academicJournals

Vol. 8(25), pp. 2436-2448, 18 June, 2014
DOI: 10.5897/AJMR2013.6511
Article Number: 1C5CA3445417
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research paper

Growth enhancement in vegetable crops by multifunctional resident plant growth promoting rhizobacteria under tropical Island Ecosystem

Krishna Kumar*, Kuttum Madhuri, Vel Murugan, Krishnan Sakthivel, Anbalagan Anantharaj, Awnindra Kumar Singh, Raj Kumar Gautam and Sibnarayan Dam Roy

Central Agricultural Research Institute, Port Blair-744105, Andaman and Nicobar Islands, India.

Received 20 November, 2013; Accepted 8 January, 2014

Plant growth promoting rhizobacteria have been used to improve crop production. A total of 114 bacterial isolates were recovered from the rhizosphere soils of healthy plantation and vegetable crops grown in Andaman Islands. These isolates were evaluated *in vitro* for plant growth promoting traits, hydrolytic enzyme production and antagonistic activity. Among the isolates, NPB6 and MNB1 were positive to 8 out of 10 properties tested followed by NFB3, MKP3, NNB4 and NTB2. In the dual culture assay, NFB3 showed highest inhibition against the plant pathogen *Macrophomina* spp (33.3%) and *S. rolfsii* (23%). Six most promising isolates were selected and identified as *Bacillus* and *Pseudomonas* spp. on the basis of Microbial Identification System and 16S rDNA. These isolates significantly increased the seed germination, vigor index, radical and plumule length however, the individual isolates effect varies with crop. All the six isolates enhanced the root and shoot length of brinjal, chilli and okra seedlings while *Bacillus cereus* (NPB6) in brinjal, *B. stratosphericus* (NFB3) in chilli and *Pseudomonas fluorescens* (NNB4) in okra were most effective. The promising isolates can be used in combination with other beneficial native microbes for plant growth promotion even under abiotic stresses and promotion of organic agriculture.

Key words: Biolog, plant growth promotion, rhizobacteria, vegetables.

INTRODUCTION

Soil micro-organisms belonging to different groups are known to play prominent role in plant-soil interactions at the rhizosphere (Mantelin and Touraine, 2004). Among them bacteria that colonize the rhizosphere (rhizobacteria) are very important and beneficial for plant health. They are referred to as plant growth promoting bacteria (PGPB) or plant-growth promoting rhizobacteria (PGPR) (Kloepper, 1993). The principal mechanisms of growth

*Corresponding author. E-mail: kkpath@gmail.com.

promotion include production of growth stimulating phytohormones, solubilization and mobilization of phosphate and siderophore production whereas antibiosis, inhibition of plant ethylene synthesis and induction of plant systemic resistance against pathogens are favourable health effects (Gutierrez-Manero et al., 2001; Whipps, 2001; Idris et al., 2007; (Gutierrez-Manero et al., 2001; Whipps, 2001; Idris et al., 2007; Richardson et al., 2009). However, only 1–2% of rhizobacteria are known to promote plant growth, which are associated with many plant species and commonly present in varied environments (Antoun and Kloepper, 2001). Some of the most promising genera reported to exhibit plant growth promotion are *Bacillus*, *Pseudomonas, Azospirillum*, *Azotobacter*, *Enterobacter* and *Serratia* (Hurek and Reinhold-Hurek, 2003).

The interactions of rhizobacteria in the rhizosphere soil play a pivotal role in transformation, mobilization, solubilization of nutrient, from the nutrient pool in the soil and subsequent uptake by the crops. As a result, PGPR increases germination rate, root length, leaf area, chlorophyll content, protein content, nitrogen content, tolerance to drought, shoot and root weight and delayed leaf senescence (Cakmakci, 2005; Babalola et al., 2006). Therefore, soil microorganisms including PGPR play prominent role in the maintenance of soil health (Lucy et al., 2004).

