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Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid.

Subramaniam Gopalakrishnan* · Pagidi Humayun · Bandru Keerthi Kiran · Iyer Girish Kumar Kannan · Meesala Sree Vidya · Kanala Deepthi · Om Rupela

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

*Corresponding author. E-mail: s.gopalakrishnan@cgiar.org

Tel.: +91 40 3071 3610; Fax: +91 40 3071 3074

Abstract A total of 360 bacteria, isolated from the rhizospheres of a system of rice intensification (SRI) fields, were characterized for the production of siderophore, fluorescence, indole acetic acid (IAA), hydrocyanic acid (HCN) and solubilization of phosphorus. Of them, seven most promising isolates (SRI-156, -158, -178, -211, -229, -305 and -360) were screened for their antagonistic potential against *Macrophomina phaseolina* (causes charcoal rot in sorghum) by dual culture assay, blotter paper assay and in greenhouse. All the seven isolates inhibited *M. phaseolina* in dual culture assay, whereas six isolates solubilized phosphorous (except SRI-360), all seven produced siderophore, four produced fluorescence (except SRI-178, -229 and -305), six produced IAA (except SRI-305) and five produced HCN (except SRI-158 and -305). In the blotter paper assay, no charcoal rot infection was observed in SRI-156-treated sorghum roots, indicating complete inhibition of the pathogen, while the roots treated with the other isolates showed 49–76% lesser charcoal rot infection compared to the control. In the antifungal activity test (in green house on sorghum), all the isolates increased shoot dry mass by 15–23% and root dry mass by 15–20% (except SRI-158 and -360), over the control. In order to confirm the plant growth-promoting (PGP) traits of the isolates, the green house experiment was repeated but, in the absence of *M. phaseolina*. The results further confirmed the PGP traits of the isolates as evidenced by increases in shoot and root dry mass, 22–100% and 5–20%, respectively, over the control. The sequences of 16S rDNA gene of the isolates SRI-156, -158, -178, -211, -229, -305 and -360 were matched with *Pseudomonas plecoglossicida*, *Brevibacterium antiquum*, *Bacillus altitudinis*, *Enterobacter ludwigii*, *E. ludwigii*, *Acinetobacter tandoii* and *P. monteilii*, respectively in BLAST analysis. This study indicates that the selected bacterial isolates have the potential for PGP and control of charcoal rot disease in sorghum.

Keywords: Biocontrol · Antagonistic bacteria · Charcoal rot · *Macrophomina phaseolina* · Sorghum · PGPR

Abbreviations

ANOVA	Analysis of variance
CFU	Colony forming unit
HCN	Hydrocyanic acid
IAA	Indole acetic acid
M. phaseolina	*Macrophomina phaseolina*
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PGP	Plant growth promoting
PGPR	Plant growth-promoting rhizobacteria
SRI	System of rice intensification

Introduction

Charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid., soil-and seed-borne disease of sorghum, is endemic to tropical and temperate regions of the world (Wyllie 1998). Significant losses of yield (up to 64%) have been observed in India under conditions favoring the incidence of the disease in post-rainy sorghum (*Sorghum bicolor*) occupying more than 5 million ha in Maharashtra, Karnataka and Andhra Pradesh (Das et al. 2008). In addition to severely damaging the crop, the pathogen also produces a toxin called

"phaseolinone" in the diseased stalk that causes anemia in mice (Bhattacharya et al. 1994). *M. phaseolina* can be effectively controlled by fumigating the soil with methyl bromide; however, with the ever increasing cost and concern over environmental pollution, major efforts are being taken to develop environment-friendly methods of control (Duniway 2002). These include use of pathogen, antagonist or competitor populations of a third organism and botanicals to suppress the pathogen population, making it less abundant and thus less damaging than it would be otherwise.

Plant growth-promoting rhizobacteria (PGPR) has been reported not only to improve plant growth but also to suppress the plant pathogens, of which *Pseudomonas* spp. and *Bacillus* spp. are important as these are aggressive colonizers of the rhizosphere of various crops and have broad spectrum of antagonistic activity against many pathogens (Weller et al. 2002). Biocontrol bacterial species generally employ an array of mechanisms such as antibiosis, competition, production of hydrocyanic acid, siderophore, fluorescent pigments and antifungal compounds to antagonize pathogens (Singh et al. 2006). It is a well-known fact that actively growing microbes are greater in number in the rhizosphere as crop plants release root exudates that contribute, in addition, to simple and complex sugars and growth regulators, contain different classes of primary and secondary compounds including amino acids, organic acids, phenolic acids, flavonoids, enzymes, fatty acids, nucleotides, tannins, steroids, terpenoids, alkaloids and vitamins (Uren 2000). Researchers around the world attempted to isolate PGPR organisms from the rhizospheres of crop plants and the compost (Khalid et al. 2004). An unpublished study suggested that microbial strains isolated from the rhizospheres of upland rice and system of rice intensification (SRI) fields were antagonistic to plant pathogenic fungi. This study was therefore undertaken to know if it was true for *M.*

