NRC Research Press

ARTICLE

Evaluation of *Streptomyces* spp. for their plant-growth-promotion traits in rice

Subramaniam Gopalakrishnan, Srinivas Vadlamudi, Shravya Apparla, Prakash Bandikinda, Rajendran Vijayabharathi, Ratna Kumari Bhimineni, and Om Rupela

Abstract: Five strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) were previously reported to have potential for charcoal rot control and plant growth promotion (PGP) in sorghum. In this study, those 5 *Streptomyces* strains were characterized for their enzymatic activities and evaluated for their PGP capabilities on rice. All the *Streptomyces* strains were able to produce lipase and β-1,3-glucanase; grew in NaCl (up to 8%), at pH 5–13, and at temperatures 20–40 °C; and were resistant to ampicillin, sensitive to nalidixic acid, and highly sensitive to chloramphenicol, kanamycin, streptomycin, and tetracycline. They were highly tolerant to the fungicide bavistin but were highly sensitive to benlate, benomyl, and radonil. When evaluated on rice in the field, *Streptomyces* significantly enhanced tiller and panicle numbers, stover and grain yields, dry matter, root length, volume and dry weight, compared with the control. In the rhizosphere at harvest, microbial biomass carbon and nitrogen, dehydrogenase activity, total nitrogen, available phosphorus, and % organic carbon were also found significantly higher in *Streptomyces*-treated plots than in the control plots. This study further confirms that the selected *Streptomyces* have PGP activities.

Key words: plant growth promotion, rice, *Streptomyces*, field evaluation.

Résumé : On a préalablement rapporté que 5 souches de *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26 et KAI-27) démontraient un potentiel pour lutter contre la pourriture charbonneuse et pour favoriser la croissance végétale (FCV) du sorgho. Dans la présente étude, on a caractérisé l'activité enzymatique de ces 5 souches de *Streptomyces* et on a évalué leur capacité à favoriser la croissance du riz. Tous les *Streptomyces* avaient la capacité de produire de la lipase, de la β-1,3-glucanase, croissaient dans du NaCl (jusqu'à 8 %), sous un pH de 5 à 13 et une température de 20–40 °C. Ils résistaient à l'ampicilline, étaient sensibles à l'acide nalidixique et hautement sensibles au chloramphénicol, à la kanamycine, à la streptomycine et à la tétracycline. Ils étaient fortement tolérants au fongicide bavistine mais étaient très sensibles au benlate, au bénomyle et au radonil. Lorsque leur impact sur le riz fut évalué sur le terrain, on a constaté que les souches de *Streptomyces* augmentaient significativement le développement de tiges secondaires, des panicules, le rendement en tiges et en grains, la masse sèche, la longueur racinaire, le volume sec et la masse sèche, par rapport au témoin. Au moment de la récolte, la rhizosphère présentait des taux significativement supérieurs de carbone et d'azote associés à la biomasse microbienne, d'activité déshydrogénase, de N total, de P disponible et de carbone organique dans les parcelles traitées avec des *Streptomyces* comparativement au témoin. Cette étude confirme une fois de plus que les *Streptomyces* sélectionnés exercent une activité FCV. [Traduit par la Rédaction]

Mots-clés : promotion de la croissance végétale, riz, *Streptomyces*, évaluation sur le terrain.

Introduction

The use of plant-growth-promoting (PGP) microorganisms for sustainable agriculture has increased tremendously in many parts of the world, with significant increases in the growth and yield of agriculturally important crops being widely reported (Biswas et al. 2000; Asghar et al. 2002; Vessey 2003; Figueiredo et al. 2008; Gopalakrishnan et al. 2012*a*, 2012*b*). PGP microorganisms may facilitate plant growth either by direct stimulation (such as fixing nitrogen (N), solubilizing phosphate, chelating iron, and modulating phytohormones) or by indirect stimulation (such as inhibiting phytopathogens). Strains of *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. were found effective in helping the plants not only to mobilize and acquire nutrients (Perner et al. 2006) but also to control phytopathogens (Postma et al. 2003; Khan et al. 2004; Gopalakrishnan et al. 2011*a*, 2011*b*, 2011*c*, 2012*a*, 2012*b*). *Streptomyces* are a group of Gram-positive bacteria with a high G+C content belonging to the order *Actinomycetales*, which form branched mycelia and hence have sometimes been classified as Fungi imperfecti. The PGP potential of *Streptomyces* was reported on bean (Nassar et al. 2003), tomato (El-Tarabily 2008), pea (Tokala et al. 2002), wheat (Sadeghi et al. 2012), and rice (Gopalakrishnan et al. 2012*a*, 2012*b*). *Streptomyces* promote plant growth either by producing indole-3-acetic acid (Aldesuquy et al. 1998) or siderophores (Tokala et al. 2002). *Streptomyces* has also been extensively studied and used for biocontrol of soil-borne fungal pathogens (Mahadevan and Crawford 1997; Trejo-Estrada et al. 1998; Macagnan et al. 2008).

