Pak. J. Bot., 45(5): 1781-1788, 2013.

FUNCTION AND PYLOGENETIC CHARACTERIZATION OF RHIZOSPHERIC BACTERIA ASSOCIATED WITH GM AND NON GM MAIZE

NASEER AHMAD, ZABTA KHAN SHINWARI*, SAMINA BASHIR AND MUHAMMAD YASIR

Department of Biotechnology,
Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.
Corresponding author's e-mail: shinwari2002@yahoo.com

Abstract

Rhizospheric bacterial community associated with GM and Non GM maize crop were studied for PGP characteristics and cell wall hydrolytic enzymatic production**.** Twelve bacterial strains were isolated from GM and non GM maize rhizosphere and was identified by 16S rRNA gene sequencing. The isolated strains were belong to phyla's *Firmicutes* and *Proteobacteria*, whereas 50% strains belonged to genus *Bacillus,* 33.3% belong to genus *Pseudomonas* and 16.7% strains belong to genus *Enterobacteriaceae*. Majority of the isolates showed plant growth promotion (PGP) activity by producing indole acetic acid (IAA) and increased root and shoot elongation as well as biomass of Z.*mays* as indicated crop. A major proportion of the isolates also demonstrated other ecologically important activities like production of hydrolytic enzymes including cellulase, chitinase, protease, pectinase and lipase. Plant growth promoting traits of these rhizobacteria indicated beneficial relationship between rhizobacteria and Z.*mays* plant. There were no significant difference between the isolates of GM and Non GM maize rhizosphere in term of plant growth parameters and activities.

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crop in terms of world production. In Pakistan, maize is one of the most imperative food and feed crop. The area of cultivation was 935.1 million ha and production was 3261.5 million tones in 2010-1011 (SPA). As world cereal consumption have a tendency to increase due to a constantly growing population, productivity should be drastically enhanced through different strategies that allow an optimization of yields without implicating an increased the cultivation area (Braun, 2010). Maize production is severely affected by different factors like insects, diseases and salinity. Synthetic chemical pesticides and fertilizers are drastically used to overcome on these challenges but they are not unsafe so a more environmentally friendly alternatives is required to protect biodiversity and sustainability of agroecosystems and natural systems all over the world. Due to its importance, maize was one of the first crops to be genetically modified and commercially released. Transgenic maize expressing insect resistance and herbicide tolerance gene has been cultivated for more than a decade in the world (James, 2009). Transgenic maize crop resistant to different factors like biotic and non biotic offer several advantages over their corresponding non transgenic cultivars. For example, transgenic maize (Bt) is self-protected against corn borer, so it reduced the specific insecticide use and alternately decreased the risk of pollution due to chemical insecticides application. Crop root microflora is very sensitive to environmental factors (Gomes *et al.*, 2001). However transgenic crops may have side effects which can alter rhizospheric bacterial community. The effects of transgenic crops on the rhizosphere community have been the subject of many recent studies (Dohrmann *et al.*, 2013; Romeis *et al.*, 2013).

Plants influence the rhizosphere bacterial community through direct physical interaction and secretion of different chemicals in the root exudates (Bednarek *et al.*, 2010). Although the soil characteristics and plant species in shaping the rhizosphere bacterial community (Singh *et al.*, 2007). Rhizosphere bacteria and host plants influence each other by many physico-chemical and biological interactions. Plant exudates secreted in the rhizosphere influence the diversity and activity of surrounding bacteria. In turn, the soil bacteria play important ecological role in plant growth support. Several rhizobacteria belonging to heterogeneous groups are known to improve plant growth by mechanisms like production of plant growth regulators and facilitating nutrient uptake (Babalola, 2010).

Rhizospheric bacteria improve plant growth and health using several direct and indirect mechanisms, such as phytohormone productiona (O'Sullivan & O'Gara, 1992), raising the solubilisation of nutrients with consequent increase in the supply of bioavailable phosphorous and other trace elements for plant uptake nitrogen cycle (Ahn *et al*., 2007) and producing or changing the concentration of plant growth regulators like indole acetic acid (IAA) (Ahmad *et al*., 2008). PGPR increase seed germination, seedling vigour, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, early flowering and yield of grain, fodder, or fruit (Van *et al.*, 1998). Lytic enzyme and biocontrol activities against deleterious plant pathogens. Synthesis of antibiotic and other pathogen-depressing substances such as siderophores, cyanide and chelating agents that protect plants from diseases (Kamnev & Lelie, 2000). The indirect mechanisms involve inhibition of phytopathogens by producing fungal cell wall degrading enzymes and thus promoting plant growth (Padalalu & Chopade, 2006).

Pakistan is an agricultural country and is characterized by a wide diversity of soil types and climates but the cultivated land is decreased due to urbanization. Therefore transgenic crops commercialization is under debate and different concern like transgenic crops effect on soil bacterial community which is important source for crop development is still the major concern. Therefore, the purpose of this work was to isolate and characterize plant growth-promoting rhizobacteria and cell wall hydrolytic enzymatic production associated with GM and Non GM maize plants.

Material and Methods

Sampling: A field experiment was conducted in a randomized complete block design (RCBD) with three replicates at National Agriculture Research Council (NARC) Islamabad-Pakistan (Latitude 33° 43' N; longitude 73° 04' E). The rhizosphere soil samples of the one GM and two Non-GM maize varieties were collected at crop three developmental stages. The rhizosphere soil samples of each at stage were pooled together and transfer to lab. The samples were processed for cultural analysis based on 16S rRNA gene.

Cultural analysis: Rhizospheric soil samples were processed: 10 g of rhizosphere soil was weighed aseptically and added into 90 ml of sterile autoclaved water, in a 250 ml flask. Flasks were kept in shaking condition at 150 rpm at room temperature (25±2°C) for 1h. Samples were serial diluted up to 10^{-6} in 9 ml sterile autoclaved water. From the each dilution 100 µl were spread on culturing medias, Trypticase soy agar (TSA, Oxoid Ltd, Basingstoke, Hampshire, England), R2A (Sccharlab.S.L.Barcelona,Spain) and nutrient agar medium. All media were supplemented with (40µg/ml) antifungal agent amphotericin B to prevent fungal growth. Plates were incubated at 25±°C up to 6-7 days. After incubation colonies having different morphology were picked randomly and sub cultured on respective media for further purification. The bacterial isolates were preserved in LB medium containing glycerol (25 % v/v) at -80°C.