phaseolina that causes charcoal rot of sorghum. In the SRI method of rice cultivation a set of agronomic practices are followed such as transplanting young seedlings, wider spacing, less synthetic fertilizer and growing plants in no standing water except transplanting (Kumar et al. 2010; Uphoff 2001). The objective of this study was to evaluate bacterial isolates, from the rice-rhizosphere, for their ability to suppress *M. phaseolina*, which causes charcoal rot in sorghum and to improve the growth of sorghum with and without the disease-causing agent.

Materials and methods

Collection of rhizosphere soil samples

Samples were randomly collected from the SRI field trial at ICRISAT, Patancheru, during the post-rainy season of 2008. The field trial was laid out in a split-plot design with cultivar "Krishna Hamsa", a long duration (135 days) variety of paddy. The top 0–15cm soils contained high organic carbon (0.76–1.27%) and were neutral to alkaline in pH (7.5–8.3). One whole paddy plant, after chopping off the shoots, was carefully uprooted (along with the adhering soil; without breaking the secondary and tertiary roots), placed in a polythene bag, labeled and tied (in order to minimize the evaporation loss), and further placed in a box containing ice. The ice box was transported to a lab where the roots were shaken to dislodge and separate loosely adhering soil aggregates around primary, secondary and tertiary roots, and the adhering soils were collected and stored in a refrigerator at 4^{o}C for further studies.

Isolation of bacterial strains

Ten grams of soil from each sample were separately 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 soil samples were serially diluted up to 10^6 dilutions with physiological saline. Dilutions 10^4–10^6 were plated on potato dextrose agar (PDA) by spread plate technique and incubated at 28°C for 48 h. The most prominent colonies were isolated and maintained on PDA slants at 4°C for further studies.

In vitro plant growth-promoting attributes of the bacterial isolates

Siderophore production:

It was determined according to the methodology described by Schwyn and Neilands (1987). Bacteria were streaked on chrome azurol S (CAS) agar media and incubated at 28±2°C for 48 h. When the bacteria consume iron, present in the blue-colored CAS media, orange halos are produced around the colonies, which indicate the presence of siderophores.

Fluorescence production:

The protocol of King et al. (1954) was used for fluorescence production. Bacteria were streaked on King's B agar and incubated at 28±2°C for 48 h. At the end of the incubation, the plates were observed under UV light for production of fluorescence.

Indole acetic acid (IAA) production:

It was done as per the protocols of Patten and Glick (1996). The bacteria were grown in Luria broth supplemented with L-tryptophan (1 µg ml^{-1}) for 72 h. At the end of the incubation, cultures were centrifuged at 10,000*g* for 10 min and the supernatants collected. One ml of this culture filtrate was allowed to react with 2 ml of Salkowsky reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) at 28±2°C for 30 min. At the end of the incubation, pink color

developed which indicates the presence of IAA. Quantification of IAA was done by measuring the absorbance in a spectrophotometer at 530 nm. A standard curve was plotted to quantify the IAA (µg ml^{-1}) present in the culture filtrate.

Hydrocyanic acid (HCN) production:

HCN was estimated qualitatively by sulfocyanate colorimetric method (Lorck, 1948). The bacteria were grown in Kings B agar amended with glycine (4.4 g L^{-1}). One sheet of Whatman filter paper no. 1 (8 cm diameter) was soaked in 1% picric acid (in 10% sodium carbonate; filter paper and picric acid were sterilized separately) for a minute and struck underneath the Petri dish lids. The plates were sealed with Parafilm and incubated at 28±2°C for 48 h. Development of reddish brown color on the filter paper indicated positive for HCN production.

Phosphorus (P) solubilization:

All the isolates were screened for their phosphate-solubilizing ability on Pikovskaya agar (Pikovskaya 1948). The bacteria were streaked on Pikovskaya agar and incubated for 72 h. at 28±2°C. The presence of halo zone around the bacterial colony indicated positive.

In vitro antifungal activity

Bacterial isolates were evaluated for their antifungal activity against *M. phaseolina* by dual culture assay. For this, a fungal disk of 6 mm diameter was placed on one edge of the PDA plate (1 cm from the corner) and bacterial isolate was streaked on the other edge of the plate (1 cm from the corner), followed by incubation at 28±2°C for 96 h or till the pathogen

covered the entire plate in control. Inhibition of fungal mycelium (halo zone) around the bacterial colony was scored positive and inhibition zone measured.

