CHAPTER FIVE

DEVELOPMENT OF BROAD-SPECTRUM ACTINOMYCETES FOR BIOCONTROL AND PLANT GROWTH PROMOTION OF FOOD CROPS

S. Gopalakrishnan, G. V. Ranga Rao, B. Ratna Kumari, R. Vijayabharathi, V. Srinivas & C. L. L. Gowda
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.
*E-mail: s.gopalakrishnan@cgiar.org

Abstract

The current investigation is aimed at identifying actinomycetes and their metabolites with multiple actions against insect pests and pathogens, including plant growth promotion (PGP). We characterized 137 actinomycetes, isolated from 25 different herbal vermi-composts, for their antagonistic potential against charcoal rot in sorghum (caused by *Macrophomina phaseolina*) and wilt in chickpea (caused by *Fusarium oxysporum* f. sp. *ciceri* [FOC], respectively) by dual culture assay. Of the isolates, the three most promising *M. phaseolina* antagonistic strains (CAI-21, CAI-26 and MMA-32) and the five most promising *F. oxysporum* f. sp. *ciceri* (FOC) antagonistic strains (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) were further evaluated for their antagonistic and PGP potential by blotter paper assay, greenhouse and field conditions. All eight strains were characterized for their physiological traits (tolerance to salinity, temperature, pH and compatibility to antibiotics and fungicides) and further evaluated in the field for their PGP on rice. Ten strains (CAI-8, CAI-13, CAI-70, CAI-85, CAI-87, CAI-132, CAI-133, CAI-155, SAI-25 and BCA-508) were also found to be effective in suppressing *Helicoverpa*, *Spodoptera* and *Chilo* spp. under laboratory and greenhouse conditions. The sequences of the 16S rDNA gene of all eighteen strains matched with *Streptomyces*, but the species appeared to be different. This study confirms

that the selected *Streptomyces* strains have broad-spectrum biocontrol and PGP properties.

Introduction

Plant pathogens such as *Rhizoctonia bataticola*, *Fusarium oxysporum*, *Sclerotium rolfsii* and *Phytophthora* spp. have a broad host range, affecting several crops such as sorghum, chickpea, groundnut and pigeon pea leading to significant yield losses. For example, the dry root rot of chickpea caused by *R. bataticola* is not only a serious threat to chickpea but also to sorghum where its morph form *Macrophomina phaseolina* causes charcoal rot. On the other hand, some of the insect pests such as *Helicoverpa armigera*, *Spodoptera litura* and *Chilo partellus* (sorghum stem borer) cause serious damage to a number of food crops in dry-land agriculture, including chickpea, pigeon pea, groundnut and sorghum. The management of these key pathogens and insect pests was mainly addressed through chemical means for several decades, leading to the development of insecticidal resistance to a range of chemicals (Kranthi et al. 2002) and environmental contamination (Rao et al. 2009). Due to the broad host range of these biotic constraints, the farmers are finding it difficult to grow these crops profitably. Chemical control of these pathogens and insect pests is possible, but resource-poor farmers of semi-arid tropics who own small farms (75% of Indian farmers own 1.4−2.4 ha [Chadha et al. 2004]) cannot afford expensive chemical inputs such as fungicides and pesticides. Also, with increasing concern over environmental pollution as a result of injudicious usage of synthetic chemicals, there is a need for environment-friendly methods of pest management. Breeding for host-plant resistance is one of the promising areas; however, extensive screening of chickpea, groundnut and sorghum germplasm in the past has resulted in the identification of lines with only low to moderate levels of resistance to these biotic constraints (Pande et al. 2010; Das et al. 2008). Hence, there is an urgent need to identify alternate, environmental-friendly management options to control these important pathogens and insect pests.

Broad-spectrum antifungal and anti-insect pest biocontrol organisms are required for use in different cropping systems and for the control of multiple diseases and pests in a single crop. It is difficult to breed cultivars with resistance to a wide range of pathogens and pests in any crop species. Hence, we initiated a holistic approach to identify biocontrol agents that are effective against multiple pathogens and pests. A few of the available biocontrol agents mostly belonging to *Pseudomonas* spp. show a wide spectrum antifungal activity by virtue of volatile and diffusible antibiotics

(Haas & Keel 2003; Viji et al. 2003). Bacterial strains from diverse habitats of groundnut with broad spectrum antifungal activity have been isolated, identified and applied as seed treatment for control of collar rot in groundnut with or without Thiram (Kishore et al. 2005). Secondary metabolites of *P. aeruginosa* possess antifungal, plant-growth promoting, and biocontrol activities (Bano & Musarrat 2003). Spinosad (a product of soil actinomycete, *Sacchropolyspora spinosa*) causes significant reduction in the population of *H. armigera* and other pests (Mandour 2009; Wang et al. 2009). The main objective of the current investigation is to develop and evaluate broad-spectrum biocontrol agents with multiple actions against pathogens (charcoal rot in sorghum and wilt in chickpea caused by *M. phaseolina* and *F. oxysporum* f. sp. *ciceri* [FOC]) and insect pests (*H. armigera*, *S. litura* and *C. partellus*) so that one bio-control application can address more than one problem.

Materials and Methods

Preparation of herbal vermicompost

Foliage of 25 different botanicals (see Table 5.1 below) were collected from an International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) farm and were air-dried at room temperature (30 ± 2°C). The container for vermicomposting was constructed by cutting a 200 L plastic barrel into two halves. A metal grill was placed at the bottom of the barrel and the air-dried foliages of herbals were composted on top of the grill with earthworms (*Eisenia foetida*). When the herbal compost was ready, in about 2 months, about 100 g of the sample was collected and stored in a refrigerator at 4°C for further studies.