Identification of the bacterial strains by 16S rRNA gene sequencing: We have isolated 52 strains, 25 from GM and 27 from Non GM maize rhizosphere. On the basis of initial *in vitro* screening 12 strains showed positive results and were selected for 16S rRNA gene analysis. Genomic DNA was extracted from the isolates by using the Promega Wizard® Genomic DNA purification Kit (Lot # 323556) according to the manufacturer's instructions. The genomic DNA pellet was resuspended in 50-100µl of nuclease-free water and conformation was done by 1% (w/v) agarose gel electrophoresis according to Sambrook *et al*., (1989).

The partial 16S rRNA genes were amplified from extracted DNA by PCR (Applied Biosystems, USA) using bacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Rainey *et al*., 1996). Amplifications were performed (25 µl reaction) as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles, denaturation at 94°C for 30s, annealing at 55C^{0} for 30s and extension 72°C for 1 min while final extension at 72°C for 10 min. The PCR products (estimated size about 1500 bp) were analyzed by running 3 µl aliquots of the reaction mixtures in 1% (w/v) agarose gels along with 1kb Plus DNA ladder (Invitrogen Corporation, USA).

PCR products were purified by Wizard® SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's instructions and were sequenced by Macrogen, Korea.

Phylogenetic analysis: Sequence were blast in RDP (Ribosomal Database Project, USA) using version 10 (Cole *et al*., 2005). Strains identification were achieved by similarity rank analysis function at EzTaxon-e. The sequences were aligned using the Clustal W (Larkin *et al.*, 2007). The phylogenetic tree based on 16S rRNA gene sequences was constructed by using the neighbour-joining method with the Jukes and Cantor model in a MEGA4 Program with bootstrap values based on 1000 replications (Tamura *et al.,* 2007). The partial 16S rRNA gene sequences of all the isolates (some data is not presented) in this study have been deposited in NCBI GenBank database (http://www.ncbi.nlm.nih.gov/nuccore) under accession numbers RS-A-2-KC430943, RS-A-6-KC430947, RS-A-10-KC430951, RS-A-15-KC430956, RS-BIG-38- KC430979, RS-BIW-27-KC430968, RS-BIW-32- KC430973, RS-BGM-22-KC430963, RS-CIG-50- KC430991, RS-CIW-46-KC430987, RS-CGM-42- KC430983 and RS-CGM-43-KC430984.

Screening of plant growth promoting traits: We have isolated 52 strains, 25 from GM and 27 from Non GM maize rhizosphere. All the isolates were *in vitro* screened on filter paper assays for PGPR and hydrolytic enzymes. On the basis of their efficiency total 12 strains 9 from Nom GM and 3 from GM maize rhizosphere were selected for this study. Different functional assays of the isolated bacterial strains from GM and Non-GM maize rhizosphere were conducted, Which are mentioned as bellow.

Indole acetic acid (IAA) production: The indole-3-acetic acid (IAA) production of the selected bacterial strains were conducted by using Patten and Glick (2002) method with slight modification. About 20 µl aliquots of an overnight grown bacterial culture were used to inoculate 5 ml TSB without and with tryptophan (500 µg ml^{-1}) and incubated at 30°C for 24 hours .After 24 hours incubation the cultures were centrifuged for 30 min and 1 ml supernatant was mixed with 4 ml Salkowski's reagent. The mixture was incubated for 20 min at room temperature before the absorbance was measured at 535 nm. The concentration of each sample was calculated from a standard plot which was prepared from different dilution of pure IAA (Sigma) ranging from 0.01 to 0.4 µg ml^{-1} .IAA value was calculated by the formula (x= ax+b) and the values was express in $ugml^{-1}$.

Greenhouse experiment

Plant growth promotion traits and in vitro plant growth promotion assay

Inoculation procedure and plant growth: The evaluation of plant growth promotion ability of the isolates on maize was conducted in pot experiment (greenhouse) under exonic condition with 16/8 photoperiod and 24-28°C temperature. The maize seeds were surface sterilized with 70% ethanol for 5 min, and 0.1% mercuric chloride ($HgCl_2$) for 15 min. The sterilized seeds were rinsed three times with sterilized distilled water. Pure cultures were grown in TSB medium agitated on a rotary shaker (120 rpm; 37C) for two days and before harvesting centrifuged and resuspended. The surface sterile seeds were inoculated by immersion in the appropriate PGPR suspension for 30 min on a rotary

shaker (140 rpm), air-dried, and sown in 10 cm diameter pots contained sterilized loamy sand in greenhouse (24-28°C) under control condition. The inoculation treatments and control (without bacterial inoculation), were set-up in a randomized design with three replicates. Six seeds of maize were sown per pot and after germination; plants were thinned to four per pot. For each treatment, the plants were harvested 6 weeks after the emergence of seedlings and washed; morphological characteristics of each plant were recorded: shoot length, root length, fresh shoot and root weights, dry shoot and root weights. The total root number per plant was recorded after washing away the soil from the roots. The Data were statistically analyzed by analysis of variance using STATISTIX 8.1 Software, and means were compared using the least significant difference (LSD) at $p \leq 0.05$

Enzymatic characterization: Different enzymatic activities of the isolated bacterial strains from transgenic and non transgenic maize rhizosphere were conducted. The protease activity of selected bacterial strains was determined by Smibert & Krieg (1994) using skim milk agar medium, which include (L): 5 g pancreatic digest of casein, 1 g glucose, 2.5 g yeast extract, 1% skim milk solution and 15 g of agar. Bacterial strains were spot inoculated on the media plate containing skim milk. After 2 days incubation at 28°C proteolytic activity was indicated by clear zone around the bacterial strain. Cellulase activity of the bacterial strains were analyzed by Cattelan *et al.,* (1999) method. After incubation up to 2 days at 28°C, the colonies surrounded by clear halos were considered positive for cellulose production. Lipase activity on TSB medium with 1% Tween-20. After 48 hours incubation at 28°C. Strains surrounded by white precipitation were considered positive for lipase activity. Chitinase activity was determined by using Renwick *et al.,* (1991) method, in which carbon was the sole source in a defined medium having colloidal chitin. The pectinase activity was determined by Raju & Divakar (2013) method. After 48 hours incubation at 28°C, the plates were flooded with 50 mM iodine solution and incubated for 15 min at 37°C. Strains surrounded by clear halos around colonies were considered positive for pectinase activity.