In vivo antifungal activity

Determination of in vivo antifungal activity of the seven most potential bacterial isolates against *M. phaseolina* was done by blotter paper assay technique (Nene et al. 1981). Inoculum of *M. phaseolina* was prepared by homogenizing (with a tissuemizer; Techmar type T 25, Japan) a 5-day-old *M. phaseolina* culture grown in potato dextrose broth (PDB) at 28±2°C. Two-week-old seedlings of sorghum (raised in sterilized vermiculite in 12 cm pots; 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 X 25 cm with one fold) so that only the roots were covered. Bacterial isolates (SRI-156, -158, -178, -211, -229, -305 and -360) and their consortium were inoculated (5 ml $plant^{-1}$) separately into plants. The consortium was prepared by mixing all the seven isolates in equal proposition (all the seven isolates were compatible to each other, data not shown). Fifteen plants per replicate and three replications were made for each bacterial isolate. Positive and negative controls were made by inoculating the plants only with *M. phaseolina* and sterile water respectively. The blotter paper was kept moist all the time with sterilized water and incubated at 28±2°C for 8 days with a 12-h day length provided by fluorescent lights (120 µ mol m^{-2} s^{-1}). At the end of the incubation, the disease symptoms of charcoal rot (black-colored microselerotia infection on the root surface) in the 0–4 rating scale was noted (0 represents no visible charcoal rot symptom, while 4 represents maximum disease symptoms), and the percentage of infected roots in bacteria inoculated treatments compared with control was calculated.

In vivo antifungal activity in a greenhouse

Seven potential antagonistic bacterial isolates (SRI-156, -158, -178, -211, -229, -305 and -360) and their consortium (mixture of all the seven isolates in equal proposition, all the seven isolates were compatible to each other, data not shown) were evaluated in greenhouse for their antagonistic potential against *M. phaseolina*. A total of 10 treatments (seven bacteria + one consortium + *M. phaseolina* inoculated- positive control + water inoculated- negative control) were made with six replications. *M. phaseolina* inoculum was mass multiplied in sorghum grains (variety R16) as per the protocols of Gupta et al. (2002). Pot mixture (800 g) was prepared by mixing red soil, sand and farm yard manure at 3:2:2 and filled in 8″ plastic pots followed by inoculation with *M. phaseolina* inoculum (20% of pot weight, 200 g pot^{-1}). Inoculum was mixed thoroughly with the pot mixture. 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^{o}C for 15 days for charcoal rot sick conditions to be developed. Two weeks later, sorghum seeds (variety R16) were surface sterilized with sodium hypochlorite (2.5% for 5 min) and rinsed with sterilized water (8 times) before being allowing it to sprout in a Petri plate overnight. The sprouted seeds were transferred into test bacterial isolates (grown in PDB separately) for an hour before being sown in the pots (six seeds/pot but thinned to three after one week). Booster doses of bacterial isolates (five ml per seedling, 10^{8} CFU ml^{-1}) were applied twice (at 15 and 30 days after sowing) by soil drench method. Growth parameters including root length, root dry weight, shoot dry weight, shoot root ratio, percentage of root and shoot dry weight increase over the control and the disease incidence were determined at day 60 after sowing.

In vivo PGPR activity in a greenhouse

For evaluating the PGPR potential of the seven biocontrol potential (against *M. phaseolina*) bacterial isolates, the above-explained greenhouse experiment was done once again but without adding *M. phaseolina*. However, one new treatment was included in which *Azotobacter chroococcum* HT-54 (phosphate solubilizing and phyto-hormone producing strain known for its PGP attributes; Kumar et al. 2001), was inoculated and positive control (only *M. phaseolina* inoculated) was removed. A total of 10 treatments (seven antagonistic bacteria + one consortium + *A. chroococcum* HT-54 + negative [water] control) were made with six replications. Growth parameters including root length, root volume, root dry weight, shoot dry weight, shoot root ratio and % root and shoot dry weight increase over the control were determined at day 60, after sowing.

Molecular identification of the isolates

Pure cultures of potential *M. phaseolina* antagonistic bacteria were grown until log phase and genomic DNA were isolated essentially according to Bazzicalupo and Fani (1995). The amplification of 16S rDNA gene was done by using universal bacterial primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCMTGGCTC AG-3') as per the conditions described by Pandey et al. (2005). The 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), aligned with using the Clustal W software (Thompson et al. 1997) and phylogenetic trees inferred using the neighbor-joining method (Saitou and Nei, 1987).

Statistical analysis

All the green house experiments were arranged in randomized block design with six replications in each treatment and repeated twice. The data were analyzed statistically by ANOVA (Genstat 10.1 version) to evaluate the efficiency of biocontrol agent's application in all the green house studies. Mean values were compared at significant levels of 1% and 5%.