Previously, we reported a set of 8 *Streptomyces* strains (CAI-21, CAI-26, MMA-32, CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) isolated from herbal vermicompost, with the potential for PGP and control of charcoal-rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid., in sorghum (Gopalakrishnan et al. 2011*b*). The first 3 of the 8 *Streptomyces* strains (CAI-21, CAI-26, and MMA-32) were also reported to have potential for PGP in rice (Gopalakrishnan et al. 2012*a*). The objective of this study was to further characterize the remaining 5 *Streptomyces* strains (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) for their enzymatic activities (cellulase, lipase, and β-1,3-glucanase), physiological traits (salinity, temperature, pH,

Received 6 May 2013. Revision received 3 June 2013. Accepted 3 June 2013.

S. Gopalakrishnan, S. Vadlamudi, S. Apparla, P. Bandikinda, R. Vijayabharathi, R.K. Bhimineni, and O. Rupela. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.

Corresponding author: Subramaniam Gopalakrishnan (e-mail: s.gopalakrishnan@cgiar.org).

and resistance to antibiotics and fungicides) and to evaluate, under field conditions, their PGP traits in rice grown using the system of rice intensification (SRI; Uphoff 2001; Kumar et al. 2010) method.

Materials and methods

Streptomyces strains

Five strains of *Streptomyces* isolated from herbal vermicompost, CAI-17 (*Streptomyces* spp. from *Chrysanthemum morifolium* foliage compost; NCBI acc. No. JQ682619), CAI-68 (*Streptomyces* spp. from *Nerium indicum* foliage compost; NCBI acc. No. JQ682622), CAI-78 (*Streptomyces* spp. from *Parthenium hysterophorus* foliage compost; NCBI acc. No. JQ682623), KAI-26 (*Streptomyces* spp. from rice straw compost; NCBI acc. No. JQ682624), and KAI-27 (*Streptomyces* spp. from rice straw compost; NCBI acc. No. JQ682625), reported earlier by us as having potential for PGP and biocontrol traits in sorghum (Gopalakrishnan et al. 2011*b*), were further studied.

Evaluation of *Streptomyces* for their enzymatic activities

Production of cellulase and lipase

The standard protocols of Hendricks et al. (1995) were used to evaluate the cellulase production for all the 5 strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27). Lipase production was done as per the methodologies of Bhattacharya et al. (2009). In brief, the streptomyces were streaked on Tween 80 agar and incubated at 28 °C for 5 days. The plates were observed for a halo zone around the streptomyces colonies, which indicate the presence of lipase. Treatments were replicated 3 times and the experiment was conducted 3 times.

Response of the 5 *Streptomyces* strains to cellulase and lipase were recorded on a 0–5 rating scale as follows: 0, no change; 1, positive; 2, halo zone of 1–3 mm; 3, halo zone of 4–6 mm; 4, halo zone of 7–9 mm; and 5, halo zone of ≥10 mm.

Production of β-1,3-glucanase

To determine the production of β-1,3-glucanase by *Streptomyces* strains, we followed the protocols of Singh et al. (1999). The strains were cultured individually in tryptic soy broth, supplemented with 1% (*m*/*v*) colloidal chitin, at 28 °C for 4 days. Treatments were replicated 3 times and the experiment was conducted 3 times. At the end of the incubation period, the cultures were centrifuged at 10 000*g* for 12 min and the supernatants collected. One millilitre of the culture filtrate was allowed to react with 0.1 mL of laminarin solution (2%, *m*/*v*) in 0.2 mol·L^{-1} acetate buffer (pH 5.4) at 40 °C for 1 h. The reaction was stopped by adding 3 mL of dinitrosalicylic acid to the mixture, and the color of the end product was developed by boiling for 10 min. At the end of the incubation, development of dark red color indicated the presence of β-1,3-glucanase, and the concentration of the reducing sugar was determined by measuring the absorbance at 530 nm using a spectrophotometer. Calibration standards were prepared using glucose at 0–1 mg·mL^{-1} at an interval of 0.2 mg·mL^{-1}. One unit of β-1,3-glucanase activity was defined as the amount of enzyme that liberated 1 μmol glucose·h^{-1} at defined conditions.

Evaluation of *Streptomyces* for their physiological traits

Salinity, pH, temperature, and resistance to antibiotics and fungicides

The 5 strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) were streaked on Bennett's agar with various concentrations of NaCl ranging from 0% to 12% at an interval of 2%. The plates were incubated at 28 °C for 5 days and the intensity of growth was measured at the end of incubation. For pH, the 5 strains were streaked on Bennett's agar, adjusted to pH 5, 7, 9, 11, and 13, and incubated for 5 days at 28 °C, whereas for pH 3, the Bennett's broth was inoculated, and at the end of the 5-day incubation, the intensity of growth was measured at 600 nm in a spectrophotometer. For temperature, the *Streptomyces* were streaked on Bennett's agar and incubated at 20, 30, and 40 °C for 5 days, while for 50 °C, the Bennett's broth was inoculated, and at the end of the 5-day incubation, the intensity of growth was measured at 600 nm in a spectrophotometer. Treatments were replicated 3 times and the experiment was conducted 3 times.

Table 1. Enzymatic activities by 5 *Streptomyces* strains.

Enzyme	CAI-17	CAI-68	CAI-78	KAI-26	KAI-27
Cellulase	3	4	4	3	0
Lipase	5	4	5	5	4
β-1,3-Glucanase (units)[a]	0.61	0.66	2.92	0.35	0.20

Note: The rating scale for cellulase and lipase was as follows: 0, no change; 1, positive; 2, halo zone of 1–3 mm; 3, halo zone of 4–6 mm; 4, halo zone of 7–9 mm; and 5, halo zone of ≥10 mm.