Results

Isolation and phylogenetic analysis of rhizospheric bacteria on the basis of 16S rRNA gene sequence: Bacteria were isolated from GM and Non GM maize rhizosphere at three developmental stages. Total 52 isolate, 25 from GM and 27 from Non GM maize rhizosphere were screened for PGPR and hydrolytic enzymes production. On the basis of their efficiency total 12 strains, 9 from Nom GM and 3 from GM maize rhizosphere were selected for 16S rRNA gene sequencing. PCR with 16S rRNA gene were conducted and sequenced through Macrogen, Korea. The 12 rhizospheric bacteria, 4 from each stage that were screened for plant growth promoting characteristics and cell wall hydrolytic enzymatic production. All the isolates were identified by partial 16S rRNA gene sequence analysis, five different genera were identified and where in turn assigned to tow major phylum: *Firmicutes* (n=6; 50%) and *Proteobacteria* (n=6; 50%), whereas five isolates (n=5; 42%) belong to genus *Bacillus,* four isolates (n=4; 34%) belong to genus *Pseudomonas* while three isolates one from each (n=1; 8%) belong to *Solibacillus*, *Klebsiella* and *Enterobacter* genus respectfully (Fig. 1). A phylogenetic tree was generated using the neihbor-joining method in a MEGA 4 software (Fig. 2). For 16S rRNA gene data, branching pattern remained consistent, depending on which sequence of related type strains of different genera and related sequence were included in the data set. Sequence identity within 93.7% -100% and their phylogentic relationships to the representative type strains are shown in Fig. 2. Representative strains of phylum *Firmicutes* were placed in the cluster recovered with bootstrap value higher then 49%. Strains RS-A-6 and RS-GGM- 22 were almost identical to each other with high bootstrap value (100%), whereas RS-A-2 and RS-CIW-46 closely related with each other (97%). In addition RS-CGM-43 were closely related with RS-A-6 and RS-BGM-22 with bootstrap value (94%). These isolates mainly belonged to genus *Bacillus.* Where isolate RS-A-6 and RS-BGM-22 closely related to the type strains *Bacillus anthracis* while RS-A-2, RS-BIW-27, RS-CIW-46 and RS-CGM-43 were almost identical to type strains *Bacillus aerophilus, Bacillus isronensis, Bacillus idriensis* and *Bacillus aryabhattai* respectfully. The phylum *Proteobacteria* were placed in tow distinct cluster where strains RS-CGM-42 and RS-A-15 were closely identical to each other with high bootstrap value (100%), and belong to type strain *Enterobacter cloacae*. Whereas strain RS-BIW-32 closely related to type strains *Pseudomonas lini*, while RS-A-10 and RS-BIG-38 were closely identical to each other with bootstrap value (86%) which belonged to closest type strains *Pseudomonas taiwanensis* and *Pseudomonas cremoricolorata* respectfully (Fig. 1).

***In vitro* screening of isolates for plant growth promoting potential:** The inoculation with the isolates from rhizosphere of transgenic and non transgenic maize were done with maize. The shoot fresh weight was significantly increase by all applied isolated as compare to control. The results of pot study showed that inoculation of maize seeds with bacterial strains significantly enhanced seedling vigor. However, the rate of enhancement varied with inoculated bacterial strains which was isolated from three developmental stages of GM and Non GM maize rhizosphere. All bacterial isolates (RS-A-2,RS-A-6, RS-A-10, RS-A-15, RS-BIG-38, RS-BIW-27, RS-BIW-32, RS-BGM-22, RS-CIG-50, RS-CIW-46, RS-CGM-42 and RS-CGM-43) increased shoot fresh weight of maize seedling as compared to control (Fig. 1). The maximum shoot length was recorded in RS-BIW-27 (isolated at vegetative state) and RS-CIW-46 (Isolated at harvesting stage from Islamabad white maize variety rhizosphere respectively) ($p<0.05$). While there was no significant difference in the shoot length among the isolates of the GM maize with pre sowing as well as with the isolates of Non GM Islamabad gold variety.

In contrast, root fresh weight significantly increased by inoculation with RS-A-2, RS-A-6, RS-A-10 and RS-BIG-38 as compared to control ($p<0.05$).While the isolates of GM maize rhizosphere have no significant effect on the root fresh weight as compared to the isolates of Non GM rhizosphere except RS-BIG-38 shown in the Fig. 1.