Results

A total of 360 bacteria, the most prominent ones in the PDA plate, were isolated from the rhizosphere soil samples of SRI field trials. When the bacterial isolates were evaluated for their plant growth-promoting traits, 57, 19, six and five isolates produced siderophore, fluorescence, IAA and HCN, respectively, whereas 51 isolates solubilized phosphorous. Seven most positive isolates (SRI-156, -158, -178, -211, -229, -305 and -360; selected on the basis of having multiple PGP traits) were further screened for their antagonistic potential against *M. phaseolina* by in vitro dual culture assay. All the seven bacterial isolates inhibited the pathogen in the dual culture assay, whereas isolates SRI-156, -158 and -178 showed the maximum inhibition with inhibition zones of 13 mm, 14 mm and 9 mm, respectively (Table 1; Fig. 1). Of the seven bacterial isolates, 7/7, 4/7 (except SRI-178, -229 and -305) and 5/7 (except SRI-158 and -305) were positive for siderophore, fluorescence and HCN production, respectively, and 6/7 (except SRI-360) solubilized phosphorous (Table 1). Isolates SRI-211 and -229 produced higher IAA (8.06 and 8.86 µg ml^{-1}, respectively), whereas all the other isolates (except SRI-305) produced IAA between 2 and 4 µg ml^{-1} (Table 1). When all the seven bacterial isolates were evaluated for their in vivo antifungal potential against *M.*

phaseolina by blotter paper assay, neither charcoal rot disease symptoms (rating 0 in 0–4 visual rating scale) nor root infections (0%) were observed in the SRI-156-treated sorghum roots, whereas very little disease symptoms (rating 1–2) and lesser root infection (49–76% lesser than control) were observed in the other SRI isolates and consortium-treated sorghum roots (Fig. 2).

The isolates were further evaluated for their in vivo antagonistic potential against *M. phaseolina* in green house on sorghum crop. Root length was found greater in 6/7 isolates (except SRI-158), the maximum being found in two isolates viz. 413 cm in SRI-156 and 414 cm in SRI-178, compared to 250 cm in control plant roots (Table 2). All the seven isolates increased (15–23%) sorghum shoot biomass over control whereas 5/7 isolates (except SRI-158 and -360) increased (15–20%) sorghum root biomass over the control (Table 2). The highest increase of both shoot and root biomass (23% and 20%, respectively) was found in SRI-178 (Table 2). No negative effect of isolates was found even when their consortium (mixture of all the seven isolates) was used, where an increase of 18% shoot and 14% root biomass was found (Table 2). Shoot root ratio was found higher in SRI-158, -211, -229, -305, and -360 and lower in two isolates viz. SRI-156 and -178 (Table 2).

In order to confirm the PGP traits, all the isolates were further evaluated in green house but without inoculating *M. phaseolina* on sorghum crop. All the isolates increased sorghum shoot biomass between 22% and 100%, of which SRI-211 increased 100% (Table 3) followed by SRI-178 (96%), -156 (68%), -229 (32%), -305 (29%), -360 (28%) and -158 (22%). Root biomass was increased between 5% and 20% for all the isolates; however, 5/7 isolates (SRI-156, -178, -211, -229 and -305) increased more than 17% (Table 3). The consortium and *A. chroococcum* HT-54 increased 18% and 45%, respectively, for shoot

biomass, and 11% and 5%, respectively, for root biomass (Table 3). Root length, root volume and shoot root ratios were also found greater in all the isolates in comparison to the control (Table 3).

In order to determine the identity of the seven potential PGP and antagonistic (against *M. phaseolina*) bacteria, its 16S rDNA was sequenced and analyzed. A neighbor-joining dendrogram was generated using the sequence from the seven SRI isolates (1400 bp) and representative sequences from the databases. Phylogenetic analysis of 16S rDNA sequences of the seven SRI isolates showed that SRI-156 and -360 had maximum sequence similarities with *Pseudomonas* but different spp. (*P. plecoglossicida* and *P. monteilii*, respectively) whereas two other isolates (SRI-211 and -229) showed maximum sequence similarities with *Enterobacter ludwigii* (Fig. 3). The sequences of the other three isolates SRI-158, -178 and -305 were found similar to *Brevibacterium antiquum*, *Bacillus altitudinis* and *Acinetobacter tandoii*, respectively (Fig. 3).

Discussion

Microorganisms isolated from the rhizosphere soil may be better adapted to crop plants and provide better disease control than organisms isolated from the other sources such as composts, harsh environments, etc. as these are already closely associated with the plant system as well as adapted to the local environment (Cook 1993). The beneficial effects of rhizobacteria on plant health management have been demonstrated for several host pathogen systems (Rosales et al. 1986; Weller and Cook 1986; Sakthivel et al. 1986). Hence, in the present study, it was decided to isolate plant growth-promoting antagonistic bacteria from the

rhizosphere soils of SRI fields that have the potential to inhibit *M. phaseolina* which causes charcoal rot in sorghum.