Isolation of actinomycetes

Ten grams of herbal vermicompost were suspended in 90 ml of physiological saline (0.85% of NaCl) in a flask and placed on an orbital shaker (at 100 rpm) at room temperature (28 ± 2°C) for 1 h. At the end of shaking, the vermicompost samples were serially diluted up to 10^6 dilutions with physiological saline. Dilutions 10^4–10^6 were plated on starch casein agar (SCA) by spread plate technique and incubated at 28 ± 2°C for 4 d. The most prominent colonies were isolated and maintained on SCA slants at 4°C for further studies.

Antifungal activity of the actinomycetes

Actinomycetes were evaluated for their antifungal activity against FOC and *M. phaseolina* by dual-culture assay. A fungal disk (*M. phaseolina*/ FOC) of 6 mm diameter was placed on one edge (1 cm from the corner) of the glucose casamino acid yeast extract (GCY agar) plate, and actinomycete isolate was streaked on the other edge of the plate (1 cm from the corner), followed by incubation at 28 ± 2°C for 4 d, or until the pathogen covered the entire plate in the control plate. Inhibition of fungal mycelium (halo zone) around the actinomycete colony was noted as positive and the inhibition zone was measured.

Enzymatic activities and secondary metabolite production by the actinomycetes

Siderophore production was determined according to the methodology described by Schwyn & Neilands (1987) and Gopalakrishnan et al. (2011b). Colloidal chitin was freshly prepared and used in the chitin agar as per the standard protocols of Hirano & Nagao (1988), and the chitinase production assay was conducted as described by Gopalakrishnan et al. (2011a). The standardized protocols of Hendricks et al. (1995) and Gopalakrishnan et al. (2011b) were used to evaluate the cellulase production. Protease production was done as per the protocols of Bhattacharya et al. (2009) and Gopalakrishnan et al. (2011b). Hydrocyanic acid (HCN) production was estimated qualitatively by the sulfocyanate colorimetric method (Lorck 1948). Observations were recorded on a 0−3 rating scale (based on the intensity of the reddish brown colour) as follows (Gopalakrishnan et al. 2011b): 0 = no colour change; 1 = light reddish brown; 2 = medium reddish brown, and 3 = dark reddish brown. Indole acetic acid (IAA) production was done as per the protocols of Patten & Glick (1996) and Gopalakrishnan et al. (2011b). Quantification of IAA was done by measuring the absorbance in a spectrophotometer at 530 nm and expressed as µg/ml in the culture filtrate.

Physiological traits of the actinomycetes

This was carried out as per the protocols of Gopalakrishnan et al. (2012). In brief, actinomycetes were streaked on Bennet's agar with various concentrations of NaCl ranging from 0% to 16% at an interval of 2% and incubated at 28°C for 5 d. For pH, the actinomycetes were streaked on Bennet's agar adjusted to pH 5, 7, 9, 11, and 13 and incubated at 28°C for

5 d. For pH 3, Bennet's broth was inoculated with the three actinomycetes and at the end of a 5-day incubation the intensity of growth was measured at 600 nm in a spectrophotometer. The influence of temperature on the actinomycetes was measured by streaking on Bennet's agar and incubated at 20, 30, and 40°C for 5 d. For 50°C, Bennet's broth was inoculated with the three actinomycetes, and at the end of a 5-day incubation the intensity of growth was measured at 600 nm in a spectrophotometer. A total of seven antibiotics (chloramphenicol, kanamycin, trimethoprim, nalidixic acid, streptomycin, ampicillin and tetracycline) and six fungicides (thiram, bavistin, benlate, captan, benomyl and radonil at field application level) were studied for their resistance/susceptible pattern against the actinomycetes. The required quantities of antibiotics/fungicides were dissolved in sterilized Milli Q water and mixed into Bennet's agar just before being poured into the Petri plates. Upon solidification, the actinomycetes were streaked and incubated at 28°C for 5 d.

Antifungal (against *M. phaseolina*) activity of the actinomycetes

Determination of *in vivo* antifungal activity of the three actinomycetes against *M. phaseolina* (CAI-21, CAI-26 and MMA-32) was done by blotter paper assay (Nene et al. 1981). Inoculum of *M. phaseolina* was prepared by homogenizing a 5-day-old culture grown on potato dextrose broth at 28 ± 2°C. Two-week-old seedlings of sorghum (variety R16-susceptible to charcoal-rot) were dipped in the inoculum of *M. phaseolina* for 30 min and placed side by side on a blotter paper (45 × 25 cm) so that only the roots were covered. Actinomycete isolates were inoculated (5 ml/plant) separately into plants. Fifteen plants per replicate and three replications were made for each actinomycete. Positive and negative controls were made by inoculating the plants only with *M. phaseolina* and sterile water, respectively. The blotter paper was kept moist with sterilized water and incubated at 28 ± 2°C for 8 d with a 12-h day length provided by fluorescent lights (120 μmol/m^2/s). The disease symptoms of the charcoal-rot (black-coloured microselerotia infection on the root surface) were recorded on a 0−4 rating scale (0 represents no visible charcoal-rot symptom, while 4 represents maximum disease symptoms), and the percentage of infected roots in actinomycete inoculated treatments compared with the control was calculated.

Antifungal (against *M. phaseolina*) and PGP activity of the actinomycetes on sorghum under greenhouse conditions

The three antagonistic actinomycetes against *M. phaseolina* (CAI-21, CAI-26, and MMA-32) were evaluated in a greenhouse. A total of 4 treatments (three actinomycetes + *M. phaseolina* inoculated-positive control) were made with six replications. *M. phaseolina* inoculum was mass multiplied on sorghum grains (R16). A pot mixture (800 g) was prepared by mixing red soil, sand and FYM at 3:2:2 and filled in 8" plastic pots followed by inoculation with *M. phaseolina* inoculum (20% of pot weight, 200 g/pot). Water (100 ml) was added to each pot to wet the potting mixture, and the pots were covered with polythene sheets. The whole set up was incubated at 32 ± 2°C in a greenhouse for 15 d for charcoal-rot symptoms to develop. Two weeks later, surface sterilized and sprouted seeds were transferred into test actinomycetes for an hour before being sown in the pots (six seeds/pot but thinned to three after one week). Booster doses of actinomycetes (5 ml/seedling, 10^8 cfu/ml) were applied twice (at 15 and 30 d after sowing) by the soil drench method. Growth parameters including root length, root dry weight, shoot dry weight, shoot root ratio, percentage of root and shoot dry weight increased over the control and the disease incidences were determined at day 60 after sowing. For evaluating the PGP potential of the three actinomycetes, the above-explained greenhouse experiment was repeated without applying *M. phaseolina*. However, one new treatment was included in which only water was added and the positive control (only *M. phaseolina* inoculated) was removed. Growth parameters including root length, root volume, root dry weight, shoot dry weight, shoot root ratio, and % root and shoot dry weight and length increase over the control were determined at day 60, after sowing.