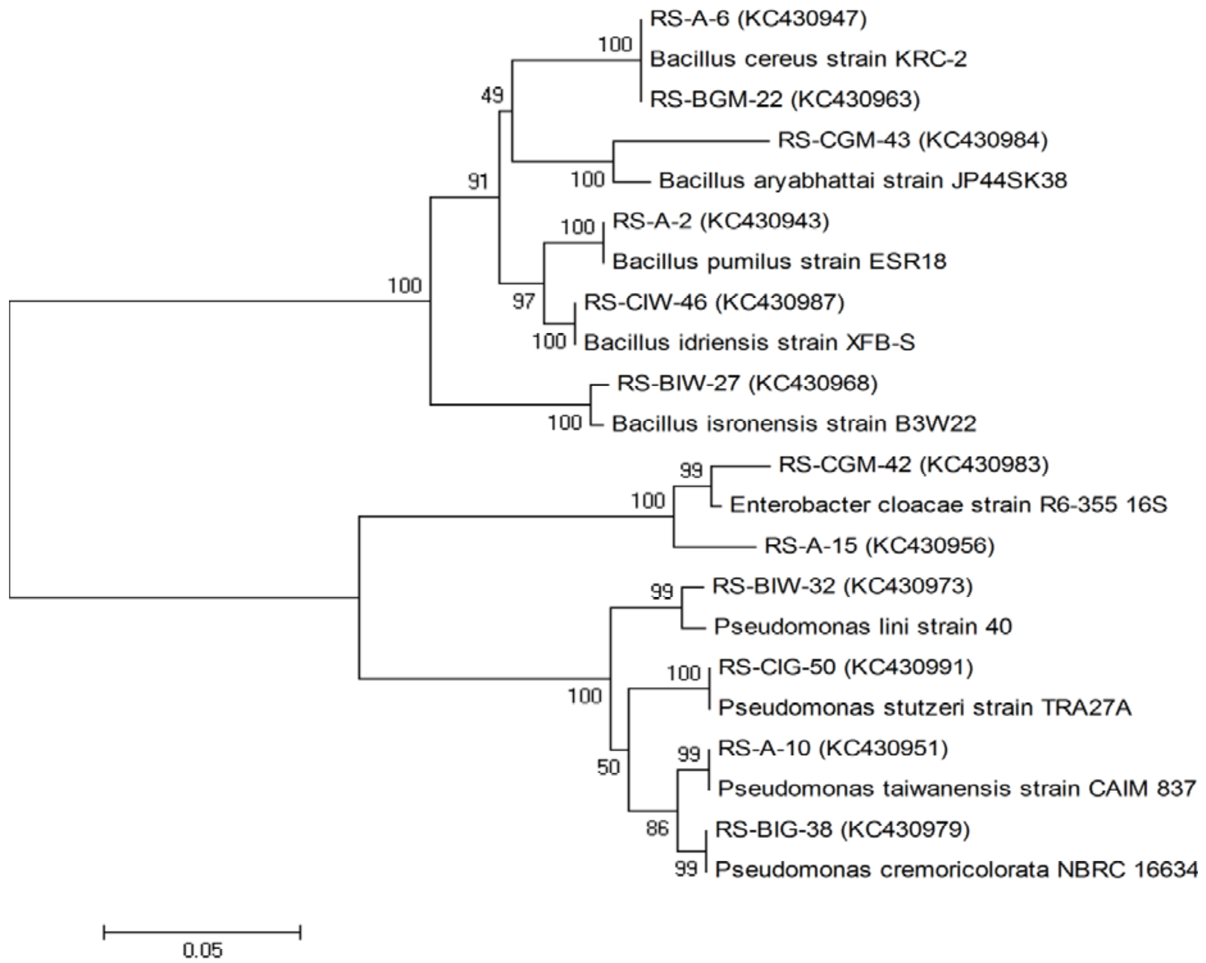

Fig. 1. Phylogenetic tree of bacteria isolated from GM and Non GM maize rhizospheric on the basis of 16S rRNA gene partial sequences and closely related sequence of the type strains. The phylogenetic relationships among taxa were constructed from the 16S rRNA gene sequences by using the neighbor-joining method from distances computed with the Jukes-Cantor algorithm. Bootstrap values (1,000 replicates) are shown next to the branches. NCBI GenBank accession numbers for each sequence are shown in Parentheses. Bar, 0.05 changes per nucleotide position.

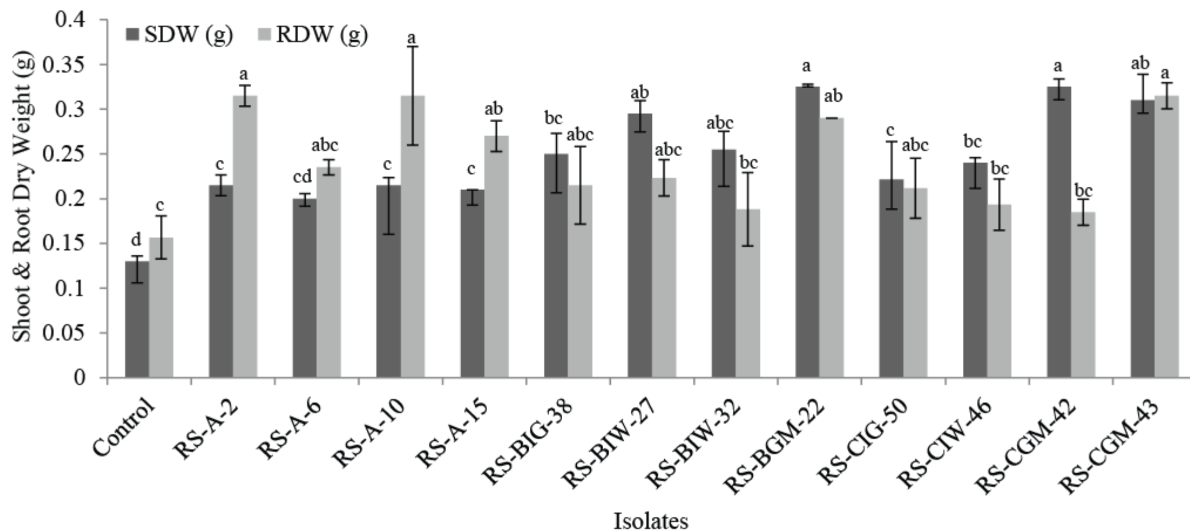

Fig. 2. Effect of bacterial strains (isolated at three stages from GM and Non GM maize Rhizosphere) on maize shoot (SDW) and root (RDW) dry weight.

