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Plant growth-promoting traits of *Streptomyces* with biocontrol potential isolated from herbal vermicompost

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Three strains of *Streptomyces* (CAI-21, CAI-26 and MMA-32) were earlier reported by us as having potential for biocontrol of charcoal rot of sorghum, caused by *Macrophomina phaseolina* (Tassi) Goid., and plant growth promotion (PGP) of the plant. In the present investigation, the three *Streptomyces* were characterized for their physiological traits (tolerance of salinity, temperature, pH and resistance to

antibiotics) and further evaluated in the field for their PGP of rice, grown by a system of rice intensification (SRI) methods. All three *Streptomyces* were able to grow in NaCl concentrations of up to 12% (except MMA-32), at pH values between 5 and 13 and temperatures between 20 and 40^{o}C. They were highly resistant to ampicillin and trimethoprim (>800 ppm), sensitive to chloramphenicol, kanamycin and nalidixic acid (50−100 ppm) and highly sensitive to streptomycin and tetracycline (5−25 ppm). When evaluated for their PGP activity on seedlings of rice, % germination and shoot and root lengths were significantly enhanced over the control. In the field, the *Streptomyces* strains significantly enhanced the panicle length, filled grain numbers and weight, panicle weight, 1000 seed weight, tiller numbers, total dry matter, root length (39−65%), root volume (13−30%), root dry weight (16−24%), grain yield (9−11%) and stover yield (11−22%) over the control. In the rhizosphere soil (0−15cm from root) at harvest, the population of actinomycetes was significantly enhanced as was microbial biomass carbon (27−83%) and nitrogen (24−43%), dehydrogenase activity (34−152%), available P (13−34%) and N (30−53%) and % organic carbon (26−28%). This study further confirms that the selected *Streptomyces* have plant growth promoting properties.

Keywords: Actinomycetes, field evaluation, rice, plant growth promotion, *Streptomyces*

Introduction

Plant growth-promoting (PGP) microorganisms are beneficial soil microorganisms which may facilitate plant growth both directly and indirectly. Direct stimulation may include providing plants with fixed nitrogen, soluble phosphate, iron chelators and phytohormones, while indirect stimulation includes inhibiting phytopathogens (biocontrol), thus promoting plant growth and development. Novel microorganisms with PGP and biocontrol traits are found at much higher incidences in forests, pasture soils and herbal compost than in arable soils (Torsvik et al. 2002; Tinatin and Nurzat 2006). Vermicompost and vermiwash prepared from herbals not only benefit crop plants, as they contain beneficial microorganisms that help the plants to mobilize and acquire nutrients, but also inhibit many plant pathogenic microorganisms and promote plant growth (Postma et al. 2003; Suthar et al. 2005; Nath and Singh 2009; Gopalakrishnan et al. 2010; 2011a,c).

Earlier, we reported a set of seven *Streptomyces* strains isolated from herbal vermicompost, including the three *Streptomyces* strains reported in this study viz. CAI-21, CAI-26 and MMA-32, which had the potential for PGP and control of charcoal-rot disease, caused by *Macrophomina phaseolina* (Tassi) Goid., in sorghum (Gopalakrishnan et al. 2011b). Also, the selected *Streptomyces*

strains produced siderophore, hydrocyanic acid, protease, chitinase (except CAI-21) and indole acetic acid (Gopalakrishnan et al. 2011b). The objective of this study was to characterize the three *Streptomyces* strains for their physiological traits (salinity, temperature, pH and antibiotic resistance) and to further evaluate under field conditions, their PGP traits in rice grown using the system of rice intensification (SRI) method. In the SRI method of rice cultivation a set of agronomic practices are followed involving transplanting young (8−12d old) seedlings, wider spacing, less synthetic fertilizer and growing plants in no standing water except at transplanting (Uphoff 2001; Kumar et al. 2010).