Antifungal activity (against FOC) of the actinomycetes on chickpea under wilt-sick field conditions

The five most potential antagonistic actinomycetes against FOC (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) from the *in vitro* and greenhouse studies were further evaluated individually for their antagonistic potential in *Fusarium* wilt sick field at ICRISAT, Patancheru, during the 2009−10 cropping seasons. The field had been maintained as a wilt sick plot from 1980. Each actinomycete was inoculated by two different methods: M1 = inoculation of the seeds by soaking in the respective actinomycete culture for 1 h and M4 = inoculation of the seedlings after emergence with the

respective actinomycete culture (5 ml/seedling, 10^8 cfu/ml). Thus, the combination of actinomycete isolates × two methods of inoculation constituted 10 independent treatments in addition to one positive control, where no actinomycete was inoculated. Each treatment was replicated three times in a randomized complete block design (RCBD) and the plot size was 3 rows of 2 m long with a row spacing of 30 cm and a plant-to-plant spacing of 10 cm. Chickpea seeds of a highly susceptible cultivar to *Fusarium* wilt, JG-62 (acquired from Legumes Pathology Division, ICRISAT) were surface-sterilized with sodium hypochlorite (2.5% for 5 min) and rinsed with sterilized water (8 times) before being sown into the field. During the cropping season, a maximum temperature range of 30.1°C and 34.3°C, and a minimum temperature range of 9.2°C and 16.2°C, were recorded. Incidence of *Fusarium* wilt disease (number of plants showing wilt symptoms to total number of plants in a plot) was recorded on 17, 21, 24 and 28 DAS until the susceptible check showed 100% mortality. The actinomycete population was also enumerated, as explained earlier, from the rhizosphere soils at 28 DAS for all treatments.

PGP activity of the actinomycetes on rice under field conditions

The experiment was laid out in a completely randomized block design with three replicates and with subplot sizes of 10 × 7.5 m. Rice was grown by the rice intensification (SRI) method proposed by the Central Rice Research Institute (crri.nic.in). The eight actinomycetes (CAI-21, CAI-26, MMA-32, CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) were grown on a starch casein broth at 28°C for 5 d and further evaluated for their PGP traits. Control contained no actinomycetes. A nursery was established adjacent to the experiment field. Twelve-day-old single seedlings were uprooted from the nursery, their roots dipped in the respective actinomycete strains broth (containing 10^8 cfu/ml) for 45 min and transplanted at a spacing of 25 × 25 cm. Rice plants were inoculated with the actinomycetes (1000 ml) once in 15 d until the flowering stage along with the irrigation water. The recommended dose of NPK (120, 60, and 40 kg/ha, respectively) was supplied through compost, vermicompost and organic manures mixed with cow dung and straw. The plots were weeded by Cono-weeder at 10, 20 and 30 days after transplanting (DAT). Water management was done as recommended for the SRI method. After panicle initiation, all the plots were kept flooded with a thin layer of water (1−2 cm), and all were drained 15 d before harvest. The crop was harvested manually and all required observations were made. Root samples were collected from the top 15 cm soil profile and analyzed for root length

density, volume, and dry weight. Soil samples were collected from a 0−15 cm soil profile at 75 DAT and at harvesting. These were analyzed for soil chemistry (% organic carbon, available phosphorous and total nitrogen) and biological analysis (dehydrogenase activity, microbial biomass nitrogen and microbial biomass carbon).

Evaluation of actinomycetes for their entomopathogenic traits

A total of 96 actinomycetes were screened for their entomopathogenic traits against *Helicoverpa armigera* (Hubner), of which 10 were found to be promising. All 10 isolates were tested for their efficacy against 2nd and 3rd instar larvae of *H. armigera* (repeated thrice), 3rd instar larvae of *Spodoptera litura* (F.) (repeated twice), and seven-day-old larvae of sorghum stem borer, *Chilo partellus* (Swinhoe) (repeated thrice). The actinomycetes were cultured in starch casein broth for 8 d at 28°C. At the end of the incubation, the cultures were centrifuged at 10,000 g for 10 min and the supernatants were concentrated on a rotary evaporator at 35°C and assayed. Biomass was extracted with acetonitrile and concentrated on a rotary evaporator at 35°C and assayed. The efficacy of actinomycete cultures were tested in a diet impregnation bioassay, a detached leaf bioassay, and a greenhouse experiment (Sharma et al. 2005).

Molecular identification of the actinomycetes

Pure cultures of the actinomycetes were grown in starch casein broth until log phase (4 d) and genomic DNA was isolated. The amplification of the 16S rDNA gene was done by using universal primer 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') and 27F (5'- AGA GTT TGA TCM TGG CTC AG-3'). The PCR product was sequenced at Macrogen Inc. Seoul, Korea. The sequences obtained were compared with those from the GenBank using the BLAST program, aligned using the Clustal W software, and phylogenetic trees were inferred using the neighbour-joining method in the MEGA version 4 program (Tamura et al. 2007).