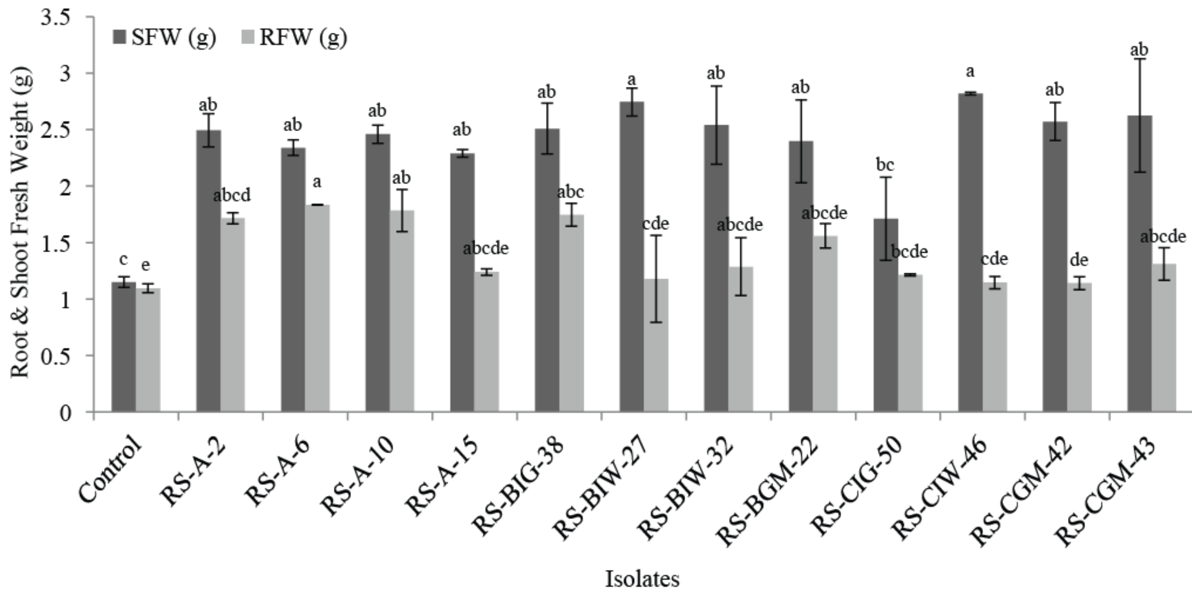

Fig. 3. Effect of bacterial strains (isolated at three stages from GM and Non GM maize Rhizosphere) on maize shoot (SFW) and root (RFW) fresh weight.

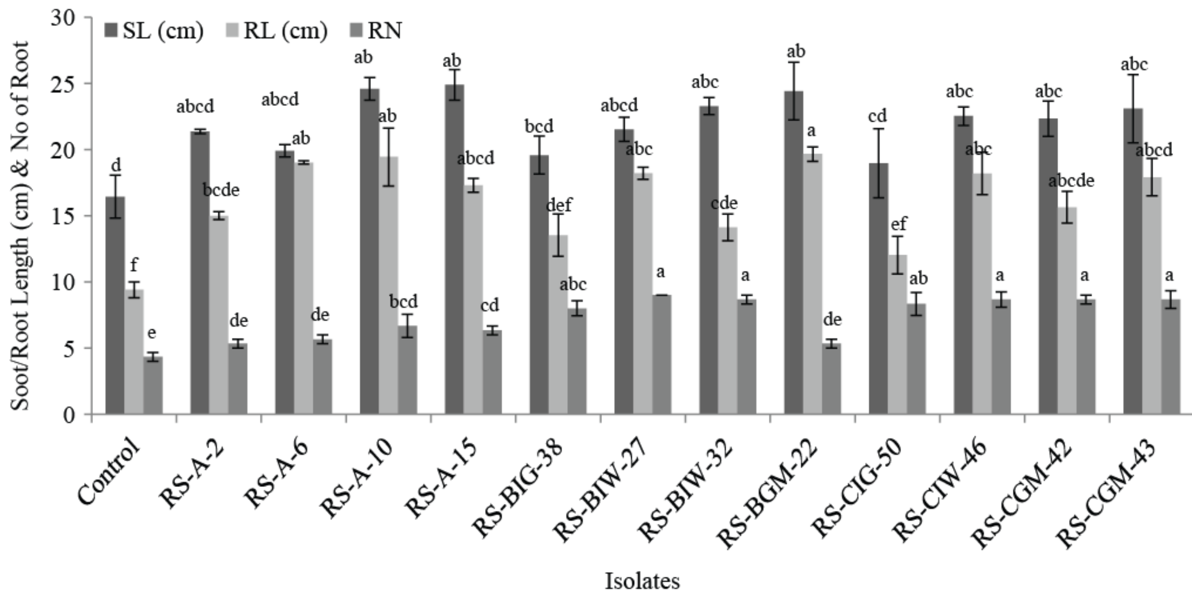

Fig. 4. Effect of bacterial strains (isolated at three stages from GM and Non GM maize Rhizosphere) on maize shoot (SL), root (RL) length and No of roots (RN) per plant.

IAA production: The ability of the tested twelve rhizospheric bacterial strains for produce IAA was determined using quantitative method. The results in Fig. 4 indicates that the different rhizospheric bacterial strains varied greatly in their efficiency for IAA production, both in absence and presences of L-TRP. Without added L-TRP, the IAA reached its highest amount (4.7 mgl-1) in RS-A-10 which belong to genus *Pseudomonas*, followed by RS-CIW-46 (3.4 mgl-1) and RS-CGM-43 (3.3 mgl-1). On the other hand, bacterial strains RS-BIW-32 (1.523 mgl-1) and RS-CIG-50 (1.524 mgl-1) produced the lowest IAA. The bacterial strains isolated from harvesting stage of GM maize crop produces maximum (without L-TRP addition) IAA production RS-CGM-43 (3.3 mgl-1), RS-CGM-42 (2.3 mgl-1) as compared to vegetative stage isolate RS-BGM-22 (1.9 mgl-1). When L-TRP was added to the medium, the maximum IAA level reached (12.6 mgl-1) in case of RS-A-10 (pre sowing isolate) followed by RS-CGM-43 (5.5 mgl-1) and RS-CIW-46 (5.05 mg l) while the lowest value was recorded in RS-BIW-32 (2.5 mgl-1). comparatively the GM maize rhizosphere isolates of harvesting stages RS-CGM-43 and RS-CGM-42 produced maximum amount of IAA (with L-TRP) as compared to the isolates of vegetative stage (RS-BGM-22) showed in Table 1.