Materials and methods

Actinomycete strains

Three strains of *Streptomyces* isolated from herbal vermicompost, CAI-21 (*Streptomyces* spp.; NCBI Accession Number: JQ682620), CAI-26 (*Streptomyces* spp.; NCBI Accession Number: JQ682621) and MMA-32 (*Streptomyces* spp.; NCBI Accession Number: JQ682626), reported earlier by us as potential for biocontrol and PGP traits in sorghum (Gopalakrishnan et al. 2011b), were further studied in this investigation.

Evaluation of actinomycetes for their physiological traits

Salinity

Streptomyces strains (CAI-21, CAI-26 and MMA-32) were streaked on Bennet's agar with various concentrations of NaCl ranging from 0% to 16% at the interval of 2% and incubated at 28^{o}C for five days.

pH

The three *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) were streaked on Bennet's agar adjusted to pH 5, 7, 9, 11 and 13 and incubated at 28^{o}C for five days. For pH 3, Bennet's broth was inoculated with the three actinomycetes and at the end of five-day incubation the intensity of growth was measured at 600nm in a spectrophotometer.

Temperature

The three *Streptomyces* strains were streaked on Bennet's agar and incubated at 20, 30, and 40^{o}C for five days. For 50^{o}C, Bennet's broth was inoculated with the three actinomycetes, and at the end of five-day incubation, the intensity of growth was measured at 600nm in a spectrophotometer.

Antibiotic resistance/susceptible pattern

A total of seven antibiotics viz. chloramphenicol, kanamycin, trimethoprim, nalidixic acid, streptomycin, ampicillin and tetracycline were studied for their resistance/susceptible pattern against the three *Streptomyces* strains. The required quantities of antibiotics were dissolved in sterilized Milli Q water and mixed into Bennet's agar just before pouring into the Petri plates (when the temperature of the media was about 50^{o}C). Upon solidification, the actinomycetes (CAI-21, CAI-26 and MMA-32) were streaked and incubated at 28^{o}C for five days.

Responses of the three *Streptomyces* strains to salinity, pH, temperature and antibiotics were recorded as follows: - = no growth; + = slight growth; ++ = moderate growth and +++ = good growth.

Influence of actinomycetes on germination of rice seed

Seeds of rice (variety Sampada) were surface sterilized with 2.5% sodium hypochlorite solution for 5min and rinsed with sterilized water (8 times). Samples of the surface-sterilized seeds were soaked separately with the three *Streptomyces* strains for 1h (10^{8} CFU ml^{-1}). Growth promoting activity was evaluated by the "Rag-Doll" method as described by Chamblee and Green (1995). In brief, 100 rice seeds inoculated with actinomycetes were placed in one half of a wet paper towel, folded and rolled into a moderately tight tube and tied at the end to make a rag doll. The rag doll was positioned so that the tube was upright (in order to allow roots to grow down and shoots to grow up so that seedlings were more easily removed during counting) and kept in a warm place (at 30^{o}C) for five days. At the end of the incubation period % germination and root and shoot length were measured.

Evaluation of actinomycetes for PGP traits on rice under field conditions

Experimental site and soil

The experiment was conducted in 2010−11 at the Directorate of Rice Research Farm in ICRISAT, Patancheru, Hyderabad, Andhra Pradesh, India (17^{o}53'N latitude, 78^{o}27'E longitude and 545 m altitude) during the dry season (December−May) 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. Organic carbon content was moderate (0.76−1.27%). The mineral content of the top 15cm layer was as follows: available nitrogen 292kg ha^{-1}, available phosphorus 26.8kg ha^{-1} and available potassium 527kg ha^{-1}.

Experimental design and treatments

The experiment was laid out in a completely randomized block design with three replicates and subplot sizes of 10x7.5m. Rice was grown by the system of rice intensification (SRI) method proposed by the Central Rice Research Institute (http://crri.nic.in). All plots were surrounded by 1.5m wide bounds to prevent lateral water seepage and nutrient diffusion between plots. The three *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) were grown on a starch casein broth at 28°C for five days and further evaluated for their PGP traits. Control contained no actinomycetes.