A significant increase in growth and yield of cowpea and other important crops in response to seed bacterization with PGPRs have been reported by many workers (Amara and Dahdoh, 1997; Asghar et al., 2002; Minaxi et al., 2012). This considerably reduces the input cost on fertilizers and pesticides. In the tropical Islands of Andaman and Nicobar brinjal, tomato, chilli and okra are the major vegetable crops cultivated and consumed. Out of the total consumption of fertilizers and pesticides, a significant amount is used for these crops (DES, 2011). Keeping this in view, field survey was conducted to collect rhizosphere soil samples from different locations followed by *in vitro* and green house experiment was conducted to find out the promising native PGPR strains with multifunctional properties, which can be used for increasing the productivity of vegetable crops in these islands, in particular and other parts of the world, in general.

MATERIALS AND METHODS

Sampling site

The tropical island of Andaman and Nicobar is located in the Bay of Bengal 1200 km off the eastern coast of main land India. It experiences annual mean temperature of 32°C, annual rainfall of 3180 mm and high evaporation during the summer months. The rhizosphere soil samples from vegetable and plantation crops were collected from across North (13° 22'02.48''N-92° 58'05.61''E) and Middle Andaman Islands (12° 30'16.21''N-92° 55'34.13''E) and their cropping history were recorded. The soil samples were brought to the laboratory in an icebox and processed further for bacterial isolation.

Isolation of bacteria

Bacteria from the rhizosphere soil samples were isolated using serial dilution method on King's B agar and Nutrient agar medium. This was incubated at 28°C for 48 h. A total of 114 colonies were recovered on King's B agar and nutrient agar, which were purified with repeated culturing and maintained in 20% glycerol at -20°C. These isolates were used to study its plant growth promoting properties and antagonistic activities.

Assessment of plant growth promoting traits

Indole 3-Acetic Acid (IAA) production by the isolates was estimated by qualitative method (Naik et al., 2008) and it was further confirmed by quantitative estimation (Benizri et al., 1998). Bacteria were cultured overnight in Luria-Bertani broth in the dark at 30°C. The bacterial cells were removed from the culture medium by centrifugation at 8,000 *g* for 10 min. 1 ml of supernatant was mixed vigorously with 2 ml of Salkowski's reagent and incubated at room temperature in the dark for 30 min. The absorbance at 535 nm was measured and the concentration of IAA produced was estimated from a standard IAA graph and expressed as micrograms per milliliter (Patten and Glick, 1996).

Two days old pure bacterial culture grown in nutrient agar was streaked on King's B medium amended with an indicator dye. Change of blue color of the medium to yellow halo surrounding the bacterial growth indicated the production of siderophore. The reaction of each bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands, 1987).

All bacterial isolates were screened for inorganic phosphate solubilization according to the method suggested by Verma et al. (2001). A loopful of fresh bacterial culture was streaked onto Pikovaskaya's medium amended with inorganic phosphate and the plates were incubated at 28±2°C for 4 days. A clear halo around the bacterial colony indicated solubilization of mineral phosphate.

Analysis of extracellular enzyme activity

Bacterial isolates were analyzed for production of six enzymes *viz.* amylase, cellulase, chitinase, lipase, protease and pectinase by plate method. Proteolytic activities of the cultures were screened qualitatively in a medium containing skimmed milk (HiMedia, Mumbai). Zones of precipitation of paracasein around the colonies appearing over the next 48 h were taken as evidence of proteolytic activity. For cellulase activity, mineral-salt agar plate containing 0.4% $(NH_4)_2SO_4$, 0.6% NaCl, 0.1% K_2HPO_4, 0.01% $MgSO_4$, 0.01% $CaCl_2$ with 0.5% carboxy methyl cellulose (CMC) and 2% agar (Hi-Media) were surface inoculated. Iodine solution was used to detect cellulase activity as described by Kasama et al. (2008). The clear zone formation around the growing colony was considered as positive. The lipase activity of bacterial isolates was determined according to the diffusion agar methods in which nutrient agar medium was supplemented with $CaCl_2.H_2O$ 0.01%. Tween 80 was sterilized for 20 min at 121°C and added to the molten agar medium at 45°C to give a final concentration of 1%. The medium was shaken until the Tween 80 had dissolved completely and then poured onto Petri dishes. For positive test, an opaque halo that occurred around the colonies was considered as positive.