Plant growth-promoting bacteria, isolated from rhizosphere soils, stimulate growth directly by nitrogen fixation (Han et al. 2005), solubilization of nutrients (Rodriguez and Fraga 1999), production of growth hormones, 1-amino-cyclopropane-1-carboxylate (ACC) deaminase (Correa et al. 2004) and indirectly by antagonizing pathogenic fungi by the production of siderophores, chitinase, β-1,3-glucanase, antibiotics, fluorescent pigments and cyanide (Pal et al. 2001). Significant population of siderophore and HCN producers present in this study (57 out of 360 SRI isolates including all the seven promising SRI isolates produced siderophore while five out of seven [except SRI-158 and-305] produced HCN) reveals that rhizospheres of SRI soils provides a conducive environment for proliferation of antagonistic bacteria that promote plant growth. In the present study, 4/7 promising SRI isolates (SRI-156, -158, -211 and -360) produced fluorescent pigments (Table 1). Fluorescent pigments producing Pseudomonads are known to have a significant role in the suppression of fungal pathogens, apparently via the production of antifungal metabolites such as phenazine-1-carboxylate (Pierson and Thomashow 1992), 2, 4-diacteylphloroglucinol (Keel et al. 1992), siderophore (Hamdram et al. 1991) and HCN (Defago and Haas 1990). Siderophores produced by a number of *Pseudomonas* spp. are attracted for their possible role in the biological control of number of plant pathogens (Mishaghi et al. 1988; Budzikiewicz 1988). Hence, siderophores can act as antimicrobial compounds by increasing the competition for available iron in the rhizosphere.

IAA-producing bacteria are known to promote root elongation and plant growth (Patten and Glick 2002). In the present investigation, 6/7 SRI isolates (except SRI-305)

produced IAA suggesting that these isolates could be used for plant growth promotion. The entire promising SRI isolates, san SRI-360, showed good solubilization zone on Pikovskaya medium supplemented with insoluble tricalcium phosphate, indicating its potential role as a P-solubilizer. Phosphate-solubilizing microorganisms convert insoluble phosphates into soluble forms through the process of acidification, chelation, exchange reactions and production of gluconic acid (Chung et al. 2005). Artursson et al. (2006) reported that free-living P-solubilizing bacteria release phosphate irons from sparing soluble inorganic and organic P compounds in soil and thereby contribute to an increased soil phosphate pool available for the plants.

In the dual culture assay, all the seven most positive and promising SRI isolates (based on their in vitro PGP and biocontrol traits) inhibited *M. phaseolina*, of which SRI-156 and -158 inhibited the pathogen the most. This inhibition could be due to the production of hydrolytic enzymes or antibiotics by the SRI isolates which were disseminated through the media. Similar results were obtained when the isolates were evaluated by blotter paper assay on sorghum plants where SRI-156 completely inhibited *M. phaseolina* (no infection and charcoal rot disease symptom was found in the sorghum roots), while all the other isolates also inhibited the fungus greatly (49−76% lesser infection compared to control; Fig. 2).

When the isolates were evaluated in the green house for their antagonistic and PGP potential on sorghum plants, shoot dry mass (18−100% increase), root dry mass (5−20% increase), root length (up to 13% increase), root volume (5−18% increase) and shoot root ratio (14−87% increase) were found higher in comparison with the control and the reference strain *A. chroococcum* HT-54. The mechanism by which the SRI isolates enhanced sorghum seedlings vigour possibly could be its PGP attributes (phosphate solubilization, IAA and

siderophore production), as in the present study, all the seven SRI isolates produced siderophores and IAA (except SRI-305) and solubilized phosphorous (except SRI-360; Table 1). Similar results were reported by Hameeda et al. (2006) in maize under greenhouse conditions where two P-solubilizing bacteria (*Serratia marcescens* EB-67 and *Pseudomonas* spp. CDB-35) increased the biomass of maize by 99% and 96%, respectively. SRI-305 was effective in the promotion of both shoot (29%) as well as roots (18%) dry mass though it did not produce IAA. Hence it can be concluded that there was no correlation between IAA production and plant growth; similar results were found by Kishore et al. (2005). Root colonization is very much essential to deliver the beneficial bacteria at the right place and time on the root, as poor root colonization may result in decreased biocontrol activity (Schippers et al. 1987). Observation on root colonization was not done in this study, however, upon looking at the data on biomass of plants (22–100% increase in shoot mass and 5–20% increase in root mass in SRI isolates inoculated treatments) it can be hypothesized that SRI isolates might have multiplied and colonized on sorghum roots, a property desirable for survival and functioning of a biocontrol agent.