Experimental protocol

A nursery was established adjacent to the experimental field so that transplanting could be performed rapidly in order to minimize seedling injury. Twelve-day-old single seedlings were uprooted from the nursery, their roots dipped in the respective *Streptomyces* strains broth (containing 10^8 CFU ml^{-1}) for 45min and transplanted on 3rd Jan 2011 at a spacing of 25x25cm. Rice plants were inoculated with the actinomycetes (1000ml; 10^8 CFU ml^{-1}) once in 15 days until the flowering stage along with the irrigation water. The recommended dose of NPK (120, 60 and 40kg ha^{-1}, 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, i.e. the alternate wetting and drying method of irrigation. After panicle initiation, all the plots were kept flooded with a thin layer of water (1−2cm), and all were drained at 15 days before harvest.

The crop was harvested manually on 31st May 2011 and observed for plant height, number of tillers m^{-2}, panicle length, panicle weight, filled grain number, filled grain weight, 1000 seed weight, grain yield, stover yield and total dry matter. Root samples were collected from the top 15cm soil profile and analyzed for root length density (EPSON expression1640x, Japan), volume and dry weight (dried in an oven at 70°C for 48h). Soil samples were collected from 0 to 15cm soil profile at 75 DAT

and at harvesting. These were analyzed for soil chemistry (% organic carbon, available phosphorous and total nitrogen as per the protocols of Nelson and Sommers 1982; Olsen and Sommers 1982 and Novozamsky et al. 1983, respectively) and biological analysis (dehydrogenase activity, microbial biomass nitrogen and microbial biomass carbon as per Casida 1977, Brooks et al. 1985 and Anderson and Domsch 1989, respectively). For actinomycete counts, the soil samples (10g) were serially suspended in 90ml of physiological saline (0.85% NaCl) in a flask and placed on an orbital shaker (at 100 rpm with 25mm throw) at room temperature (28±2^{o}C) for 1h. At the end of shaking, the soil samples were serially diluted ten-fold up to 10^6 dilution with physiological saline. Dilutions 10^4–10^6 were plated on starch casein agar by the spread plate technique and incubated at 28±2^{o}C for five days. At the end of incubation, the actinomycete colonies were enumerated and compared with the control.

Statistical analysis

The field experiment data were subjected to ANOVA (GenStat 10.1 version 2007, Lawes Agricultural Trust, Rothamsted Experimental Station) to evaluate the efficiency of the biocontrol agents application. Significance of differences between the treatment means were tested at $P = 0.01$ and 0.05.

Results

Evaluation of actinomycetes for their physiological traits

All three *Streptomyces* strains were able to grow in NaCl up to 12% (except MMA-32 which grew only up to 6% NaCl) and they were all able to grow at pH values between 5 and 13 (acidic to highly alkaline) and temperatures between 20 and 40^{o}C (Table 1). However, the optimum conditions for good growth were 0−4% NaCl, pH values of 7−13 pH and temperatures of 20−30^{o}C (Table 1). All three strains were highly resistant to ampicillin and trimethoprim (>800 ppm), sensitive to chloramphenicol, kanamycin and nalidixic acid (50−100 ppm) and highly sensitive to streptomycin and tetracycline (5−25 ppm; Table 1).

Influence of actinomycetes on germination of rice seed

The three *Streptomyces* strains significantly enhanced the germination of rice seedlings as determined by the Rag-Doll method (Table 2). Also root and shoot length of seedlings were increased significantly by 13−14% and 25−37%, respectively (Table 2).

Evaluation of actinomycetes for PGP traits on rice under field conditions

Under field conditions, the *Streptomyces* strains significantly enhanced plant height (cm plant^{-1}), tillers m^{-2}, panicle length (cm plant^{-1}), panicle weight (g plant^{-1}), filled grain number (plant^{-1}), filled grain weight (plant^{-1}), 1000 seed weight (g), stover yield (g m^{-2}), grain yield (g m^{-2}) and total dry matter (g m^{-2}) over the control (Table 3). Grain and stover yield were enhanced by 9−11% and 11−22%, respectively, over un-inoculated controls (Table 3). Root length (mm^{-2}), volume (cm^{-3} m^{-2}) and dry weight (g m^{-2}) were also increased by 39−65%, 13−30% and 16−24%, respectively (Table 4). Among the three *Streptomyces* strains, CAI-21 caused greater increases of the root system and yield than the other two isolates (Table 4).