[a]One unit of β-1,3-glucanase is defined as the amount of enzymes causing the release of 1 μmol of glucose equivalent per hour under the conditions described in materials and methods.

A total of 6 antibiotics namely ampicillin, chloramphenicol, kanamycin, nalidixic acid, streptomycin, and tetracycline were studied for their resistance pattern against the 5 strains as per the standard protocols of Gopalakrishnan et al. (2012*a*). The 5 strains were also evaluated for their tolerance to fungicides at a field application level. The fungicides studied include Thiram (dimethylcarbamothioylsulfanyl *N*,*N*-dimethylcarbamodithioate), Bavistin (carbendazim 50%; methyl benzimidazol-2-yl-carbamate), 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 ppm, respectively. The required quantities of antibiotics and fungicides were dissolved in sterilized Milli-Q water and mixed into Bennett's agar just before pouring into the Petri plates (when the temperature of the media was about 50 °C). The plates were incubated at 28 °C for 5 days, and the intensity of growth was measured at the end of incubation. There were 3 replications for each test and the experiment was done thrice.

Response of the 5 *Streptomyces* strains to salinity, pH, temperature, and fungicide tolerance was recorded as follows: 0, no growth; 1, slight growth; 2, medium growth; and 3, good growth.

Evaluation of *Streptomyces* for PGP potential on rice under field conditions

The experiment was conducted in 2011–2012 (post rainy season) at ICRISAT, Patancheru, Andhra Pradesh, India, with a medium-duration rice variety, Sampada (135 days), which normally yields 6.5–7.0 t·ha^{-1}. Soils at the experimental site are classified as sandy loam in texture (55% sand, 17% silt, and 28% clay) with alkaline pH of 8.5–9.4 and organic carbon (C) content of 0.76%–1.27%. The mineral content of the rhizosphere (top 15 cm layer) was as follows: available N, 292 kg·ha^{-1}; available P, 26.8 kg·ha^{-1}; and available potassium, 527 kg·ha^{-1}. The experiment was laid out in a randomized complete block design with 3 replicates and subplot sizes of 10 m × 7.5 m. Rice was grown by the system of rice intensification (SRI) method proposed by the Central Rice Research Institute (http://crri.nic.in), Cuttack, Orissa, India. The 5 strains of *Streptomyces* (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) were grown on a starch casein broth at 28 °C for 5 days and further evaluated for their PGP traits. The control contained no *Streptomyces* strains.

The field experiment was conducted as described previously (Gopalakrishnan et al. 2012*a*). In brief, the 12-day-old single seedlings were uprooted from the nursery, their roots dipped in the respective *Streptomyces* spp. broth (containing 10^8 CFU·mL^{-1}) for 50 min and transplanted on 27 December 2011 at a row-to-row spacing of 25 cm and a plant-to-plant spacing of 25 cm. The strep-

Table 2. Effect of salinity, pH, temperature, and tolerance to antibiotics and fungicides on the growth of *Streptomyces* strains.

Trait	CAI-17	CAI-68	CAI-78	KAI-26	KAI-27	Mean
Salinity						
0%	3	3	3	3	3	3
2%	3	3	3	3	3	3
4%	3	3	3	3	3	3
6%	3	3	3	3	3	3
8%	3	1	3	3	1	2
10%	2	0	2	2	0	1
12%	0	0	0	0	0	0
SE±			0.1 (0.1)***			0.06***
CV%			12			
pH						
3	0	0	0	0	0	0
5	2	1	1	1	1	1
7	3	3	3	3	3	3
9	3	3	3	3	3	3
11	3	3	3	3	3	3
13	3	3	3	3	3	3
SE±			0.1 (0.1)***			0.03***
CV%			5			
Temperature						
20 °C	3	3	3	3	3	3
30 °C	3	3	3	3	3	3
40 °C	3	3	3	3	3	3
50 °C	0	0	0	0	0	0
SE±			0.003 (0.003)***			0.002***
CV%			1			
Fungicide tolerance						
Thiram (3000 ppm)	1	1	1	1	0	1
Bavistin (2500 ppm)	3	3	3	3	3	3
Benlate (4000 ppm)	0	0	0	0	0	0
Captan (3000 ppm)	2	1	0	1	1	1
Benomyl (3000 ppm)	0	0	0	0	0	0
Radonil (3000 ppm)	0	0	0	0	0	0
SE±			0.09 (0.09)***			0.04***
CV%			18			
Antibiotic resistance pattern (ppm)						
Ampicillin	1800	100	1800	1800	1800	1460
Chloramphenicol	25	25	25	5	5	17
Kanamycin	50	5	5	4	4	14
Nalidixic acid	50	50	50	50	50	50
Streptomycin	15	100	15	5	5	28
Tetracycline	2	15	1.5	9	9	7
SE±			0.5 (0.5)***			0.2***
CV%			1			

Note: Responses of the 5 actinomycetes to salinity, pH, temperature, and fungicide tolerance were recorded as follows: 0, no growth; 1, poor growth; 2, medium growth; 3, good growth. ***, statistically significant at 0.001. Standard errors (SE) in parentheses are to compare means within same treatment. CV, coefficient of variation.