Antagonistic activities against plant pathogenic fungi

The antagonistic effects of bacterial isolates were tested against

two fungal plant pathogens namely: *Scelerotium rolfsii* and *Macrophomina* sp. For this, the bacterial isolates were streaked at a distance of 3.5 cm from rim of individual Petri plate containing potato dextrose agar (PDA) medium. 6 mm mycelial disc from a 7-day old PDA culture of fungal pathogens were then placed on the other side of the Petri dish and the plates were incubated at 28°C for 4 days. The percent inhibition was calculated by using the formula:

$I = (C – T)/C * 100$

Where, I is percent inhibition of mycelial growth over the control, C is mycelial growth of fungal pathogen in control plate and T is mycelial growth of fungus in bacteria inoculated plate. The experiment was carried out in three independent replicates.

Identification of the selected isolates with Biolog system

Six potential bacterial isolates were selected and tested for preliminary biochemical characterization as per standard methodologies (Collins and Lyne, 1980). The identities of the bacterial isolates were revealed on the basis of Biolog carbon source utilization. Bacterial suspensions were inoculated into Biolog GENIII Micro plates and incubated at 30°C for 24 h. The results were interpreted with Biolog Micro Log^{TM}, Release 4 software (Biolog, Hayward, CA).

16S rDNA amplification and phylogenetic analysis

Genomic DNA was extracted using the method described by Chen and Kuo (1993). The extracted DNA was dissolved in 20 µl TE buffer and used as a template for the Polymerase Chain Reaction (PCR). The PCR mix included 3 U of *Taq* DNA polymerase, 10X Taq buffer A, 2.5 mM concentrations of each dNTP (Bangalore GeNeI, Bangalore India), 1 µl each of pA 5'-AGAGTTTGATCCTGGCTCAG-3' and pH 5'-AAGGAGGTGATCCAGCCGCA-3' primers (Edwards et al., 1989) and 20 ng of the template DNA in a total volume of 50 µl. Amplification of 16S rDNA was performed in a Gene Amp-PCR system 9700 (Applied Biosystems) under the following conditions: 130 s of denaturation at 92°C followed by 35 cycles of amplification with a 60 s denaturation at 92°C, 30 s of annealing at 48°C and 130 s of extension at 72°C. An extra extension step of 360 s at 72°C was added after completion of the 35 cycles. Amplified PCR products were sequenced and were aligned with 16S rDNA gene sequences available by the BLASTN search in the NCBI, GenBank database (http://www.ncbi.nlm.nih.gov). Phylogenetic tree was generated after performing multiple alignments (CLUSTAL W version). The method of Jukes and Cantor (1969) was used to calculate evolutionary distances, phylogenetic dendrogram was constructed by the neighbour-joining method and the tree topologies were evaluated by performing bootstrap analysis of 1,000 dataset using MEGA 3.1 software (Kumar et al., 2004). The sequence obtained in this study was deposited in the GenBank nucleotide sequence database under the accession number JX885487, JX960423-26 and JX9604230 (6 sequences).

Seed germination rate and seedling vigour index

Brinjal (CARI Brinjal-1), chilli (Cv. K2) and okra (Cv. Kashi Lalima) seeds were surface-sterilized with 70% ethanol for 2 min and in 2% sodium hypochlorite for 2 min followed by ten times washing in sterile distilled water. The surface sterilized seeds were inoculated by soaking in the respective rhizobacterial suspension (10^8 cfu/ml) for 45 min at 28 ± 2°C. The coated seeds were incubated in petri dishes lined with moist filter paper and incubated at 28 ± 2°C. Seeds immersed in sterilized distilled water served as control. The seed germination rate and vigor index were calculated after five days of incubation using the formula given below (Zucconi et al., 1981).

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seed}}{\text{Number of total seed tested}} \times 100\%$$

Plume and radicle lengths were recorded for the calculation of vigor index

Vigor index = (mean of plumule + radical lengths) x germination rate (%).

Pot experiment

Soil analysis

Physico-chemical characterization of experimental soil collected from the vegetable block of Central Agricultural Research Institute, Port Blair was carried out before filling the pots. The soil pH, EC, available potassium, phosphorous and nitrogen were determined as per the standard procedure (Jackson, 1973). Briefly, pH and EC was measured both in supernatant and in the suspension using pH and Electrical Conductivity meter. The soil was medium in 0.51% organic carbon content, available N (base hydrolysable N), available phosphorus (P) (Bray 1-P, 0.03 mol L^{-1} NH_4F + 0.025 mol l^{-1} HCl extractable), available potassium (K) (1 mol L^{-1} NH_4OAc extractable) were determined.