The seven isolates used in this study were apparently well adapted to the sorghum rhizosphere environment as it not only controlled the charcoal rot disease in sorghum but also enhanced the plant growth. Adhikari et al. (2001) reported that bacterial strains isolated from rice rhizosphere have the potential to control of the seedling disease of rice (caused by *Achlya klebsiana* and *Pythium spinosum*) and for plant growth promotion. Similar results were obtained with plant growth-promoting *Bacillus subtilis* BN1 from the rhizospheres of chir pine (*Pinus roxburghii*; Singh et al. 2008), fluorescent *Pseudomonas* GRC_2 from potato rhizosphere (Gupta et al. 2002) and *Pseudomonas chlororaphis* SRB 127 from sorghum

rhizosphere (Das et al. 2008) that showed strong antagonistic effect against *M. phaseolina*, a charcoal rot pathogen of peanut and sorghum. Bacteria belonging to genera *Bacillus*, *Pseudomonas*, *Serratia* and *Enterobacter* are reported to solubilize the insoluble phosphatic compounds and aid in plant growth (Rodriguez and Fraga 1999), but in the present study bacteria belonging to two other genera *Acinetobacter* and *Brevibacterium* were also reported to solubilize phosphorous. *Pseudomonas* and *Bacillus* species generally employ an array of mechanisms like antibiosis, site competition, HCN production, siderophore production, fluorescent pigments and antifungal compounds to antagonize pathogens (Singh et al. 2006; Validov et al. 2005). The two *Pseudomonas* spp. (SRI-156 and -360) and one *Bacillus* spp. (SRI-178) isolated in this study were positive for siderophore, HCN and IAA production and P-solubilization (except SRI-360), and hence it can be concluded that one of these mechanisms could be the reason for their antagonistic and PGP potential.

The present study was successful in selecting effective isolates of bacteria, from rice rhizosphere, that can be a useful component of integrated disease management. All the seven SRI isolates could be used as biocontrol agents for the control of charcoal rot of sorghum. In the absence of high level of genetic resistance in high-yielding varieties, these bio-agents could be effective in controlling charcoal rot disease and related loss in grain and stover quality of sorghum. Further experiments are needed to determine the effectiveness of these isolates under different field conditions and to understand the nature of interaction with the pathogen and the host plant.

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Table and Figure Legends

Table 1 The seven most potential SRI isolates based on their plant growth promotion and biocontrol traits

Table 2 Evaluation of the seven most potential SRI isolates for their antagonistic potential against *M. phaseolina*, in sorghum under green house conditions

Values are means of six replications and data calculated per plant after 60 DAS

* = Statistically significant at 0.05 (*P* values), *** = statistically significant at 0.001 (*P* values), NS = not significant, LSD = least significant difference, SE = standard error, CV = coefficient of variance, CT = consortium

Table 3 Evaluation of the seven most potential SRI isolates for their plant growth-promoting potential, in sorghum under green house conditions

Values are means of six replications and data calculated per plant after 60 DAS

*** = Statistically significant at 0.001 (*P* values), NS= not significant, CT = consortium, $^{@}$= *Azotobacter chroococcum* HT-54, cont. = control, LSD = least significant difference, SE = standard error, CV = coefficient of variance.

Fig. 1 Influence of SRI 158 isolate on *M. phaseolina* by dual culture assay

Fig. 2 Influence of the seven most potential SRI isolates on *M. phaseolina* by blotting paper assay

Fig. 3 Phylogenetic relationship between SRI bacterial isolates and representative species based on full length 16S rDNA sequences constructed using the neighbor-joining method

Isolate	Phosphorous solubilization	Siderophore production	Fluorescence Production	IAA Production (µg ml^{-1})	HCN production	Antagonistic to *M. phaseolina* (inhibition zone)
SRI 156	+	+	+	3.69	+	+ (13mm)
SRI 158	+	+	+	2.79	–	+ (14mm)
SRI 178	+	+	–	1.98	+	+ (9mm)
SRI 211	+	+	+	8.06	+	+
SRI 229	+	+	–	8.86	+	+
SRI 305	+	+	–	0	–	+
SRI 360	–	+	+	2.75	+	+

Table 1 Gopalakrishnan et al. 2010

Treatment	Root length (cm)	Root weight (g)	Shoot weight (g)	Shoot root ratio	%Root weight increase over control	%Shoot weight increase over control
Control	250	1.05	5.00	4.60	–	–
SRI 156	413	1.22	5.93	4.37	15.9	18.6
SRI 158	218	1.11	5.77	4.70	5.5	15.4
SRI 178	414	1.26	6.13	4.41	19.8	22.6
SRI 211	331	1.24	5.98	4.90	17.6	19.6
SRI 229	308	1.20	5.87	4.83	14.5	17.4
SRI 305	350	1.23	5.77	5.00	17.1	15.4
SRI 360	260	1.11	5.82	5.65	5.8	16.4
CT	289	1.20	5.89	5.03	13.9	17.8
Mean	315	1.18	5.80	4.83	13.8	17.9
LSD (5%)	147.8	0.169	0.639	0.507		
SE±	51.5^{NS}	0.059^{*}	0.223^{*}	1.761^{***}		
CV%	40	12	9	9		