tomyces (1000 mL; 10^8 CFU·mL^{-1}) were applied once in 15 days until the flowering stage along with the irrigation. Irrigation was done as recommended for the SRI method, i.e., the alternate wetting and drying method. Weeding was done 4 times by conoweeder to incorporate weeds into the soil at 10, 20, 30, and 40 days after transplanting. No serious insect pests or phytopathogen attacks were observed during the cropping period. The crop was harvested manually on 23 May 2012 and observed for plant height (cm), total panicles (plant^{-1}), panicle length (cm), filled grain number and mass (g), total tillers (m^{-2}), stover yield (g·m^{-2}), grain yield (g·m^{-2}), and total dry matter (g·m^{-2}). Root samples were collected from 0 to 30 cm soil profile and analyzed for root length (mm^{-2}; EPSON expression 1640×, Japan), volume (cm^3·m^{-2}), and dry mass (g·m^{-2}) dried in an oven at 70 °C for 48 h. Soil samples were collected from 0 to 15 cm soil profile, at harvest, and subjected to soil chemistry analysis (total N (ppm), available phosphorous (ppm), and % organic C as per the protocols of Novozamsky et al. (1983), Olsen and Sommers (1982), and Nelson and Sommers (1982), respectively) and biological analysis (microbial biomass C (μg·(g soil)$^{-1}$) by the fumigation method, microbial biomass N (μg·(g soil)$^{-1}$) by the Kjeldahl distillation method, and dehydrogenase activity (μg TPF·(g soil)$^{-1}$·24 h^{-1}) by the triphenyl formazan production method as per the protocols of Anderson and Domsch (1989), Brooks et al. (1985), and Casida (1977), respectively).

Statistical analysis

Data were analyzed by using analysis of variance (ANOVA) technique, by SAS GLM (General Linear Model) procedure (SAS Institute 2002-08, SAS version 9.3) considering isolates and replication as fixed in randomized complete block design. Depth-wise ANOVA was performed for the traits root length, volume, and dry mass. Isolate means were tested for significance and compared using Fisher's protected least significant difference.

Table 3. Effect of *Streptomyces* strains on the morphology of rice cultivation.

Treatment	Plant height (cm)	Total panicles (plant^{-1})	Panicle length (cm)	Filled grain number	Filled grain mass (g)	Total tillers (m^{-2})
CAI-17	73	39	22	128	2.04	573
CAI-68	72	40	23	135	2.10	539
CAI-78	81	47	22	152	2.42	565
KAI-26	85	45	23	147	2.18	607
KAI-27	73	46	23	149	2.38	564
Control	72	36	21	120	2.03	484
Mean	76	42	22	138	2.19	555
SE±	0.5***	0.7***	0.3*	3.2**	0.032***	12.4***
LSD (5%)	1.6	2.6	1.0	11.7	0.116	39.0
CV%	1	3	2	3	2	4

Note: SE, standard error; LSD, least significant difference; CV, coefficient of variation; *, statistically significant at 0.05; **, statistically significant at 0.01; ***, statistically significant at 0.001.

Results

Evaluation of *Streptomyces* for their enzymatic activities and physiological traits

When the 5 *M. phaseolina* antagonistic *Streptomyces* were evaluated for their enzymatic activities, all strains produced β-1,3-glucanase, while *Streptomyces* strains CAI-17, CAI-68, CAI-78, and KAI-26 were able to produce cellulase (Table 1). *Streptomyces* strains CAI-17, CAI-78, and KAI-26 were able to grow at up to 10% NaCl conditions and none grew at 12% NaCl. *Streptomyces* grown under a gradient of pH indicated that none of the isolates grew at pH 3 and all of them grew well at pH 7–13. A pH of 5 was discriminatory for the strains; strain CAI-17 showed medium growth, while others exhibited poor growth. Temperatures between 20 and 40 °C were found optimum for growth of all *Streptomyces* strains, whereas none of them grew at 50 °C. With regard to antibiotic resistance pattern studies, *Streptomyces* strains CAI-17, CAI-78, KAI-26, and KAI-27 were found highly resistant to ampicillin (>1800 ppm), while all strains were found sensitive to nalidixic acid (<50 ppm) and highly sensitive to chloramphenicol, kanamycin, streptomycin, and tetracycline (<25 ppm). The *Streptomyces*, at field application level, were found highly tolerant to fungicide bavistin, slightly tolerant to thiram (except KAI-27) and captan (except CAI-78) but highly sensitive to benlate, benomyl, and radonil (Table 2).

Evaluation of *Streptomyces* for PGP potential on rice under field conditions

Under field conditions, when the 5 *M. phaseolina* antagonistic *Streptomyces* were evaluated for their PGP potential against an untreated control, the *Streptomyces*-treated plots significantly enhanced plant height, total panicles, filled grain numbers and mass, total tillers (11%–25%), stover yield (16%–91%), grain yield (1%–25%), and total dry matter (14%–58%) compared with the untreated control (Tables 3 and 4). In addition, the plots treated with *Streptomyces* significantly enhanced the root development at both 0–15 and 16–30 cm depths, including the root length (16%–34%), root volume (29%–53%) and root dry mass (14%–58%) over the control (Table 5). Of the 5 *Streptomyces* studied, CAI-78 most enhanced the yield parameters (including stover yield, grain yield, and total dry matter) and root development (including root length, root volume, and root dry mass).