Preparation of inoculum and seed bacterization

Pure colony of each multi trait plant growth promoting (PGP) bacteria was inoculated in 100 ml of Nutrient Broth and incubated at 30 ± 2°C in an orbital incubator shaker for 3 days at 130 rpm. Seed bacterization was carried as per standard procedure of Silva et al. (2003). Three different varieties of vegetable crops *viz*., brinjal, chilli and okra were used in this study. Seeds of brinjal, chilli and okra were surface-sterilized as mentioned above. Approximately, 500 seeds from each vegetable crop were immersed in appropriate inoculum of PGP bacterial for 1 h at a concentration of 10^8 cfu/ml in sterile saline water (0.85%). Bacterized seeds were then air dried in laminar air flow hood and sown immediately.

Experimental design

The treatments were arranged in a Completely Randomized Block Design (CRBD) and were placed on a platform in the greenhouse. The treatments were as follows: T1, control (seeds coated with media); T2, seeds coated with NFB3; T3, NPB6; T4, MKP3; T5, MNB1; T6, NNB4 and T7- NTB2. Ten seeds of each brinjal, chilli and okra were sown in each plastic pots of 15 cm diameter containing 1 kg of sterile field soil. The seedlings were grown at a temperature of 28-32°C and 85% relative humidity in a greenhouse under a day-night cycle of 11–13 h natural light. The soil was

moistened to 60% water-holding capacity and was maintained at this moisture content by watering to weight every day. Uninoculated seeds sown in pots served as control. In each of the treatment, the plants were harvested 3 weeks after the emergence of the seedlings. The morphological characteristics of each plant were recorded *viz.* root length, shoot length and total number of secondary root of each plant.

Statistical analysis

Data of morphological characteristics of seedlings were statistically analyzed using the general linear model software Agres (3.01) and Agdata. The means were compared using the least significant difference (LSD) method at $P \leq 0.05$.

RESULTS

Distribution of rhizobacteria

There was a significant variation in the distribution of rhizobacteria in the soils samples of different crops. The total bacterial count in Middle Andaman samples ranged from 0.6 to 77 x 10^3 cfu ml^{-1} and most of the samples belong to the rhizosphere of spices. In North Andaman samples, the value ranged from 1.6 to 52 x 10^3 cfu/ml wherein most of the samples were collected from the rhizosphere of vegetable crops. The number of cfu was more in coconut + vegetables with regular supply of organic manures whereas regular use of only inorganic fertilizers in rice-vegetable resulted in the reduction of microbial count. A total of 114 bacterial isolates were isolated from the rhizosphere soils from Middle and North district of Andaman and Nicobar Islands, India (Table 1). In this study, all the isolates were screened for *in vitro* PGP, extracellular enzyme and antagonistic activity.

Traits of rhizobacterial isolates

Out of 114 rhizobacteria, it was found that 110, 107, 67 and 59 isolates produced siderophore, ammonia, P-solubilization and IAA, respectively, whereas none of the isolates produced HCN. In addition, some isolates produced extracellular enzyme such as protease (92), amylase (73), cellulase (58), chitinase (37), lipase (24) and pectinase (14). Six most promising isolates (NFB3, NPB6, MKP3, MNB1, NNB4 and NTB2) were selected on the basis of having multi-functional properties. The PGP, extracellular enzyme and antagonistic activity of the selected isolates are shown in Table 2. All the isolates utilized a significant amount of iron in siderophore production showing a yellow zone on the CAS agar medium plate, solubilized P in the plate-based assay as evidenced by the formation of a clear halo zone around the colony and tested positive for ammonia production. All the promising isolates exhibited IAA production in qualitative assay. Further the quantitative determination revealed that bacterial strain NTB2 showed maximum significant concentration of IAA followed by NFB3, NNB4, NPB6, MNB1 and MKP3.