Table 1. *In vitro* screening of isolates for cell wall hydrolytic enzymatic and IAA production.

Strains	Growth Stages	Cellulase	Chitinase	Protease	Pectinase	Lipase	IAA (mgl-1)	
							Without Tryp	With Tryp
RS-A-2	Pre sowing	-	++	++	-	+	2.94 BC	4.56 CD
RS-A-6		+	+	+++	+++	+	2.12BCD	2.66 D
RS-A-10		-	++	-	++	-	4.72 A	12.65 A
RS-A-15		-	-	++	+	-	2.56BCD	3.89 CD
RS-BIG-38	Vegetative	-	-	++	-	-	2.93 BC	4.006CD
RS-BIW-27		-	-	-	+	-	1.84 CD	3.64 CD
RS-BIW-32		-	+	++	++	+	1.52 D	2.50 D
RS-BGM-22		+	+	+	+++	-	1.90CD	3.79 CD
RS-CIG-50	Harvesting	-	-	+++	++	+	1.52 D	7.654 B
RS-CIW-46		+	-	+++	-	+	3.43 AB	5.0557C
RS-CGM-42		-	-	-	-	+	2.38BCD	4.054CD
RS-CGM-43		-	-	-	-	+	2.94 BC	4.569CD

Enzymatic activities were estimated by measuring the diameter of the clear zones around bacterial colonies.
Symbols: -, No activity; +, activity (1-2cm); ++, (2-3cm) and +++, above 3cm diameter

Characterization of hydrolytic enzymes: All the bacterial 12 bacterial isolates from GM and Non GM maize rhizosphere were further characterized for their *In vitro* production of fungal cell wall hydrolyzing enzymes such as cellulase, chitinase, protease, pectinase and lipase (Table 1). The proportion of the rhizospheric bacteria with cellulase activity (n=3; 25%), 3 out of 12 bacterial strains RS-A-6, RS-BGM-22 and RS-CIW-46 which were belong to genus *Bacillus* showed positive cellulase activity. Chitinase production was observed (n=5; 41.7%), 5 out of 12 bacterial strains RS-A-2, RS-A-6, RS-A-10, RS-BIW-32 and RS-BGM-22 shown chitinase activity, highest activity (2-3cm) was recorded in RS-A-2 and RS-A-10 isolates which belonged to genera *Bacillus* and *Pseudomonas* respectfully. Only one bacterial strains of GM rhizosphere RS-BGM-22 showed activity while other tow did not showed any activity. The highest activity was recorded in the pre sowing bacterial isolates RS-A-2 and RS-A-10 respectively. While none of the harvesting stage isolates showed chitinase activity. The protease activity was recorded in maximum number of bacterial strains (n=8; 66.7%) with different zone diameter. Among these strains RS-A-6, RS-CIW- 46 and RS-CIG-50 which belong to genera *Bacillus, Pseudomonas* respectfully showed highest protease activities (> 3cm). Pectinase activities of the selected bacterial strains was conducted in which 7 out of 12 showed pectinase activity. The pectinase activity was recorded in different bacterial strains (n=7; 58.3%), RS-A-6 and RS-BGM-22 which belonged to genus *Bacillus* shown maximum pectinase activity (>3cm). whereas strains RS-A-10, RS-BIW-32 and RS-CIG-50 showed relatively low pectinase activities (2-3cm) respectively. Lipase activity was conducted for selected bacterial strains (n=7; 58.3%), in which 7 out of 12 bacterial strains shown lipase activity. There is no distinct variation in production of cell wall hydrolyzing enzymes among the isolates of GM and Non GM rhizospheric.

Discussion

GM maize is grown worldwide and is the most widely grown crop in the world (James, 2005). But the release of GM maize remain a long term concern because of the presumed potential ecological and environmental risk like impact on non-target organisms (Sanvido *et al*., 2007; Ibrahim *et al*., 2013). Growing GM maize can effect soil microorganism is the possible release of transgenic gene into soil, where it could be transferred to rhizospheric bacterial cells. However, there was no evidence of transfer transgenic gene to soil bacteria (Ma *et al.,* 2011). The insecticidal cry toxins released from root exudates and biomass of GM crops have been shown to accumulate in soil. However these toxins do not appear to affect soil microorganism (Icoz *et al.,* 2008). Such observations recorded in our study that there is no variation in the function of the isolates of GM with that of Non GM maize rhizosphere.

Plant rhizosphere is the main source for microbial growth and major microbial activities in the soil (Ana *et al.*, 2010). In the present study, 12 bacterial isolates 4 from each growth stage of GM and Non-Gm maize rhizosphere were screened in vitro for their plant promoting potential (PGPR) and cell wall hydrolytic enzymatic production. The neighbor-joining phylogenetic method yielded consensus tree topology that grouped all 16S rRNA gene sequences obtained from GM and Non GM maize rhizosphere into two different groups: *Firmicutes* (*Bacillus aerophilus, Bacillus anthracis, Bacillus isronensis, Bacillus idriensis* and *Bacillus aryabhattai*) and *Proteobacteria*: (*Pseudomonas taiwanensis, Enterobacter cloacae* subsp, *Pseudomonas cremoricolorata, Pseudomonas lini and Pseudomonas stutzeri*). Our results indicated that overall phylogenetic distribution of bacterial isolates of GM and Non GM maize rhizosphere belong to genera *Bacillus and Pseudomonas* which are predominate for Plant growth promotion activity. The antagonistic properties of *Bacillus*