The soil biological activities in the top 15 cm of rhizosphere soils, at harvest, including microbial biomass C, microbial biomass N, and dehydrogenase activity, were significantly enhanced (23%–48%, 7%–321%, and 14%–278%, respectively) in the *Streptomyces*-inoculated plots compared with the untreated control plots. Further, in the *Streptomyces*-inoculated plots the soil mineral nutrients, such as total N, available P, and % organic C, were also found significantly higher than the untreated control (8%–82%, 13%–44%, and 17%–39%, respectively) in the top 15 cm of rhizosphere at rice harvest (Table 6).

Table 4. Effect of *Streptomyces* strains on the yield potential of rice cultivation.

Treatment	Stover yield (g·m^{-2})	Grain yield (g·m^{-2})	Total dry matter (g·m^{-2})
CAI-17	1452	788	2240
CAI-68	1493	787	2280
CAI-78	2133	872	3005
KAI-26	1302	981	2283
KAI-27	1344	813	2157
Control	1118	782	1899
Mean	1474	837	2311
SE±	62.6***	11.4***	65.2***
LSD (5%)	197.4	35.9	205.4
CV%	7	2	5

Note: SE, standard error; LSD, least significant difference; CV, coefficient of variation; ***, statistically significant at 0.001.

Discussion

Five *Streptomyces* strains reported in this study (CAI-17, CAI-68, CAI-78, KAI-26, and KAI-27) were known to be good biocontrol agents of sorghum charcoal rot disease and promoters of sorghum plant growth by producing siderophores and indole acetic acid (Gopalakrishnan et al. 2011*b*). Siderophores produced by bacteria bind Fe^{3+} from the environment and make it available to the plants and bacteria for their growth (Wang et al. 1993), whereas microorganisms producing indole acetic acid are known to stimulate growth of the plants, particularly roots (Patten and Glick 2002); these 2 traits can be exploited by the host plants for their PGP. Therefore, in the present study, the 5 *Streptomyces* strains were characterized for their enzymatic activities and physiological traits and further evaluated for their PGP traits under field conditions on rice grown by SRI methods.

In the present investigation, all the 5 *Streptomyces* strains were able to produce lipase and β-1,3-glucanase, and strains CAI-17, CAI-68, CAI-78, and KAI-26 produced cellulase. Cellulose and lipid present abundantly in the plant biomass can be degraded by enzymes such as cellulase and lipase (Lynd et al. 2002). The cell wall of higher pathogenic fungi, such as *Fusarium oxysporum*, is composed of layers of β-1,3-glucan and lysis of this by a β-1,3-glucanase-producing microbe leads to leakage of cell contents and collapse of the pathogenic fungi (Singh et al. 1999; Macagnan et al. 2008). Hence, microorganisms having these traits can be exploited for degradation of organic residues and (or) biological control of plant pathogens.

The 5 *Streptomyces* strains antagonistic to *M. phaseolina* were able to grow in NaCl up to 8%, at pH values between 5 and 13, at temperatures between 20 and 40 °C, and were found highly resistant to ampicillin and tolerant to fungicide bavistin. The ability of *Streptomyces* to tolerate abiotic stresses, including salinity, pH, and

Table 5. Effect of *Streptomyces* strains on the root development of rice, at harvest, of rice cultivation.

	Root length (mm^{-2})			Root volume ($cm^3 \cdot m^{-2}$)			Root dry mass ($g \cdot m^{-2}$)		
Treatment	0–15 cm	16–30 cm	Mean	0–15 cm	16–30 cm	Mean	0–15 cm	16–30 cm	Mean
CAI-17	7106	950	4028	2108	189	1148	117.3	8.3	62.8
CAI-68	6474	1416	3945	2078	194	1136	136.0	9.7	72.9
CAI-78	7415	1341	4378	2501	194	1348	163.0	10.2	86.6
KAI-26	7180	1117	4148	2090	203	1147	117.0	8.2	62.6
KAI-27	8119	950	4535	2211	158	1184	144.2	7.3	75.8
Control	6355	425	3390	1692	72	882	106.2	3.6	54.9
SE±	126.9 (29.3)***		125.2***	47.5 (44.2)***		35.8***	3.34 (2.97)***		2.59***
Mean	7108	1033		2113	168		130.6	7.9	
SE±	11.9***			18.0***			1.21***		
CV%	2			7			7		

Note: SE, standard error; CV, coefficient of variation; ***, statistically significant at 0.001; SE in parentheses are to compare means within the same treatment.

Table 6. Effect of *Streptomyces* strains on soil biological and chemical activities, at harvest, of rice cultivation.