It was observed that five isolates except MKP3 were found positive for protease production, four isolates except NNB4 and NTB2 produced cellulase, three isolates except NFB3, NTB2 and NNB4 produced chitinase and three isolates except NFB3, NTB2 and NNB4 produced amylase. However, only one isolates (NFB3) was found positive for pectinase production. In contrast, none of them were found to be positive for lipase production. Among all NPB6 and MNB1 were positive for maximum number of hydrolytic enzymes. Antagonistic activity of the bacterial isolates was evaluated in terms of inhibition zone diameter as an indicator of the reduction in growth of pathogenic fungi. The all selected isolates exhibited most obvious antagonistic activity *in vitro* against the tested pathogens and showed significant growth inhibition activity against *S. rolfsii* and *Macrophomina* sp. In the dual culture assay, NFB3 showed highest inhibition against the plant pathogen *Macrophomina* spp. (33.3%) and *S. rolfsii* (23%). Among the isolates, NPB6 and MNB1 were positive to many of the properties tested followed by NFB3, MKP3, NNB4 and NTB2 in PGP and extracellular enzyme activity.

Identification of isolates using Biolog system

The selected isolates were examined for their ability to oxidize different carbon sources using Biolog's automated identification system in which all the potential isolates were revealed as *Bacillus* and *Pseudomonas* species. There were large differences in the C utilizations among the *Bacillus* species, which are arranged according to the order of best C sources utilized (Table 3). Among the multi-trait isolates, the dominant ones were *B. cereus* (3 strains) followed by *Pseudomonas tolaasii* (2) and one strain of *B. pumilus.* Among the isolates *B. pumilus* (NFB3) and *P. tolaasii* (NNB4) utilized highest numbers (55) of substrates followed by NTB2 (*P. tolaasii*). Three strains of *Bacillus cereus* named NPB6, MKP3 and MNB1 utilized 14, 15 and 22 common carbon. All the strains utilized 1% sodium lactate and sodium chloride up to 4% (w/v) have the ability to grow at pH 5.0.

Sequence analysis of partial 16SrDNA and phylogenetic analysis

About 1.5 kb fragment of 16Sr RNA gene of six selected bacterial isolates were amplified by PCR using universal primers pA and pH. The PCR products were purified and sequenced. The sequence obtained was analyzed using a BLAST search in which six bacterial strains were placed into two genus viz. *Bacillus* and *Pseudomonas*.

Table 1. Effect of crop and cropping history on the CFU in rhizosphere soil samples.

Location	Cropping history	Inputs	Crop rhizosphere	CFU
Middle Andaman				
Panchwati	More than 30 years of coconut crop mixed with spices	Only manure recycling	Pepper	10-35 × 10^3
			Cinnamon	0.6-26 × 10^3
			Clove	1.9-32 × 10^3
			Nutmeg	5.2-77 × 10^3
Citrakut	More than 30 years of coconut + arecanut plantation mixed with cinnamon, pepper and recent cultivation of vegetable	FYM and compost only to vegetables	Ladies finger	33-63 × 10^3
			Paper	24-48 × 10^3
			Cinnamon	4.2-12 × 10^3
			Clove	4.2-22 × 10^3
Wavi	New plantation + pepper with intercropping of vegetable crops	Organic manures and compost only to vegetables	Pepper	21-35 × 10^3
Nimbudara	Vegetable crop rotation mainly of cucurbits	FYM and inorganic fertilizers	Cucumber	17-39 × 10^3
			Bitter gourd	18-42 × 10^3
North Andaman				
Kudirampur	Intensive vegetable cultivation with irrigation	Compost only	Bitter gourd	4.8-26 × 10^3
Keralapurm	10 year old coconut + arecanut plantation with young spices	Organic recycling and occasional FYM	Nutmeg	2.3-15 × 10^3
DB gram	10 years old coconut + arecanut plantation with vegetable cultivation in the slopes	Occasional FYM and regular inorganic fertilizers	French bean	32-52 × 10^3
			Pumpkin	1.6-25 × 10^3
			Bitter gourd	16-23 × 10^3
			Ladies finger	6.3-18 × 10^3
			Chilli	6.3-43 × 10^3
RK gram	Rice followed by irrigated vegetable crops	Regular use of inorganic fertilizers with FYM	Pumpkin	5.8-12 × 10^3
			Cauliflower	4.8-25 × 10^3
Kalipur	Rice followed by irrigated vegetable crops	Regular use of inorganic fertilizers	Tomato	4.2-18 × 10^3

Table 2. Determination of substrates utilization as carbon sources by selected strains.