Table 2 Gopalakrishnan et al. 2010

Treatment	Root length (cm)	Root volume (cm^{-3})	Root weight (g)	Shoot weight (g)	Shoot root ratio	%Root weight increase over cont.	%Shoot weight increase over cont.
Control	406	4.28	3.51	13.8	3.82	–	–
HT 54[@]	457	4.70	3.67	20.0	5.29	4.5	44.6
SRI 156	410	4.51	4.12	23.2	6.22	17.4	68.0
SRI 158	457	5.02	3.74	16.8	4.36	6.5	21.7
SRI 178	409	4.72	4.26	27.1	7.17	21.3	96.4
SRI 211	437	4.86	4.22	27.6	7.12	20.1	99.6
SRI 229	444	5.08	4.13	18.2	4.90	17.8	31.9
SRI 305	445	4.94	4.16	17.8	4.77	18.4	28.8
SRI 360	457	4.91	3.69	17.6	4.64	5.1	27.7
CT	411	4.18	3.91	16.3	5.12	11.4	18.1
Mean	436	4.77	3.94	19.8	5.34	13.6	48.5
LSD (5%)	100.6	1.055	0.304	6.04	1.581		
SE±	35.2[NS]	0.370[NS]	0.107[NS]	2.19[***]	0.555[***]		
CV%	20	19	7	25	25		