Treatment	Microbial biomass C ($\mu g \cdot (g\ soil)^{-1}$)	Microbial biomass N ($\mu g \cdot (g\ soil)^{-1}$)	Dehydrogenase activity (μg TPF$\cdot (g\ soil)^{-1} \cdot 24\ h^{-1}$)	Total N (ppm)	Available P (ppm)	Organic C (%)
CAI-17	3404	74.5	88	1766	99	1.26
CAI-68	2943	62.1	71	1866	98	1.30
CAI-78	3374	42.3	72	1544	96	1.35
KAI-26	3028	73.0	172	2595	115	1.47
KAI-27	2830	19.0	78	1855	90	1.50
Control	2300	17.7	62	1426	80	1.08
Mean	2980	48	90	1844	96	1.33
SE±	164.1**	6.66***	3.9***	33.5***	2.6***	0.060**
LSD (5%)	517.1	20.9	12.2	105.4	8.3	0.188
CV%	10	24	7	3	5	8

Note: C, carbon; N, nitrogen; TPF, triphenyl formazan; P, phosphorus; ppm, parts per million; SE, standard error; LSD, least significant difference; CV, coefficient of variation; **, statistically significant at 0.01; ***, statistically significant at 0.001.

temperature, and antibiotics and fungicides is widely reported (Waksman 1959; Gopalakrishnan et al. 2012*a*, 2012*b*; Sadeghi et al. 2012). Consequently, it can be stated that these strains have the capability to survive in harsh environments and can be used in the integrated disease management programs.

In the field, the 5 *Streptomyces* strains significantly enhanced morphological and yield traits of rice, including plant height, total panicles, filled grain numbers and mass, total tillers, root length, root volume, root dry mass, stover yield, grain yield, and total dry matter, compared with the untreated control. The efficacy of *Streptomyces* strains for PGP is extensively reported (Nassar et al. 2003; El-Tarabily 2008; Gopalakrishnan et al. 2012*a*, 2012*b*). The soil biological activities and mineral nutrients (including microbial biomass C and N, dehydrogenase, total N, available P, and % organic C) in the *Streptomyces*-treated rice plots, at harvest, were also found significantly higher compared with the untreated control plots. The mechanism by which the *Streptomyces* strains enhanced morphological and yield traits of rice could be attributed to their enzymatic activities, such as indole acetic acid and siderophores (direct stimulation of PGP), and (or) to their capability of producing chitinase, protease, hydrocyanic acid, cellulase, lipase, and β-1,3-glucanase (indirect stimulation of PGP; Gopalakrishnan et al. 2011*b*). The influence of microorganisms on PGP, including the root development of the plants, has been reported by Birkhofer et al. (2008), Uphoff et al. (2009), and Gopalakrishnan et al. (2011*a*, 2011*b*, 2012*a*, 2012*b*). Though the SRI method of rice cultivation supports the growth of PGP microbes (including phosphate-solubilizing bacteria, diazotrophs, *Azospirillium,* and *Azotobacter*) and microbial enzyme activities (Turner and Haygarth 2001; Gayathry 2002), such enhanced activities were found in the present investigation only in the *Streptomyces*-inoculated treatments. Hence, it is concluded that the 5 *Streptomyces* strains were able to survive in the rice rhizosphere and enhance soil health.

In this study, although roots were not inspected for colonization, the data on root morphology (including root volume, length, and dry mass), soil biological activity (microbial biomass C, N, and dehydrogenase), and chemical activity (total N, available P, and % organic C) in the rhizosphere (0–15 cm) strongly suggest that the 5 *Streptomyces* strains had multiplied and colonized the roots of rice plants. Therefore, it is concluded that the 5 *Streptomyces* strains used in this study were apparently well adapted not only in the sorghum rhizosphere (Gopalakrishnan et al. 2011*b*) but also in the rice rhizosphere, in the present investigation, where they promoted plant growth. Further, the 5 *Streptomyces* strains could also be used as biocontrol agents against charcoal rot disease in sorghum.

The 5 *Streptomyces* strains used in this investigation apparently contained a broad range of PGP and antifungal traits and demonstrated multiple mechanisms of action indicating *Streptomyces*' broad spectrum of activity. Broad-spectrum PGP agents offer effective novel strategies not only for crop growth but also for controlling multiple pathogens and insect pests that attack crops. In addition to suppressing plant pathogens by secretion of antibiotics, some PGP microbes can also elicit induced systemic resistance (ISR) against a broad range of pathogens, insects, and nematodes (Jetiyanon and Kloepper 2002; Ryu et al. 2007). Therefore, the 5 *Streptomyces* strains used in this investigation are likely to be the potential candidates for the discovery of novel secondary metabolites for various PGP and biocontrol applications. Further, determination of their usefulness in host plant resistance against a variety of pathogens and insect pests can assist in furthering the use of ecofriendly biopesticides and biofertilizers. The use of PGP microbes in developing such bioproducts will probably be one of the important tactics of integrated pest, disease, and nutrition management worldwide in the near future.

Acknowledgements

We thank the National Bureau of Agriculturally Important Microorganisms (NBAIM) for providing financial support. We also thank all the staff of the biocontrol unit of ICRISAT including M/s PVS Prasad, P. Manohar, B. Nagappa, D. Barath, A. Jabbar, and S. Rohini for their significant inputs in the laboratory and field studies.

References

Aldesuquy, H.S., Mansour, F.A., and Abo-Hamed, S.A. 1998. Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. Folia Microbiol. **43**: 465–470. doi:10.1007/BF02820792.

Anderson, T.H., and Domsch, K.H. 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil Biol. Biochem. **21**: 471–479. doi:10.1016/0038-0717(89)90117-X.

Asghar, H.N., Zahir, Z.A., Arshad, M., and Khalig, A. 2002. Plant growth regulating substances in the rhizosphere: microbial production and functions. Adv. Agron. **62**: 146–151.