Substrate Group	Substrate	Isolates					
		NFB3	NPB6	MKP3	MNB1	NNB4	NTB2
Carbohydrates (28)	D-Maltose	-	-	±	±	-	-
	D-Trehalose	+	-	-	±	+	+
	D-Cellobiose	+	-	-	-	-	-
	Gentiobiose	+	-	-	-	-	-
	Sucrose	+	-	-	-	-	-
	D-Turanose	-	-	-	-	-	-
	Stachyose	+	-	-	-	-	-
	D-Raffinose	+	-	-	-	-	-
	α-D-Lactose	-	-	-	-	-	-
	D-Mellibiose	+	-	-	-	-	-
	β-Methyl-D-glucoside	+	-	±	±	-	-
	D-Salicin	+	-	-	-	-	-
	N-Acetyl-D-glucosamine	+	±	±	±	+	+

Table 2. Contd.

	N-Acetyl-β-D-mannosamine	±	-	-	±	-	-
	N-Acetyl-D-galactosamine	-	-	-	-	-	-
	α-D-Glucose	+	-	-	±	+	+
	D-Mannose	+	-	-	-	+	+
	D-Fructose	+	±	±	±	+	+
	D-Galactose	+	-	-	-	+	+
	3-Methyl glucose	±	-	-	-	-	-
	D-Flucose	+	±	-	±	±	+
	L-Fucose	±	±	-	±	±	±
	L-Rhamnose	-	-	-	-	-	-
	D-Sorbitol	-	-	-	-	+	+
	D-Mannitol	+	-	-	±	+	+
	D-Arabitol	-	-	-	±	+	+
	myo-Inositol	-	-	±	±	+	+
Polymer (2)	Dextrin	+	±	-	+	-	-
	Tween 40	±	-	-	-	-	-
	B-Hydroxy-D,L- butyric acid	-	-	±	-	+	+
	D-Galacturonic acid	+	-	-	±	+	+
	L-Galactomic acid lactone	-	-	-	-	-	-
	D-Gluconic acid	+	±	±	±	+	+
	D-Glucuronic acid	±	-	-	-	+	+
	D-Lactic acid methyl ester	-	-	-	±	-	-
	p-Hydroxy-pheyl acetic acid	-	-	-	-	+	+
	N-Acetyl neuraminic acid	-	-	-	-	-	-
	γ-Amino-butyric acid	+	-	-	-	+	+
	α-Hydroxy-butyric acid	-	-	-	-	±	±
	α-Keto-butyric acid	-	-	-	-	±	±
	Acetoacetic acid	+	±	±	±	±	±
	Propionic acid	±	-	-	±	+	+
Carboxylic acids (26)	Methyl pyruvate	-	-	±	±	±	±
	Acetic acid	+	±	±	+	+	+
	Fusidic acid	-	-	-	-	+	+
	Formic acid	-	±	±	+	-	-
	L-Lactic acid	-	±	±	+	+	+
	Citric acid	+	-	-	-	+	+
	α-Keto-glutaric acid	+	-	-	-	+	+
	Bromo succinic acid	+	±	±	±	±	±
	Nalidixic acid	+	-	-	-	+	+
	D-saccharic acid	-	-	-	-	+	+
	D-Malic acid	-	-	-	-	-	-
	L-Malic acid	+	±	±	±	+	+
	Quinic acid	+	-	-	-	+	+
	Mucic acid	+	-	-	-	+	+
	L-Arginine	+	-	±	±	+	+
Amino acids (10)	L-Aspartic acid	+	±	±	±	+	+
	L-Glutamic acid	+	±	±	+	+	+
	L-Histidine	+	±	±	+	+	+
	L-Pyroglutamic acid	+	-	-	±	+	+

Table 2. Contd.