were reported previously (Berg & Hallmann, 2006). Rhizosphere isolates referred to promote maize growth including *Pseudomonas putida* (Mehnaz & Lazarovits, 2006). Different studies have also reported strains from *Bacillus species* to be effective in promoting *Z. mays* growth, such as *Bacillus subtilis* (Araujo, 2008). All the isolates increased biomass of the maize but there were no significant variation in the biomass of the seeds inoculated with isolates of GM rhizosphere as compare to that of Non-GM. Root fresh and dry weight was significantly increased by inoculation with *Bacillus, Pseudomonas sp*. as compared to control, the results confined the previous finding (Araujo, 2008; Mehnaz & Lazarovits, 2006). Whereas the root shoot length of the inoculated maize seedling was significantly increased by maximum isolates as compared to control results coincide with previous finding of (Bevivino *et al.*, 1998). Plant growth promoting bacteria enhance plant growth through various forms, such as decreasing ethylene production, which allowing plants to develop longer roots and improved establish during early growth stages, improved asymbiotic nitrogen fixation (Khan, 2005), producing plant growth regulators like indole acetic acid (Ahmad *et al*., 2008), improved nutrients solubilisation with subsequently increase in the supply of bioavailable phosphorous and other trace compound for plant uptake, production of phytohormones such as auxins, cytokinins and gibberelins (Glick, 1995) and synthesis of antibiotic and other pathogen-depressing substances and fungal cell wall hydrolyzing enzymes such as cellulase, chitinase, protease, pectinase and lipase that protect plants from diseases (Kamnev & Lelie, 2000). From the results its cleared that different isolates have the efficiency of IAA production, both in presences and absence of precursor L-TRP. Without L-TRP addition, the IAA reached its highest amount in *Pseudomonas Sp*. GM maize isolates of harvesting, Bacillus sp. also increased IAA production while with the presence of L-TRP the maximum IAA production was recorded in Pseudomonas taiwanensis. All the isolates used in this study showed IAA production, most of the isolates generating levels similar to those presented in other reports (Ahmad *et al.,* 2008). Some *pseudomonas sp*. previously obtained from rhizosphere known to produce IAA (Pedraza *et al*., 2004).

Current strategies for biological control focus on cell wall hydrolytic enzymes in the micro-organism used for seed inoculation. Among cell wall hydrolytic enzymes cellulases, proteases, chitinases, pectinase and lipase are very important for bio control. They acting in synergy were also shown to enhance decomposition of fungal cell walls (Lorito *et al.*, 1993). In the present study 3 out of 12 strains which belong to Bacillus sp. showed cellulase production, the results coincide with the previous finding (Dunn *et al.,* 1997). Biocontrol of soil-borne plant pathogens (Picard *et al.,* 2004) and the synthesis of antibiotics have also been reported in several bacterial species (Haansuu *et al.*, 1999). Another mechanism by which rhizobacteria can inhibit phytopathogens is the production of hydrogen cyanide (HCN) and fungal cell wall-s enzymes e.g., chitinase (Persello-Cartieaux *et al.*, 2003). Five strains in which 3 belong to Bacillus sp. and 2 Pseudomonas sp. shown chitinase activity. Maximum strains produced protease and pectinase while some strains also produced lipase enzyme. Some bacteria with antagonistic activity isolated from GM and Non GM maize rhizosphere were able to produce fungal cell wall hydrolytic enzymes which can be used for biocontrol agents. Growth promotion of plants is a key factor to be considered. Since the bacteria may indirectly affect the pathogenicity of fungal pathogens by simultaneous induction of systemic resistance of host plat (Manjula *et al*., 2002).The results indicated that the impact were rely on the isolates types rather than their source and stages.

References

Ahmad, F., I. Ahmad and M.S. Khan. 2008. Screening of free living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research,* 163: 173-181.

Ahn, T., J. Ka, G. Lee and H. Song. 2007. Microcosm study for revegetation of barren land with wild plants by some plant growth-promoting rhizobacteria. *J. Micobiol. Biotech.,* 17: 52-57.

Ana, P.G.C., P. Carlos, M. Helena, O.S.S. António and M.L. Paula. 2010. Assessment of the plant growth promotion abilities of sixbacterial isolates using Zea mays as indicator plant. *Soil Biology & Biochemistry,* 42: 1229-1235.

Araujo, F.F. 2008. Seed inoculation with Bacillus subtilis formulated with oyster meal and growth of corn, soybean and cotton. *Ciência Agrotecnologia Lavras,* 32: 456-462.

Babalola, O.O. 2010. Beneficial bacteria of agricultural importance. *Biotechnol. Lett.,* 32(11): 1559-1570.

Bednarek, P., C. Kwon and P. Schulze-Lefert. 2010. Not a peripheral issue: secretion in plant-microbe interactions. *Curr. Opin. Plant Biol.,* 13: 378-385.

Berg, G. and J. Hallmann. 2006. Control of plant pathogenic fungiwith bacterial endophytes: Microbial root endophytes, (Eds.): B. Schulz, C. Boyle and T. N. Sieber, Springer-Verlag, Berlin. pp. 53-69.

Bevivino, A., S. Sarrocco, C. Dalmastri, S. Tabacchioni, C. Cantale and L. Chiarini. 1998. Characterization of a free-living maize rhizosphere population of Burkholderia cepacia: effect of seed treatment on disease suppression and growth promotion of maize. *FEMS Microbiol. Ecol.,* 27: 225-237.

Braun, J.V. 2010. The world food situation. New driving forces and required actions. *International Food Policy Institute*.

Cattelan, A., P. Hartel and F. Furhmann. 1999. Screening for plant growth promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. Am. J.,* 63: 1670-1680.

Cole, R., B. Chai, R. Farris, Q. Wang, S. Kulam, D. McGarrell, G. Garrity and J. Tiedje. 2005. The ribosomal database project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res.,* 33: 294-296.

Dohrmann, A.B., M. Kuting, S. Junemann, S. Jaenicke, A. Schluter and C.C. Tebbe. 2013. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. *ISME J.,* 7: 37-49.

Dunn, C., J. Crowley, Y. Moenne-Loccoz, D. Dowling, F. Bruijn and F. O' Gara. 1997. Biological control of Pythium ultinum by Stenotrophomonas maltophilia W18 is mediated by an extracellular proteolytic activity. *Microbiology,* 143: 3921-3931.

Glick, B.R. 1995. The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology*, 41: 109-114.

Gomes, N.C.M., H. Heuer, J. Schönfeld, R. Costa, L. Medonça-Hagler and K. Smalla. 2001. Bacterial diversity of the

rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. *Plant Soil,* 232: 167-180.