The available P, total N and organic carbon % were significantly higher in the top 15cm of rhizosphere soils of *Streptomyces* treated plants (by 13−34%, 30−53% and 26−28%, respectively) at harvesting than those of controls (Table 5). The biological activities (microbial biomass carbon, microbial biomass nitrogen and dehydrogenase activity) in the top 15cm rhizosphere soils were also found to be significantly higher in the *Streptomyces* strains-inoculated treatments at harvest, over the controls (27−83%, 23−43% and 34−151%, respectively; Table 6). The biological activities were also found to be significantly higher than the controls at flowering (Table 6). Actinomycete populations at flowering and harvesting stages were considerably higher in the *Streptomyces* strains-inoculated treatments than the controls (Table 7).

Discussion

In the present investigation, the PGP activities of the *Streptomyces* strains (CAI-21, CAI-26 and MMA-32) were evaluated in rice grown under aerobic soil conditions by the SRI method. This favours the growth of microbes compared with the anaerobic conditions of paddy rice which lead to increased nitrification rates (Shooksa-nguan et al. 2009). Hence, it is likely that larger and more bio-diverse soil microbial populations under SRI conditions will give rise to a faster rate of mineralization of soil organic matter and thereby to enhanced nutrient supply. Also, changes in the dominant ammonia-oxidizing bacterial populations have the potential to change the N dynamics in the SRI system, and thereby improve yields.

When the *Streptomyces* strains were characterized for their physiological traits, all three strains were able to grow in NaCl up to 6% and at pH values between 5 and 13 and temperatures between 20 and 40^{o}C. The ability of *Streptomyces* strains to tolerate high concentrations of NaCl is well known (Waksman 1959). Sadeghi et al. (2012) have reported the beneficial role of *Streptomyces* on PGP activity under salinity stress. Hence, it can be concluded that these strains may have the ability to survive in the harsh environments such as saline and acidic to alkaline pH soils. As all three strains of *Streptomyces* were highly resistant to ampicillin and trimethoprim (>800 ppm) but highly sensitive to streptomycin and tetracycline (<25 ppm), these antibiotics could be used as markers for their identification in field evaluation studies.

In the present study, the *Streptomyces* strains significantly enhanced all the morphological observations studied: plant height, tillering, panicle length and dry weight, filled grain number and weight, 1000 seed weight, stover and grain yield. The PGP potential of *Streptomyces* strains are reported frequently in the literature (Nassar et al. 2003; El-Tarabily 2008). In the present study, the mechanism by which the *Streptomyces* enhanced all the morphological observations including rice grain and stover yield could be their PGP attributes such as indole acetic acid (IAA) and siderophore production as the strains were shown previously to produce these compounds (Gopalakrishnan et al. 2011b). IAA-producing microorganisms are known to promote root elongation and plant growth (Patten and Glick 2002) and siderophores are often produced by various soil microbes including actinomycetes (*Streptomyces* strains). Siderophores act by binding Fe^{3+} from the environment and making it available to the plant (Wang et al. 1993; Tokala et al. 2002). The interaction between soil microorganisms and roots and their possible impacts on plant growth have been studied by Birkhofer et

al. (2008) and Uphoff et al. (2009) among others. Significant differences in the root systems of rice grown by the SRI method (including root mass, length and volume) were observed by Rupela et al. (2006). When the soils were made wet and then dry, as in the case of the SRI method of rice cultivation, the levels of available P in the soil solution increased between 185% and 1900% as a result of population dynamics of species of phosphorous-solubilizing bacteria and fungi (Turner and Hayagarath 2001). Gayathry (2002) found that the counts of bacteria, such as the diazotrophs, *Azospirillum*, *Azotobacter* and phosphobacteria as well as microbial enzyme activity such as dehydrogenase, urease acid phosphatase, alkaline phosphatase and nitrogenase were significantly higher in SRI rhizospheres than those of the same variety of rice plants grown conventionally. In the present investigation, such enhanced activities were found only in the *Streptomyces*-inoculated treatments.