Bhattacharya, A., Chandra, S., and Barik, S. 2009. Lipase and protease producing microbes from the environment of sugar beet field. Ind. J. Agric. Biochem. **22**: 26–30.

Birkhofer, K., Bezemer, T.M., Bloem, J., Bonokowski, M., Chritensen, S., Dubois, D., Ekelund, F., Fliessbach, A., Gunst, L., Hedlund, K., Mader, P., Mikola, J., Robin, C., Setala, H., Tatin-Froux, F., Van der Putten, W., and Scheu, S. 2008. Long-term organic farming fosters below and above ground biota; implications for soil quality, biological control and productivity. Soil Biol. Biochem. **40**: 2297–2308. doi:10.1016/j.soilbio.2008.05.007.

Biswas, J.C., Ladha, J.K., and Dazzo, F.B. 2000. Rhizobial inoculation influences seedling vigor and yield of rice. Agron. J. **92**: 880–886. doi:10.2134/agronj2000.925880x.

Brooks, P.C., Landman, A., Pruden, G., and Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen; a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. **17**: 837–842. doi:10.1016/0038-0717(85)90144-0.

Casida, L.E. 1977. Microbial metabolic activity in soil as measured by dehydrogenase determinations. Appl. Environ. Microbiol. **34**: 630–636. PMID:339829.

El-Tarabily, K.A. 2008. Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant Soil, **308**: 161–174. doi:10.1007/s11104-008-9616-2.

Figueiredo, M.V.B., Martinez, C.R., Burity, H.A., and Chanway, C.P. 2008. Plant growth-promoting rhizobacteria for improving nodulation and nitrogen fixation in the common bean (*Phaseolus vulgaris* L.). World J Microbiol. Biotechnol. **24**: 1187–1193. doi:10.1007/s11274-007-9591-4.

Gayathry, G. 2002. Studies on dynamics of soil microbes in rice rhizosphere with water saving irrigation and in-situ weed incorporation. Ph.D. thesis submitted to Tamil Nadu Agricultural University, Coimbatore, India.

Gopalakrishnan, S., Humayun, P., Kiran, B.K., Kannan, I.G.K., Vidya, M.S., Deepthi, K., and Rupela, O. 2011*a*. Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid. World J. Microbiol. Biotechnol. **27**: 1313–1321. doi:10.1007/s11274-010-0579-0.

Gopalakrishnan, S., Kiran, B.K., Humayun, P., Vidya, M.S., Deepthi, K., and Rupela, O. 2011*b*. Biocontrol of charcoal-rot of sorghum by actinomycetes isolated from herbal vermicompost. Afr. J. Biotechnol. **10**: 18142–18152. doi:10.5897/AJB11.2710.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B.K., Sandeep, D., Vidya, M.S., Deepthi, K., and Rupela, O. 2011*c*. Evaluation of actinomycete isolates obtained from herbal vermicompost for biological control of *Fusarium* wilt of chickpea. Crop Prot. **30**: 1070–1078. doi:10.1016/j.cropro.2011.03.006.

Gopalakrishnan, S., Humayun, P., Vadlamudi, S., Vijayabharathi, R., Bhimineni, R.K., and Rupela, O. 2012*a*. Plant growth-promoting traits of *Streptomyces* with biocontrol potential isolated from herbal vermicompost. Biocontrol Sci. Technol. **22**: 1199–1210. doi:10.1080/09583157.2012.719151.

Gopalakrishnan, S., Upadhyaya, H.D., Vadlamudi, S., Humayun, P., Vidya, M.S., Alekhya, G., Singh, A., Vijayabharathi, R., Bhimineni, R.K., Seema, M., Rathore, A., and Rupela, O. 2012*b*. Plant growth-promoting traits of biocontrol potential bacteria isolated from rice rhizosphere. SpringerPlus, **1**: 71. doi:10.1186/2193-1801-1-71. PMID:23397013.

Hendricks, C.W., Doyle, J.D., and Hugley, B. 1995. A new solid medium for enumerating cellulose-utilizing bacteria in soil. Appl. Environ. Microbiol. **61**: 2016–2019. PMID:16535032.

Jetiyanon, K., and Kloepper, J.W. 2002. Mixtures of plant growth promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biol. Control, **24**: 285–291. doi:10.1016/S1049-9644(02)00022-1.

Khan, M.R., Khan, S.M., and Mohiddin, F.A. 2004. Biological control of *Fusarium* wilt of chickpea through seed treatment with the commercial formulation of *Trichoderma harzianum* and/or *Pseudomonas fluorescens*. Phytopathol. Mediterr. **43**: 20–25.

Kumar, R.M., Surekha, K., Padmavathi, Ch., Rao, L.V.S., Latha, P.C., Prasad, M.S., Babu, V.R., Ramprasad, A.S., Rupela, O.P., Goud, V.P., Raman, P.M., Somashedkar, N., Ravichandran, S., Singh, S.P., and Viraktamath, B.C. 2010. Research experiences on system of rice cultivation and future directions. J. Rice Res. **2**: 61–71.

Lynd, L.R., Weimer, P.J., Van, Z.W.H., and Pretorius, I.S. 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. **66**: 506–577. doi:10.1128/MMBR.66.3.506-577.2002. PMID:12209002.