Results

Selection of antagonistic actinomycete isolates against *M. phaseolina* and FOC

A total of 137 actinomycetes that produced pigments and inhibited the adjacent colonies in the SCA plate were isolated from the 25 different

herbal vermicompost samples (see Table 5.1 below) and further screened for their antagonistic potential against *M. phaseolina* and FOC by *in vitro* dual-culture assay. A maximum diversity of actinomycetes was found in vermi-composts prepared with chrysanthemum while no actinomycetes were found in vermicompost prepared from Datura (see Table 5.1 below). Of the 137 actinomycete isolates, only 79 were found to have the antagonistic potential against *M. phaseolina* and 33 against FOC. Of the 79 positive isolates against *M. phaseolina*, three (CAI-21, CAI-26 and MMA-32) were found to be more promising, whereas of the 33 positive isolates against FOC, five (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) were found to be more promising. Hence, these eight isolates were selected for further characterization studies.

Table 5.1. Actinomycetes population and diversity of the 25 different herbal vermicomposts used in this study

S. No	Vermi-composts of	Scientific names of the herbals	pH of the vermi-composts	Actinon--ycetes count*	Actinomycetes diversity#
1	Rice straw	*Oryza sativa*	7.0	7.41	6
2	Gliricidia foliage	*Gliricidia sepium*	8.1	7.53	6
3	Neem foliage	*Azadirchta indica*	7.8	7.51	8
4	Adhatoda foliage	*Adhatoda vasica*	8.3	7.12	4
5	Annona foliage	*Annona squamosa*	7.9	7.74	7
6	Chilli foliage	*Capsicum annuum*	8.1	6.83	2
7	Calotrophis foliage	*Calotrophis gigantea*	8.1	7.04	3
8	Chrysanthemum foliage	*Chrysanthemum morifolium*	7.6	7.40	12
9	Datura foliage	*Datura metal*	7.7	0	0
10	Garlic foliage	*Allium sativum*	8.2	7.12	4
11	Ginger foliage	*Zingiber officinale*	8.8	7.46	7

12	Sweet potato foliage	*Ipomoea batatas*	7.8	7.27	6
13	Jatropha foliage	*Jatropha curcas*	8.1	6.82	2
14	Jatropha seed	*Jatropha curcas*	7.4	6.88	1
15	Bitter guard foliage	*Momordica charantia*	8.1	7.29	3
16	Drum stick foliage	*Moringa oleifera*	7.8	6.68	2
17	Oleander foliage	*Nerium indicum*	8.0	7.25	5
18	Onion foliage	*Allium cepa*	8.7	6.33	1
19	Parthenium foliage	*Parthenium hysterophorus*	8.0	7.51	8
20	Turmeric foliage	*Curcuma aromatica*	9.1	7.20	5
21	Pongamia foliage	*Pongamia pinnata*	7.3	7.40	6
22	Yellow oleander foliage	*Thevetia peruviana*	8.0	7.17	6
23	Tobacco foliage	*Nicotiana tabacum*	8.4	2.00	2
24	Tridax foliage	*Tridax procumbens*	8.3	6.83	3
25	Vitex foliage	*Vitex negundo*	7.5	7.12	2

* expressed in Log_{10} values; # expressed in numbers

Enzymatic activities and secondary metabolite production by the actinomycetes isolates

When the three *M. phaseolina* and five FOC antagonistic actinomycetes were evaluated for their enzymatic activities and secondary metabolite production, all eight isolates produced siderophore, HCN and IAA (except KAI-90), whereas only five isolates produced chitinase (except CAI-21, CAI-121 and CAI-127), cellulase (except CAI-24, CAI-121 and CAI-127) and protease (except CAI-121, KAI-32 and KAI-90) (see Table 5.2 below). Isolate CAI-121 produced the maximum IAA with 43.7 µg/ml of culture filtrate, 8−10 times higher than the other positive isolates (see Table 5.2 below).

Table 5.2. Enzymatic activities and secondary metabolite production by the actinomycetes

Isolate	Production score for^					IAA# (µg/ml)
	Siderophore	Chitinase	Cellulase	Protease	HCN*	
CAI-21	1	0	3.8	3	3	1.13
CAI-26	2	1	2.5	4	3	1.17
MMA-32	3	1	2.2	2	2	4.66
CAI-24	3	2	0	3	3	5.90
CAI-121	3	0	0	0	2	43.70
CAI-127	4	0	0	3	3	3.50
KAI-32	3	2	3	0	3	2.30
KAI-90	3	4	3	0	3	0

^ = rating scale for siderophore, chitinase, protease and HCN was as follows: 0 = Negative; 1 = Positive; 2 = 1--3 mm; 3 = 4--6 mm; 4 = >7 mm; * = hydrocyanic Acid; # = indole acetic acid.

Physiological traits of the actinomycetes

All eight actinomycetes were able to grow in NaCl up to 6% and they were all able to grow at pH values between 5 and 13 (acidic to highly alkaline) and temperatures between 20°C and 40°C (see Table 5.3 below). However, the optimum conditions for good growth were 0%−4% NaCl, 7−13 pH and temperatures of 20°C −30°C (see Table 5.3 below). All eight strains were highly resistant to ampicillin (>100 ppm), sensitive to nalidixic acid (50−100 ppm) and highly sensitive to Kanamycin (except CAI-21 and CAI-26), chloramphenicol, streptomycin and tetracycline (< 50 ppm; see Table 5.3 below). When the actinomycetes were evaluated for their fungicide tolerance at field application level, they were found to be tolerant to Bavistin (@2500 ppm) and sensitive to all the other tested fungicides (see Table 5.3 below).