Colonization of roots by actinomycetes at the right place and time is essential for enhanced PGP activity. Successful interactions depend on sufficient population density, rhizosphere competence, root colonizing ability and PGP ability of the bacteria (Lugtenberg and Dekkers 1999). Although roots were not inspected for colonization in this study, the data on grain and stover yield, roots, chemical composition of the rhizosphere and biological and microbial activities strongly suggest that actinomycetes had multiplied and colonized the inoculated rice roots.

The three strains of *Streptomyces* used in this study were apparently well adapted not only to the sorghum rhizosphere environment (Gopalakrishnan et al. 2011b) but also to the rice rhizosphere where they promoted plant growth. Hence, these strains could be used as PGP agents in addition to biocontrol agents for the control of charcoal rot. With the ever increasing demands for chemical fertilizers and fungicides and in the absence of high level of genetic resistance in high-yielding varieties of rice and sorghum, these PGP agents could be effective alternatives to these demands. The broad range of PGP and antifungal activities of the three *Streptomyces* demonstrates multiple mechanisms of action including antibiosis and production of IAA, HCN, siderophore and cell wall degrading enzymes such as protease and chitinase (Gopalakrishnan et al. 2011b). There is a growing interest in the use of secondary metabolites such as toxins, proteins, hormones, vitamins, amino acids and antibiotics from microorganisms, particularly from actinomycetes, for plant growth promotion and control of plant pathogens as these are readily degradable, highly specific and less-toxic to non-target organisms than conventional biocides (Doumbou et al. 2001). These strains, 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 of importance in PGP and biocontrol.

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References

Anderson, T.H., and Domsch, K.H. (1989), 'Ratios of microbial biomass carbon to total organic carbon in arable soils', *Soil Biology and Biochemistry*, 21, 471−479.

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 Biology and Biochemistry*, 40, 2297−2308.

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 Biology and Biochemistry*, 17, 837−842.

Casida, L.E. (1977), 'Microbial metabolic activity in soil as measured by dehydrogenase determinations', *Applied and Environmental Microbiology*, 34, 630−636.

Chamblee, D.S., and Green, J.T. (1995), 'Production and utilization of pastures and forages in North Carolina', *North Carolina Agricultural Research Service Technical Bulletin*, 305, 153.

Doumbou, C.L., Salove, M.K., Crawford, D.L., and Beaulieu, C. (2001), 'Actinomycetes, promising tools to control plant diseases and to promote plant growth', *Phytoprotection*, 82, 85−102.

El-Tarabily, K.A. (2008), 'Promotion of tomato (*Lycopersicon esculentun* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase–producing *Streptomycete* actinomycetes', *Plant and Soil*, 308, 161−174.

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. (2011a), 'Evaluation of bacteria isolated from rice rhizosphere for biological control of charcoal rot of sorghum caused by *Macrophomina phaseolina* (Tassi) Goid', *World Journal of Microbiology and Biotechnology*, 27, 1313−1321.

Gopalakrishnan, S., Kannan, I.G.K., Alekhya, G., Humayun, P., Vidya, S.M., and Deepthi, K. (2010), 'Efficacy of Jatropha, Annona and Parthenium biowash on *Sclerotium rolfsii*, *Fusarium oxysporum* f. sp. *ciceri* and *Macrophomina phaseolina*, pathogens of chickpea and sorghum', *African Journal of Biotechnology*, 9, 8048−8057.

Gopalakrishnan,S., Kiran, B.K., Humayun, P., Vidya, M.S., Deepthi, K., and Rupela, O. (2011b), 'Biocontrol of charcoal-rot of sorghum by actinomycetes isolated from herbal vermicompost', *African Journal of Biotechnology*, 10, 18142−18152.