Macagnan, D., Romeiro, R.D.A., Pomella, A.M.V., and deSouza, J.T. 2008. Production of lytic enzymes and siderophores, and inhibition of germination of basidiospores of *Moniliophthora* (ex *Crinipellis*) *perniciosa* by phylloplane actinomycetes. Biol. Control, **47**: 309–314. doi:10.1016/j.biocontrol.2008.08.016.

Mahadevan, B., and Crawford, D.L. 1997. Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. Enzyme Microb. Technol. **20**: 489–493. doi:10.1016/S0141-0229(96)00175-5.

Nassar, A.H., El-Tarabily, K.A., and Sivasithamparam, K. 2003. Growth promotion of bean (*Phaseolus vulgaris* L.) by a polyamine producing isolate of *Streptomyces griseoluteus*. Plant Growth Reg. **40**: 97–106. doi:10.1023/A:1024233303526.

Nelson, D.W., and Sommers, L.E. 1982. Total organic carbon and organic matter', *In* Methods of soil analysis. Part 3. Chemical and microbiological properties. *Edited by* A.L. Page, R.H. Miller, and D.R. Keeney. Soil Science of America, Inc., Madison, Wis., USA. pp. 539–579.

Novozamsky, I., Houba, V.J.G., van Eck, R., and van Vark, W. 1983. A novel digestion technique for multiple element analysis. Commun. Soil Sci. Plant Anal. **14**: 239–248. doi:10.1080/00103628309367359.

Olsen, S.R., and Sommers, L.E. 1982. Phosphorus. *In* Methods of soil analysis. Part 2. Chemical and microbial properties. 2nd ed. Agronomy Monograph No. 9. *Edited by* A.L. Page. American Society of Agronomy, Madison, Wis., USA. pp. 403-430.

Patten, C., and Glick, B.R. 2002. Role of *Pseudomonas putida* in indole acetic acid in development of the host plant root system. Appl. Environ. Microbiol. **68**: 3795–3801. doi:10.1128/AEM.68.8.3795-3801.2002. PMID:12147474.

Perner, H., Schwarz, D., and George, E. 2006. Effect of mycorrhizal inoculation and compost supply on growth and nutrient uptake of young leek plants grown on peat-based substrates. HortScience, **41**: 628–632.

Postma, J., Montanari, M., and Van den Boogert, P.H.J.F. 2003. Microbial enrichment to enhance disease suppressive activity of compost. Eur. J. Soil Biol. **39**: 157–163. doi:10.1016/S1164-5563(03)00031-1.

Ryu, C.M., Murphy, C.F., Reddy, M.S., and Kloepper, J.W. 2007. A two strain mixture of rhizobacteria elicits induction of systemic resistance against *Pseudomonas syringae* and Cucumber mosaic virus coupled to promotion of plant growth on *Arabidopsis thaliana*. J. Microbiol. Biotechnol. **17**: 280–286. PMID:18051759.

Sadeghi, A., Karimi, E., Dahazi, P.A., Javid, M.G., Dalvand, Y., and Askari, H. 2012. Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil condition. World J. Microbiol. Biotechnol. **28**: 1503–1509. doi:10.1007/s11274-011-0952-7. PMID:22805932.

Singh, P.P., Shin, Y.C., Park, C.S., and Chung, Y.R. 1999. Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology, **89**: 92–99. doi:10.1094/PHYTO.1999.89.1.92. PMID:18944809.

Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F., and Morra, M.J. 2002. Novel plant–microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC108 and the pea plant (*Pisum sativum*). Appl. Environ. Microbiol. **68**: 2161–2171. doi:10.1128/AEM.68.5.2161-2171.2002. PMID:11976085.

Trejo-Estrada, S.R., Paszczynski, A., and Crawford, D.L. 1998. Antibiotics and enzymes produced by the biocontrol agent *Streptomyces violaceusniger* YCED-9. J. Ind. Microbiol. Biotechnol. **21**: 81–90. doi:10.1038/sj.jim.2900549.

Turner, B.L., and Haygarth, P.M. 2001. Phosphorous solubilization in rewetted soils. Nature, **411**: 258. doi:10.1038/35077149, 10.1038/35077146. PMID:11357117.

Uphoff, N. 2001. Scientific issues raised by the SRI: a less water rice cultivations system. *In* Water savings rice production systems. *Edited by* H. Hengsdijk and P. Bindraban. Proceedings of an International Workshop on Water Saving Rice Production, Nanjing University, China, 2–4 April 2001. PRI Report No 33. pp. 69–82.

Uphoff, N., Anas, I., Rupela, O.P., Thakur, A.K., and Thyagarajan, T.M. 2009. Learning about positive plant-microbial interactions from the system of rice intensification (SRI). Asp. Appl. Biol. **98**: 29–54.

Vessey, J.K. 2003. Plant growth-promoting rhizobacteria as biofertilizers. Plant Soil, **255**: 571–586. doi:10.1023/A:1026037216893.

Waksman, S.A. 1959. The *Streptomyces*. Vol. 1. Williams and Wilkins, Baltimore, Md., USA.

Wang, Y., Brown, H.N., Crowley, D.E., and Szaniszlo, P. 1993. Evidence for direct utilization of a siderophore, ferrioxamine B, in axenically grown cucumber. Plant Cell Environ. **16**: 579–585. doi:10.1111/j.1365-3040.1993.tb00906.x.