Table 5.3. The physiological traits of the actinomycetes

Traits	CAI -21	CAI -26	MM A-32	CAI -24	CAI -121	CAI -127	KAI -32	KAI -90
Salinity (%)								
0	+++	+++	+++	+++	+++	+++	+++	+++
2	+++	+++	+++	+++	+++	+++	+++	+++
4	+++	+++	+++	++	++	+++	+++	++
6	++	++	++	+	+	++	++	+
8	+	+	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
pH								
3	-	-	-	-	-	-	-	-
5	+++	++	+	++	+	++	+	++
7	+++	+++	+++	+++	++	+++	+++	+++
9	+++	+++	+++	+++	+++	+++	+++	+++
11	+++	+++	+++	+++	+++	+++	+++	+++
13	+++	+++	+++	+++	+++	+++	+++	+++
Temperature (°C)								
20	+++	+++	+++	+++	+++	+++	++	++
30	+++	+++	+++	+++	+++	+++	+++	+++
40	++	++	++	+++	+++	+++	++	+++
50	-	-	-	-	-	-	-	-
Antibiotics resistance pattern (ppm)								
Ampicillin	1000	800	800	200	100	100	200	200
Chloramphenicol	50	50	50	25	25	25	25	25
Kanamycin	100	100	50	1	1	1	1	1
Nalidixic acid	100	100	50	25	50	50	50	50
Streptomycin	25	25	10	50	50	50	50	20
Tetracycline	5	5	25	10	20	20	50	50
Fungicide tolerance (ppm)*								
Thiram @ 3000	-	-	+	-	-	-	-	-
Bavistin @ 2500	+++	+++	+++	+++	+++	+++	+++	+++
Benlate @ 4000	-	-	-	-	-	-	-	-
Captan @ 3000	+	+	+	-	-	-	-	-

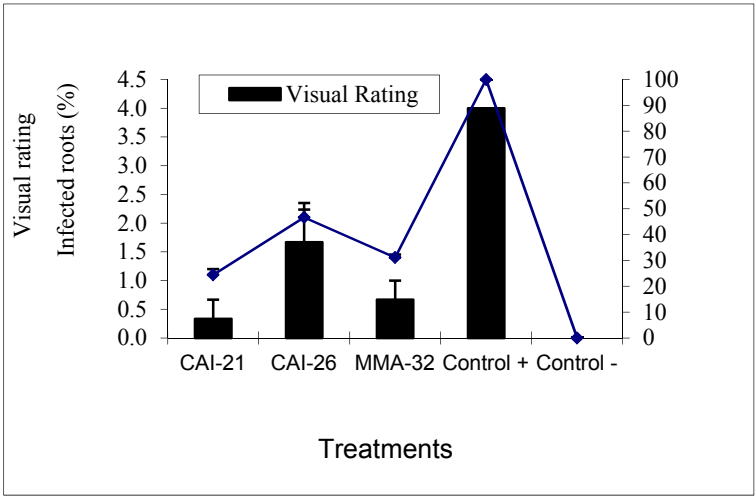
Benomyl @3000	+	+	-	-	-	-	+	+
Radonil @ 3000	-	-	-	-	-	+	+	-

+++ = good growth; ++ = medium growth; + = poor growth; - = no growth; * = at field application level.

Antifungal (against *M. phaseolina*) activity of the actinomycetes

When the three actinomycetes (CAI-21, CAI-26 and MMA-32) were evaluated for their *in vivo* antifungal potential against *M. phaseolina* by blotter paper assay, very little disease symptoms (rating < 2) and lesser root infection (47%−77% lesser than the control) were observed in the actinomycete-treated sorghum roots (see Fig. 5.1 below).

Fig 5.1. Influence of the three antagonistic actinomycetes (CAI-21, CAI-26 and MMA-32) on *M. phaseolina* by blotting paper assay



Antifungal (against *M. phaseolina*) and PGP activity of the actinomycetes on sorghum under greenhouse conditions

The actinomycetes were further evaluated for their *in vivo* antagonistic potential against *M. phaseolina* in greenhouse on sorghum crop. It was not possible to get the charcoal rot disease under positive control; however,

PGP characteristics were noticed. All the three actinomycetes increased shoot weight, root weight, root length and shoot root ratio compared to control (see Table 5.4 below). All three actinomycetes increased both sorghum shoot biomass (33%−53%) and root biomass (12%−21%) over the control (see Table 5.4 below). The highest increase of both shoot and root biomass (45% and 21% respectively) was found in CAI-26. No negative effect was found in any of the isolates.

In order to confirm the PGP traits, all three actinomycetes were further evaluated in greenhouse but without inoculating with *M. phaseolina* on sorghum crop. All three isolates increased sorghum shoot (10%−34%) and root biomass (29%−57%) (see Table 5.4 below). Root length (4%−60%) and root volume (16%−64%) were also found to be greater in comparison to the control (see Table 5.4 below). Among the three actinomycetes, MMA-32 increased all the parameters by at least 25%, i.e. shoot dry mass (27%), root dry mass (57%), root length (60%), and root volume (64%), over the control (see Table 5.4 below).

Antifungal activity (against FOC) of the actinomycetes on chickpea under wilt sick field conditions

When the five potential FOC antagonistic actinomycetes were evaluated in wilt sick field conditions, a reduction of *Fusarium* wilt incidence (4%−19%) was observed at 28 DAS over the control, where no actinomycetes were inoculated (see Fig. 5.2 below). In the control, 100% disease incidence could be noticed by 20 DAS itself. Reduction of wilt disease incidence was found to be at maximum with CAI-24 that was up to 25% at 24 DAS and 15% at 28 DAS. The next one being KAI-90 with 22% reduction at 24 DAS and 19% at 28 DAS with the M1 (seed inoculation) method. The other three isolates (CAI-121, CAI-127 and KAI-32) showed lower levels of reduction of wilt disease incidence (up to 18% at 24 DAS and 10% at 28 DAS) over the control (see Fig. 5.2 below). At 30 DAS, when the population of actinomycetes was enumerated from the rhizosphere soils, no actinomycete was found in the control plots, whereas actinomycetes (up to 10^6 log values) were found in actinomycete-inoculated plots (see Fig. 5.3 below).