Table 3 Gopalakrishnan et al. 2010

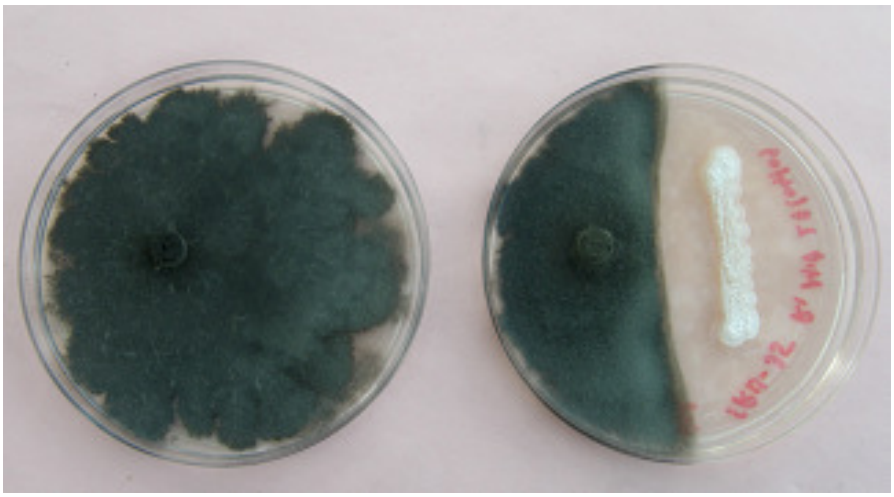

Fig. 1 Gopalakrishnan et al. 2010

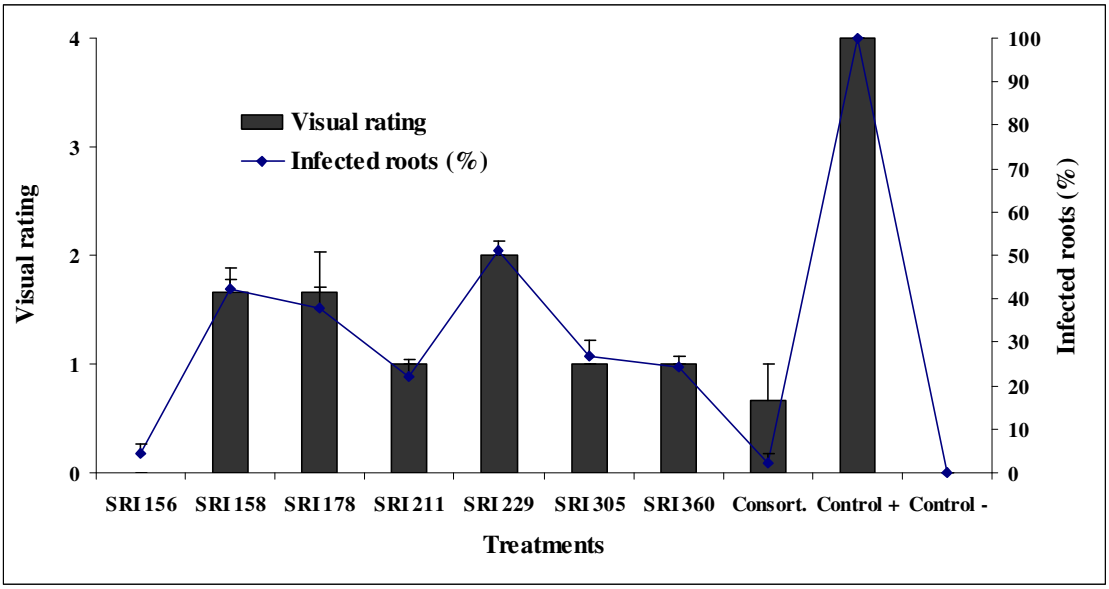

Fig. 2 Gopalakrishnan et al. 2010

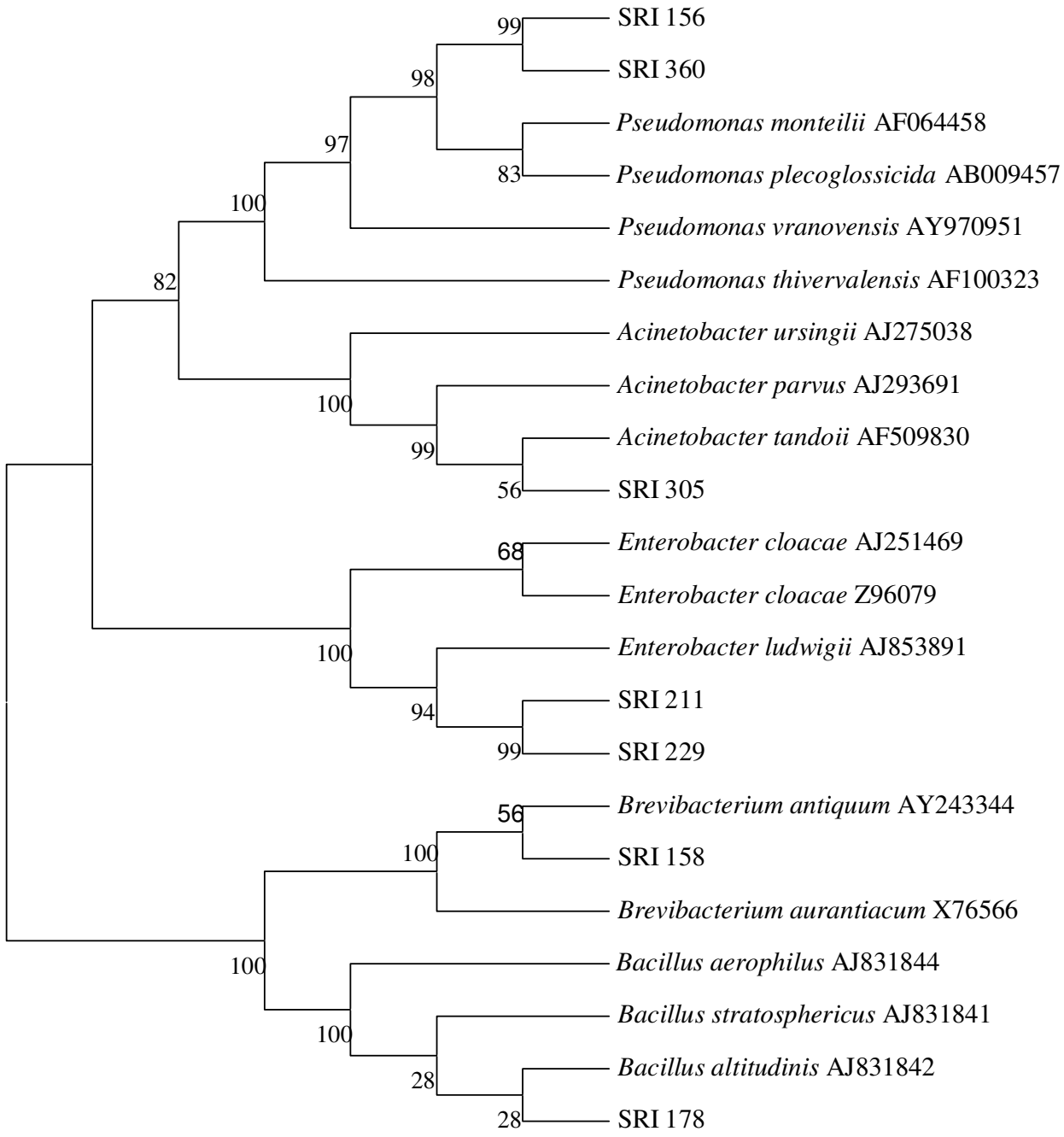

Fig. 3 Gopalakrishnan et al. 2010