta et al. 1995) and a correlation between chitinolysis and production of bioactive compounds has also been reported (Hoster et al. 2005). In the present study, five of the eight actinomycete isolates produced chitinase which could be attributed to antifungal activity. In the present study, all the isolates produced cellulase, lipase (except CAI-70) and protease (except CAI-67, CAI-70 and CAI-98). These enzymes degraded the cellulose and lipids, providing nutrition as well as posing antagonistic effect to other organisms. Based on the results, the eight antagonistic actinomycetes also possess a wide range of PGP and biocontrol traits.

The antagonistic actinomycetes exhibited excellent growth with the fungicide Bavistin and good growth with the fungicide Thiram at field application levels. This result suggests that Bavistin and Thiram can be used along with the actinomycetes in field applications, which can lower the usage of the fungicides. The 16S rDNA analysis identified eight

antagonistic actinomycetes as different species of *Streptomyces*. *Streptomyces* spp. are known to protect crop plants against pathogenic fungi (Liu et al. 1996; Gopalakrishnan et al. 2011a, b). *Streptomyces* spp. showed PGP traits as well as biocontrol activity on charcoal rot of sorghum and *Fusarium* wilt of chickpea (Gopalakrishnan et al. 2011a, b).

CONCLUSION

Eight *Streptomyces* spp. were found to have antagonistic potential actinomycetes against important diseases of chickpea and sorghum. They were also proven to have a wide range of PGP and biocontrol traits in addition to tolerance to a wide range of pH, temperature, salinity and fungicides, particularly Bavistin and Thiram. Of the eight actinomycetes, BCA-690 was found to be antagonistic against eight pathogens of chickpea and sorghum (against FOC, three strains of *R. bataticola*, *F. andiyazi* and *F. proliferatum*, *M. phaseolina* and *B. cinerea*). BCA-690 was the highest producer of PGP and biocontrol traits including siderophore, cellulase, lipase, protease, HCN and IAA and was tolerant to a wide range of fungicides. The potential of these promising isolates should be evaluated under field conditions. The isolates, BCA-690 in particular, can be exploited in integrated disease and nutrient management systems.

ACKNOWLEDGMENTS

We are thankful to DST-INSPIRE for the financial grant to G. Alekhya for her Ph.D. fellowship. We also thank all the staff of the biocontrol unit of ICRISAT, including V. Srinivas, M. Sreevidya, B. Ratna Kumari, A. Satya, R. Vijayabharathi, B. Prakash, PVS Prasad, P. Manohar, B. Nagappa, D. Barath, A. Jabbar and S. Rohini for their significant inputs in the laboratory studies.

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