Table 5.4. Antifungal (against *M. phaseolina*) and plant growth promotion (PGP) activity of the actinomycetes on sorghum under greenhouse conditions

Treatment	Shoot weight (g)	Root weight (g)	Root length (cm)	Shoot-root ratio	% increase over control	
					Shoot weight (g)	Root weight (g)
CAI-21	3.08	0.84	15.9	3.74	53	12
CAI-26	2.92	0.91	17.1	3.24	45	21
MMA-32	2.68	0.85	15.3	3.17	33	13
Control	2.01	0.75	15.0	2.74		
SE±	0195*	0.061***	1.55***	0.220***		
LSD (5%)	0.554	0.173	4.40	0.624		
CV%	17	17	23	17		

PGP potential

Treatment	Shoot weight (g)	Root weight (g)	Root length (cm)	Root volume	Shoot-root ratio	% increase over control			
						Shoot weight (g)	Root weight (g)	Root length (cm)	Root volume
CAI-21	7.36	0.88	42.11	17.02	8.58	31	40	4	16
CAI-26	6.1.6	0.81	57.55	21.23	7.65	10	29	42	45
MMA-32	7.14	0.99	64.68	23.89	7.24	27	57	60	64
Control	5.61	0.63	40.39	14.61	8.95				
SE±	0.256^{NS}	0.063^{***}	6.517^{*}	2.490^{NS}	0.851^{***}				
LSD (5%)	0.728	0.181	18.513	7.074	2.420				
CV%	9	21	33	35	22				

Note: Values are means of six replications and data calculated per plant after 60 DAS; * = Statistically Significant at 0.05; ** = Statistically Significant at <0.01; *** = Statistically Significant at < 0.001, NS = Not Significant; SE = Standard Error; LSD = Least Significant Difference; CV = Coefficient of variance.

Fig 5.2. Antifungal activity (against FOC) of the actinomycetes on chickpea under wilt sick field conditions

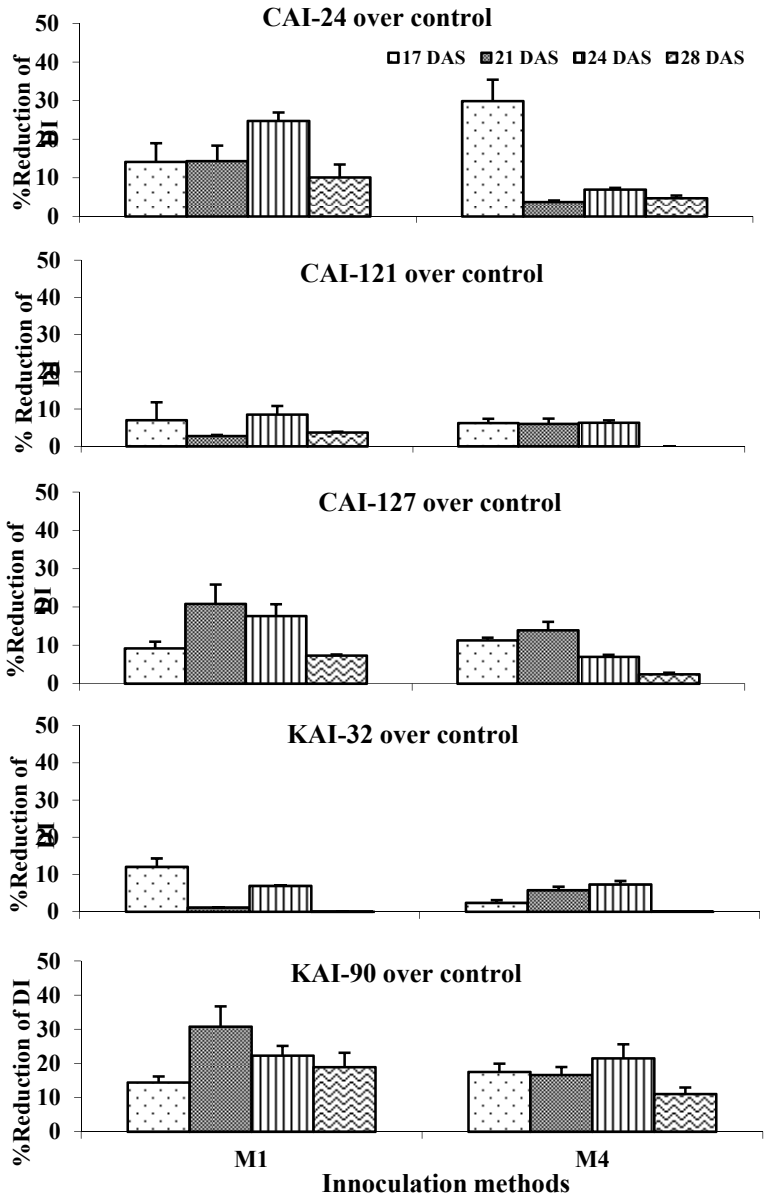
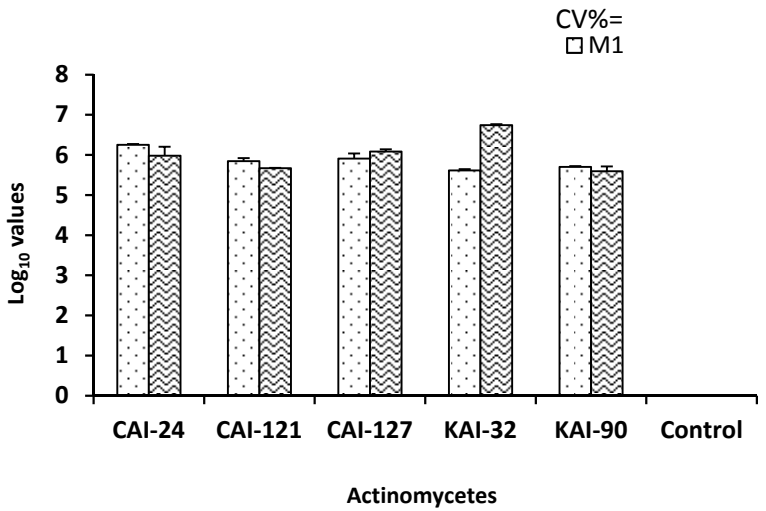