Haansuu, P., P. Vuorela and K. Haahtela. 1999. Detection of antimicrobial and 45Ca 2+ -transport blocking activity in Frankia culture broth extracts. *Pharm Pharmacol Lett*., 1: 1-4.

Ibrahim, M., N. Ahmad, Z.K. Shinwari, A. Bano and F. Ullah. 2013. Allelopathic Assessment of Genetically Modified and Non Modified Maize (*Zea Mays* L.) on Physiology of Wheat (*Triticum Aestivum* L). *Pak. J. Bot*., 45(1): 235-240.

Icoz, I., D. Saxena, D.A. Andow, C. Zwahlen and G. Stotzky. 2008. Microbial populations and enzyme activities in soil in situ under transgenic corn expressing cry proteins from *Bacillus thuringiensis*. *J. Environ. Qual.,* 37: 647-62.

James, C. 2005. Executive summary of global status of commercialized. *Biotech/GMCrops. SAAA Briefs,* pp. 34.

James, C. 2009. Global Status of Commercialized Biotech/GM Crops. *Ithaca, NY, USA: ISAAA Brief No:* 41 ISAAA.

Kamnev, A.A. and D. Lelie. 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Bioscience Reports,* 20: 239-258.

Khan, A.G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elements in Medicine and Biology,* 18: 355-364.

Larkin, M., G. Blackshields, N. Brown, R. Chenna, P. McGettigan, H. McWilliam, F. Valentin, I. Wallace, A. Wilm, R. Lopez, J. Thompson, T.J. Gibson and D. Higgins. 2007. ClustalW and ClustalX version 2. *Bioinform,* 23: 2947-2948.

Lorito, J.M., G.E. Harman, C.K. Hayes, R.M. Broadway, T. ronsmo, A. Woo, S.L. and A.D. Pietro. 1993. Chitinolytic enzymes produced by Trichoderma harzianum; Antifungal activity of purified endochitinase and chitobiosidase. *Phytopathology,* 83: 302-307.

Ma, B.L., R.E. Blackshaw, J. Roy and T. He. 2011. Investigation of gene transfer from genetically modified corn (Zea mays L.) plants to soil bacteria. *J. Environ. Sci. Health., Part B*., 46: 590-599.

Manjula, K., S.D. Singh and K.G. Kishore. 2002. Role of endophytic bacteria in biological control of plant diseases. *Ann. Rev. Plant Pathol.,* 1: 231-252.

Mehnaz, S. and G. Lazarovits. 2006. Inoculation effects of Pseudomonas putida, Gluconabacter azotocaptans and Azospirilum lipoferum on corn plant growth under greenhouse conditions. *Microbial Ecology,* 51: 326-335.

O'Sullivan, D. and F. O'Gara. 1992. Traits of fluorescent *Pseudomonas* spp., involved in suppression of plant root pathogens. *Microbiol Rev.,* 56: 662-676.

Padalalu, V. and B. Chopade. 2006. Role of plant growth promoting rhizobacteria in enhancement of plant growth. In: Proceedings of International Workshop on Fostering the Next Green Revolution: Role of Biotechnology in Advancing Indian Agriculture. *Acharya M.G. Ranga Agriculture University, Hyderabad, India*, pp. 1-30.

Patten, C.L. and B.R. Glick. 2002. The role of bacterial indoleacetic acid in the development of the host plant system. *Appl. Environ. Microbiol.,* 68: 3795-3801.

Pedraza, R., M. Xiqui and B. Baca. 2004. Aromatic amino acid aminotransferase activity and indole-3-acetic acid production by associative nitrogen-fixing bacteria. *FEMS Microbiol Letts.,* 233(1): 15-21.

Persello-Cartieaux, F., L. Nussaume and C. Robaglia. 2003. Tales from the underground: molecular plant-rhizobacteria interactions. *Plant Cell Environ.,* 26: 189-199.

Picard, F., M. Kurtev, N. Chung, N.A. Topark, T. Senawong, D.O.R. Machado, M. Leid, M. McBurney and L. Guarente. 2004. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature,* 429(77): 1-776.

Rainey, F., N. Ward-Rainey, R. Kroppenstedt and E. Stackebrandt. 1996. The Genus Nocardiopsis represents a phylogenetically coherent taxon and a distinct actinomycete lineage: Proposal of *Nocardiopsaceae* fam. nov. *Int. J. Syst. Bacteriol.,* 46: 1088-1092.

Raju, E.V.N. and G. Divakar. 2013. Screening and Isolation of Pectinase producing Bacteria from Various Regions in Bangalore. *IJRPBS.* 4(1): 151-154.

Renwick, A., R. Campbell and S. Coe. 1991. Assesment of *In vivo* screening systems for potential biocontrol agents of Gaeumannomyces graminis. *Plant Pathology Journal,* 40: 524-532.

Romeis, J., M.A. McLean and A.M. Shelton. 2013. When bad science makes good headlines: Bt maize and regulatory bans. *Nat. Biotechnol.,* 31: 386-387.

Sambrook, J., E. Fritsch and T. Maniatis. 1989. Molecular Cloning, A Laboratory Mannual, 2nd edition, CSH Publication, Vol. 1, Cold Spring Harbor Laboratory press, New York, USA.

Sanvido, O., J. Romeis and F. Bigler. 2007. Ecological impacts of genetically modified crops: ten years of field research and commercial cultivation. *Adv. Biochem. Eng. Biotechnol.,* 107: 235-278.

Singh, B.K., S. Munro, J.M. Potts and P. Millard. 2007. Influence of grass species and soil type on rhizosphere microbial community structure in grassland soils. *Appl. Soil Ecol.,* 36: 147-155.

Smibert, R. and N. Krieg. 1994. Phenotypic characterization. In Methods for General and Molecular Bacteriology. Edited by Gerhardt P, Murray RGE, Wood WA, Krieg NR. American Society of Microbiology. *Washington DC*, pp. 607-654.

Van, L.C., P.A.H.M. Bakker and C.M.J. Pieterse. 1998. Systemic resistance induced by Rhizosphere bacteria. *Annu. Rev. Phytopathol.,* 36: 453-483.

(Received for publication 29 April 2012)