Gopalakrishnan, S., Pande, S., Sharma, M., Humayun, P., Kiran, B.K., Sandeep, D., Vidya, M.S., Deepthi, K., and Rupela, O. (2011c), 'Evaluation of actinomycete isolates obtained from herbal vermicompost for biological control of *Fusarium* wilt of chickpea', *Crop Protection*, 30, 1070−1078.

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', *Journal of Rice Research*, 2, 61−71.

Lugtenberg, B.J.J., and Dekkers, L.C. (1999), 'What makes *Pseudomonas* bacteria rhizosphere competent?', *Environmental Microbiology*, 1, 9−13.

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 Regulation*, 40, 97−106.

Nath, G., and Singh, K. (2009), 'Utilization of vermiwash potential on certain summer vegetable crops', *Journal of Central European Agriculture*, 10, 417−426.

Nelson, D.W., and Sommers L.E. (1982), 'Total organic carbon and organic matter', In: Page A.L., Miller, R.H., and Keeney, D.R. (eds.), 'Methods of soil analysis', Part 3, 'Chemical and microbiological properties', Madison, WI: SSSA, pp. 539−579.

Novozamsky, I., Houba, V.J.G., Van, E.C.K.R., and vanVark, W. (1983), 'A novel digestiontechnique for multiple element analysis', *Communication in Soil Science and Plant Analysis*, 14, 239−249.

Olsen, S.R., and Sommers, L.E. (1982), 'Phosphorus', In: Page, A. L. (ed.), 'Methods of soil analysis', Agron. No. 9, Part 2, 'chemical and microbial properties', 2nd edition, 'Am. Soc. Agron., Madison, WI, 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', *Applied and Environmental Microbiology*, 68, 3795−3801.

Postma, J., Montanari, M., and Van den Boogert, P.H.J.F. (2003), 'Microbial enrichment to enhance disease suppressive activity of compost', *European Journal of Soil Biology*, 39, 157−163.

Rupela, O.P., Wani, S.P., Kranthi, M., Humayun, P., Satyanarayana, A., Goud, V., Gujja, B., Punnarao, P., Shashibhushan, V., Raju, D.J., and Reddy, P.L. (2006), 'Comparing soil properties of farmer's fields growing rice by SRI and conventional methods', In: Proceedings of 1st National SRI symposium, 17−18 November, Patancheru, Hyderabad.

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 Journal of Microbiology Biotechnology*, 28, 1503−1509.

Sooksha-nguan, T., Thies, J.E., Gypmantasiri, P., Boonkerd, N., and Teaumroong, N. (2009), 'Effect of rice cultivation systems on nitrogen cycling and nitrifying bacterial community structure', *Applied Soil Ecology*, 43, 139−149.

Suthar, S., Choyal, R., Singh, R., and Sudesh, R. (2005), 'Stimulatory effect of earthworm body fluid on seed germination and seedlings growth of two legumes', *Journal of Phytological Research,* 1, 219−222.

Tinatin, D., and Nurzat, T. (2006), 'Biodiversity of *Streptomyces* of high-mountainous ecosystems of Kyrgystan and its biotechnological potential', *Antonie van Leeuwenhoek*, 89, 325−328.

Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F., and Morra, J. (2002), 'Novel plant−microbe rhizosphere interaction involving *Streptomyces lydicus*

WYEC108 and the pea plant (*Pisum sativum*)', *Applied Environmental Microbiology*, 68, 2161−2171.

Torsvik, V., Ovreas, L., and Thingstad, T.F. (2002), 'Prokaryotic diversity: magnitude, dynamics and controlling factors', *Science*, 296, 1064−1066.

Turner, B.L., and Haygarth, P.M. (2001), 'Phosphorous solublization in rewetted soils', *Nature*, 411, 258.