Fig. 5.3. Population of actinomycetes at the end of the field experiment in the Fusarium-infested field at ICRISAT in the actinomycetes-inoculated soils over the positive control (where no actinomycetes were inoculated).
Note: M1 = seed treatment; M4 = actinomycetes applied after seedling emergence



PGP activity of the actinomycetes, antagonistic to *M. phaseolina*, on rice under field conditions

Under rice field conditions, the actinomycetes antagonistic to *M. phaseolina* CAI-121, CAI-126, and MMA-32 significantly enhanced root length (m/m^2), volume (cm^{-3}/m^2), and dry weight (g/m^2) 1,000 seed weight (g), stover yield (g/m^2), grain yield (g/m^2), and total dry matter (g/m^2) over the control (see Table 5.5 below). Grain and stover yield were enhanced by 9%−11% and 11%−22%, respectively, over uninoculated controls (see Table 5.5 below). Root length, volume and dry weight were also increased by 39%−65%, 13%−30% and 16%−24% respectively (see Table 5.5 below). Among the three actinomycetes, CAI-21 caused greater increases of the root system and yield than the other two isolates (see Table 5.5 below). The available P, total N and organic carbon % were significantly higher in the top 15 cm of rhizosphere soils of actinomycete-treated plants (by 13%−34%, 30%−53%, and 26%−28% respectively) at harvesting than those of controls (see Table 5.5 below). The biological activities (microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activity) in

the top 15 cm rhizosphere soils were also found to be significantly higher in the actinomycete-inoculated treatments at harvest over the controls (27%−83%, 23%−43% and 34%−151%, respectively; see Table 5.5 below).

PGP activity of the actinomycetes, antagonistic to FOC, on rice under field conditions

Under field conditions on rice, the five biocontrol potential (against FOC) actinomycetes CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90 significantly enhanced root length (m/m^2, 3%−15%), root volume (cm^3/m^2, 1%−35%), root dry weight (g/m^2, 2%−55%), stover and grain yield (g/m^2, 6%−25% and 0.2%−10%, respectively), total dry matter (g/m^2, 3%−18%), and test seed weight (g) over the control (see Table 5.6 below). The available P, total N and organic carbon % (chemical activities) were also found to be significantly enhanced in the top 15 cm of rhizosphere soils of actinomycete-treated plants (by 67%−122%, 32%−53% and 0%−13%, respectively) at harvesting compared to those of the controls (see Table 5.6 below). The soil biological activities (microbial biomass carbon [$\mu g/g^1$ soil, 0.5%−41%], microbial biomass nitrogen [$\mu g/g^1$ soil, 7%−52%], and dehydrogenase activity [$\mu g/TPF/g^1$ soil $24/h^1$, 2%−75%]) in the top 15 cm rhizosphere soils were found to be significantly higher in actinomycete-inoculated treatments (except CAI-24) at harvest, over the control (see Table 5.6 below).

Table 5.5. PGP activity of the actinomycetes, antagonistic to *M. phaseolina*, on rice under field conditions

Influence on roots and yield parameters

Treatment	Root length (m/m^2)	Root volume (cm^3/m^2)	Root dry weight (g/m^2)	100 seed weight (g)	Grain yield (g/m^2)	Stover yield (g/m^2)	Total dry matter (g/m^2)
A1 (CAI-21)	5453 (65.2)	1338 (30.0)	103.2(23.8)	17.02	15.9	957 (10.6)	2116
A2 (CAI-26)	5194 (57.4)	1299 (26.2)	97.4 (16.9)	21.23	15.9	942 (8.9)	2216
A3 (MMA-3)	4596 (39.3)	1159 (12.6)	96.7 (16.0)	23.89	15.6	939 (8.5)	2106
Control	3299	1029	83.3	14.61	15.1	865	1906
Mean	4636	1206	95.2		15.6	926	2086
SE±	271.5**	60.8*	3.9*	2.490^{NS}	0.80^{**}	14.4^{*}	10.1*
LSD (5%)	939.6	210.5	13	7.074	0.37	56.7	61.7
CV%	10	9	7	35	1	3	1

Influence on soil chemical and biological parameters

Treatment	Available P (ppm)	Total N (ppm)	Organic carbon (%)	Microbial biomass C (µg/g^{1} soil)	Microbial biomass N (µg/g^{1} soil)	Dehydrogenase activity (µg/TPF g^{1} soil 24 /h^{1})
A1 (CAI-21)	142 (33.9)	2978(53.1)	1.47 (25.6)	2149 (49.4)	70 (37.2)	156 (151.6)
A2 (CAI-26)	119 (12.2)	2823 (26.2)	1.50 (28.2)	1824 (26.8)	63 (23.5)	134 (116.1)
A3(MMA-3)	120 (13.2)	2535 (12.6)	1.50 (28.2)	2634 (83.1)	73	83 (38.8)
Control	106	1945	1.17	1438	51	62
Mean	122	2570	1.41	2011	64	109
SE±	5.7*	141.9*	0/052*	183.9*	2.1***	12.6*
LSD (5%)	22.2	491.2	0.179	636.2	7.8	43.7
CV%	8	10	6	16	6	20

* = Statistically significant at 0.05, ** = Statistically significant at 0.01, *** = Statistically significant at 0.001.

Table 5.6. PGP activity of the actinomycetes, antagonistic to FOC, on rice under field conditions

Influence on root and yield parameters

Treatment	Root length (m/m^2)	Root volume (cm^3/m^2)	Root dry weight (g/m^2)	100 seed weight (g)	Grain yield (g/m^2)	Stover yield (g/m^2)	Total dry matter (g/m^2)
CAI-24	2087	396	30.5	18.9	587	584	1170
CAI-121	3263	513	36.9	18.9	583	637	1221
Cai-127	3470	692	49.5	18.6	619	754	1373
KAI-32	3652	627	54.3	19.3	640	754	1394
KAI-90	3592	581	44.8	18.9	587	693	1279
Control	3182	507	35.1	18.6	582	671	1183
SE±	3208	553	41.9	18.9	600	671	1270
LSD (5%)	52.3***	8.7***	1.02***	0.03***	9.7**	27.8**	31.9**
CV%	7	7	10	1	3	7	4

Influence on soil chemical and biological parameters

Treatment	Available P (ppm)	Total N (ppm)	Organic carbon (%)	Microbial biomass (µg/g^{1} soil)	Microbial biomass N(g/g^{1} soil)	Dehydrogenase activity (µg/TPF/g^{1} soil 24/h^{1})
CAI-24	133	2456	1.49	1715	60	94
CAI-121	117	1992	1.47	3293	65	113
CAI-127	115	2644	1.52	2875	88	194
KAI-32	122	2142	1.66	4020	65	135
KAI-90	129	2160	1.62	2946	62	136
Control	87	1190	1.47	2861	58	111
Mean	117	2231	1.53	2952	66	131
SE±	5.2*	63.1*	0.032*	150.5*	3.7***	9.3*
CV%	6	4	3	9	10	12

* = Statistically significant at 0.05, ** = Statistically significant at 0.01, *** = Statistically significant at 0.001.

Evaluation of actinomycetes for their entomopathogenic traits

Of the 96 actinomycetes evaluated for their entomopathogenic traits against *H. armigera*, only 16 were found to have a mortality of more than 70% on 2nd instar larvae (data not shown). The culture filtrates of ten actinomycetes (CAI-8, CAI-13, CAI-70, CAI-85, CAI-87, CAI-132, CAI-133, CAI-155, SAI-25 and BCA-508) also showed more than 70% mortality on 3rd instar larvae (see Table 5.7 below). Of the ten actinomycetes, six (CAI-8, CAI-13, CAI-70, CAI-85, CAI-87 and SAI-25) recorded 100% mortality on 3rd instar larvae of *H. armigera* (see Table 5.7 below). Of the ten promising actinomycetes against *H. armigera*, nine (except CAI-132) were further tested (three times) against 3rd instar larvae of *S. litura* and *C. partellus*. All nine isolates were found to have induced mortality between 38% and 77% for *S. litura* and 100% for *C. partellus* (see Table 5.7 below).

Table 5.7. Evaluation of actinomycetes for their entomopathogenic traits

Isolate	% mortality of the larvae at six days after treatment		
	H. armigera	***S. litura***	***C. partellus***
CAI-8	100	73	100
CAI-13	100	77	100
CAI-70	100	54	100
CAI-85	100	46	100
CAI-87	100	38	100
CAI-132	73	ND	ND
CAI-133	73	55	100
CAI-155	98	100	100
SAI-25	100	57	100
BCA-508	86	59	100
Control	9	0	0
Mean	85	56	90

ND = Not done

Molecular identification of the actinomycetes

In order to determine the identity of the eight potential PGP and antagonistic (against *M. phaseolina*) and ten entomopathogenic actinomycetes, their 16S rDNA was sequenced and analyzed. A neighbour-joining dendrogram was generated using the sequences from the eighteen actinomycetes (1400 bp)

and representative sequences from the databases. Phylogenetic analysis of 16S rDNA sequences of the eighteen actinomycete matched with *Streptomyces* but with different species (see Table 5.8 below).

Table 5.8. Identification of the eighteen broad spectrum actinomycetes based on 16S rDNA analysis

Sl. No.	Isolate No	Closely matched with
1	CAI-21	*Streptomyces albus*
2	CAI-26	*S. champavathi*
3	MMA-32	*S. roseoviolaceus*
4	CAI-24	*S. anulatus*
5	CAI-121	*S. setonii*
6	CAI-127	*S. setonii*
7	KAI-32	*S. setonii*
8	KAI-90	*S. africanus*
9	CAI-8	*S. cremeus*
10	CAI-13	*S. albolongus*
11	CAI-70	*S. caeruleatus*
12	CAI-85	*S.* spp.
13	CAI-87	*S. cyaneofuscatus*
14	CAI-132	*S. caeruleatus*
15	CAI-133	*S. carpaticus*
16	CAI-155	*S. cyaneofuscatus*
17	SAI-25	*S.* spp.
18	BCA-508	*S. cyaneofuscatus*

Conclusion

Three actinomycetes promising action against charcoal rot disease in sorghum caused by *M. phaseolina* (CAI-21, CAI-26 and MMA-32) and five actinomycetes (CAI-24, CAI-121, CAI-127, KAI-32 and KAI-90) promising action against Fusarium wilt disease in chickpea caused by FOC were demonstrated for their biocontrol potential under greenhouse and field conditions. All eight actinomycetes were also demonstrated for their plant growth promotion (PGP) potential under field conditions on rice grown by SRI methods. Hence, it can be concluded that the eight biocontrol potential actinomycetes also have PGP traits. In addition, another set of ten actinomycetes (CAI-8, CAI-13, CAI-70, CAI-85, CAI-87, CAI-132, CAI-133, CAI-155, SAI-25 and BCA-508) were also

demonstrated for their entomopathogenic traits against not only *H. armigera* but also *S. litura* and *C. partellus*, the key insect pests of many crops including pigeon pea, chickpea, cotton, tomato and sorghum. Thus, this study confirms that the selected actinomycetes (*Streptomyces* spp.) have broad-spectrum biocontrol and PGP properties. These actinomycetes, therefore, are likely to be potential candidates for the discovery of novel secondary metabolites which may be of importance for various PGP and biocontrol applications. Furthermore, identification of the mechanisms of action of these organisms may lead to the discovery of novel phenomena in PGP and biocontrol.

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