Uphoff, N. (2001), 'Scientific issues raised by the SRI: a less water rice cultivations system', In: Hengsdijk, H. and Bindraban, P., (eds.), 'Water savings rice production systems' 'Proceedings of an international workshop on water saving rice production', Nanjing University, China, 2−4 April. 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)', *Aspects of Applied Biology*, 98, 29−54.

Waksman, S.A. (1959), 'The *Streptomyces*', vol. 1, Williams and Wilkins Co., Baltimore.

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 & Environment*, 16, 579−585.

Table 1 Effect of salinity, pH, temperature and antibiotics resistance pattern on the growth of *Streptomyces* strains CAI-21, CAI-26 and MMA-32

Traits	CAI-21	CAI-26	MMA-32
Salinity (%)			
0	+++	+++	+++
2	+++	+++	+++
4	+++	+++	+++
6	++	++	++
8	+	+	-
10	+	+	-
12	+	+	-
14	-	-	-
16	-	-	-
pH			
3	-	-	-
5	+++	++	+
7	+++	+++	+++
9	+++	+++	+++
11	+++	+++	+++
13	+++	+++	+++
Temperature (^{O}C)			
20	+++	+++	+++
30	+++	+++	+++
40	++	++	++
50	-	-	-
Antibiotics resistance/susceptible pattern (ppm)			
Ampicillin	1000	800	800
Chloramphenicol	50	50	50
Kanamycin	100	100	50
Nalidixic acid	100	100	50
Streptomycin	25	25	10
Tetracycline	5	5	25
Trimethoprim	800	800	800

+++ = Good growth; ++ = Medium growth; + = poor growth; - = no growth

Table 2: Effect of *Streptomyces* strains CAI-21, CAI-26 and MMA-32, on rice seed germination

Treatment	% Germination	Root length (cm)	Shoot length (cm)
CAI-21	98	3.18 (15.2)	0.81 (58.8)
CAI-26	94	3.21 (16.3)	0.75 (47.3)
MMA-32	99	3.17 (14.8)	0.68 (33.3)
Control	90	2.76	0.51
Mean	95	3.08	0.69
SE±	2.1*	0.111*	0.033***
LSD (5%)	6.1	0.325	0.097
CV%	7	11	15

SE = Standard error; LSD = Least significant difference; CV = Coefficient of variance;
* = Statistically significant at 0.05, *** = Statistically significant at 0.001; % increases over controls are given in parenthesis

Table 3: Effect of *Streptomyces* strains CAI-21, CAI-26 and MMA-32 on the morphology and yield potential of rice cultivation

Treatment	Plant height (cm)	Tillers (m^{-2})	Panicle length (cm)	Panicle (g $plant^{-1}$)	Filled grain wt (n $plant^{-1}$)	Filled grain (g $plant^{-1}$)	1000 wt wt (g)	Stover seed (gm^{-2})	Grain yield (gm^{-2})	Total dry yield matter (gm^{-2})
CAI-21	83	596	24	3.0	169	2.7	15.9	1159 (11.3)	957 (10.6)	2116
CAI-26	82	589	23	2.9	167	2.7	15.9	1274 (22.3)	942 (8.9)	2216
MMA-32	80	587	23	2.9	176	2.8	15.6	1167 (12.1)	939 (8.5)	2106
Control	78	588	22	2.7	152	2.3	15.1	1041	865	1906
Mean	81	590	23	2.86	166	2.59	15.6	1160	926	2086
SE±	0.41**	1.6*	0.29*	0.025***	0.65***	0.006***	0.08**	15.7**	14.4*	10.1**
LSD (5%)	1.61	6.0	1.29	0.112	2.91	0.028	0.37	70.8	56.7	61.7
CV%	1	1	2	1	1	1	1	2	3	1

*= Statistically significant at 0.05; **= Statistically significant at 0.01; ***= Statistically significant at 0.001; wt = weight; n = number; % increases over controls are given in parenthesis

Table 4: Effect of *Streptomyces* strains CAI-21, CAI-26 and MMA-32 on the root development of rice at harvesting stage of rice cultivation

Treatment	Root length (mm^{-2})	Root volume (cm^3m^{-2})	Root dry weight (gm^{-2})
CAI-21	5453 (65.2)	1338 (30.0)	103.2 (23.8)
CAI-26	5194 (57.4)	1299 (26.2)	97.4 (16.9)
MMA-32	4596 (39.3)	1159 (12.6)	96.7 (16.0)
Control	3299	1029	83.3
Mean	4636	1206	95.2
SE±	271.5**	60.8*	3.9*
LSD (5%)	939.6	210.5	13.6
CV%	10	9	7

SE = standard error; LSD = Least significant difference; CV = Coefficient of variance;
* = Statistically significant at 0.05; ** = Statistically significant at 0.01; % increases over controls are given in parenthesis

Table 5: Effect of *Streptomyces* strains CAI-21, CAI-26 and MMA-32 on soil chemical activity at vegetative (flowering) and harvesting stage of rice cultivation

	Available P (ppm)		Total N (ppm)		Organic carbon %	
	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
CAI-21	114	142 (33.9)	2364	2978 (53.1)	1.53	1.47 (25.6)
CAI-26	116	119 (12.2)	2337	2823 (45.1)	1.46	1.50 (28.2)
MMA-32	95	120 (13.2)	2556	2535 (30.3)	1.65	1.50 (28.2)
Control	98	106	2490	1945	1.65	1.17
Mean	106	122	2437	2570	1.57	1.41
SE±	4.3*	5.7*	49.2*	141.9**	0.041*	0.052*
LSD (5%)	15.0	22.2	170.3	491.2	0.140	0.179
CV%	7	8	4	10	5	6

P = Phosphorous; N = Nitrogen; ppm = Parts per million; SE = Standard error; LSD = Least significance difference; CV = Coefficient of variance; * = Statistically significant at 0.05; ** = Statistically significant at 0.01; % increases over controls are given in parenthesis

Table 6: Effect of *Streptomyces* strains CAI-21, CAI-26 and MMA-32 on soil biological activity at vegetative (flowering) and harvesting stage of rice cultivation

Treatment	MBC ($\mu g\ g^{-1}$ soil)		MBN ($\mu g\ g^{-1}$ soil)		Dehydrogenase activity (μg TPF g^{-1} soil $24h^{-1}$)	
	Flowering	Harvest	Flowering	Harvest	Flowering	Harvest
CAI-21	3180	2149 (49.4)	113	70 (37.2)	211	156 (151.6)
CAI-26	4033	1824 (26.8)	99	63 (23.5)	289	134 (116.1)
MMA-32	2767	2634 (83.1)	87	73 (43.1)	217	83 (33.8)
Control	2349	1438	75	51	177	62
Mean	3082	2011	93	64	223	109
SE±	162.9*	183.9*	5.9*	2.1**	17.7*	12.6**
LSD (5%)	733.0	636.2	231.	7.8	61.4	43.7
CV%	9	16	11	6	14	20

MBC = Microbial biomass carbon; MBN = Microbial biomass nitrogen; SE = Standard error; LSD = Least significant difference; CV = Coefficient of variance; * = Statistically significant at 0.05; ** = Statistically significant at 0.01; % increases over controls are given in parenthesis

Table 7: Actinomycetes population in *Streptomyces* strains CAI-21, CAI-26 and MMA-32 inoculated plots compared to control plots at both vegetative as well as harvesting stage of rice cultivation

Treatment	Actinomycetes (Log_{10}) at flowering	Actinomycetes (Log_{10}) at harvesting
CAI-21	5.67	5.98
CAI-26	5.69	5.99
MMA-32	5.78	6.06
Control	5.58	5.76
Mean	5.68	5.94
SE±	0.0148***	0.0423*
LSD (5%)	0.0539	0.1539
CV%	1	1

SE = Standard error; LSD = Least significant difference; CV% = Coefficient of variance; ** = Statistically significant at 0.01; *** = Statistically significant at 0.001