	L-Serine	+	±	+	+	+	+
	D-Aspartic acid	+	-	-	-		
	D-Serine	-	±	±	+	+	+
	Glycyl-L-proline	-	-	-	±	-	-
	L-Alanine	+	-	-	±	+	+
	Troleandomycin	-	-	-	-	+	+
	Rifamycin SV	-	-	+	+	+	+
Antibiotics (5)	Minocycline	-	+	+	+	-	-
	Vancomycin	-	-	-	-	+	+
	Lincomycin	-	-	-	-	+	+
Amides (1)	Gluccuronamide	+	-	-	-	+	+
	Inosine	+	±	±	±	+	+
	Glycerol	+	±	±	±	+	+
	1% Sodium lactate	+	+	+	+	+	+
	Gelatin	+	+	+	+	-	-
	D-Glucose-6-PO_4	+	±	±	+	-	-
	D-Fructose-6-PO_4	+	±	±	+	+	+
	Pectin	+	-	-	-	-	-
	Nilaproof 4	-	-	-	-	+	+
	Tetrazolium violet	-	-	-	-	+	+
	Tetrazolium blue	-	-	-	-	±	+
Miscellaneo-us (21)	Lithium chloride	+	+	+	+	-	-
	Guanidine HCL	+	+	+	+	±	+
	Potassium tellurite	+	+	+	±	+	+
	Aztreonam	+	+	+	+	-	+
	Sodium butyrate	+	+	+	+	-	-
	Sodium bromate	-	+	+	-	±	-
	pH 6	+	+	+	+	+	+
	pH 5	+	+	±	-	+	+
	1% NaCl	+	+	+	+	+	+
	4% NaCl	+	+	+	+	+	+
	8% NaCl	+	+	+	+	-	-

-, negative reaction; ±, moderate; +, positive reaction.

Among six strains, four strains MNB1, NPB6 and MKP3 were identified as *Bacillus cereus,* NFB3 was *Bacillus stratosphericus*, NNB4 was *Pseudomonas fluorescens* and NTB2 was *Pseudomonas simiae* (Table 4). Phylogenetic analyses of the strains based on the neighbor joining method resulted into three major clusters (Figure 1). Cluster I formed with *Bacillus cereus*, cluster II formed with *Bacillus stratosphericus* and cluster III formed with *P. fluorescens* and *P. simiae.*

Effect of rhizobacteria on growth parameters of vegetables

In *in vitro* seed germination test of inoculated and un-inoculated seeds, the growth performance of the seedlings were measured and the results are presented in Table 5. Inoculation of rhizobacteria such as *B. stratosphericus* and *B. cereus* had resulted in significantly higher seed germination rate in brinjal and okra whereas *P. simiae* produced similar effect in chilli. In both cases, there was 20% higher seed germination over the control. Radical length was significantly higher due to seed inoculation of all the six selected rhizobacteria and more conspicuous effect was seen in *B. cereus* in okra (4.06 cm) and *P. simiae* in brinjal (2.11 cm) and chilli (1.03). Significantly higher plumule length of 2.43, 1.44 and 1.24 cm was observed in brinjal, chilli and okra, respectively in seed bacterization with *B. cereus.* Seed bacterization also

Table 3. Characterization of selected bacterial isolates for plant growth promoting and antagonistic traits.

Properties	Isolate name					
	NFB3	NPB6	MKP3	MNB1	NNB4	NTB2
PGP Properties						
IAA Production	+++	++	+	+	++	+++
IAA Production (µg/ml)	34.91	31.79	21.6	23.19	32.68	35.26
P-Solubilization	++	+	++	+	+++	+++
Ammonia Production	+	+	+	+	+	+
Siderophore	++	+++	+++	+++	+++	++
Hydrolytic enzymes						
Protease	++	+++	-	+	+++	+++
Cellulase	+	+	+	+	-	-
Lipase	-	-	-	-	-	-
Pectinase	+++	-	-	-	-	-
Chitinase	-	++	++	++	-	-
Amylase	-	++	++	++	-	-
Antagonistic activity (% inhibition)						
Macrophomina sp	33.3	14.1	23.0	23.7	23	23.7
Sclerotium rolfsii	23.0	14.1	18.5	18.5	21.5	22.2

-, No activity; + 0.3-0.5 cm; ++, 0.6-1.0 cm; +++, >1.0 cm.

Table 4. Identification of bacterial isolates using carbon source utilization and 16SrDNA partial sequences.

Isolates	Identified organism		Accession number based on 16S rDNA
	Biolog	16S rDNA	
NFB3	*Bacillus pumilus*	*Bacillus stratosphericus*	JX960424
NPB6	*Bacillus cereus*	*Bacillus cereus*	JX960425
MKP3	*Bacillus cereus*	*Bacillus cereus*	JX960430
MNB1	*Bacillus cereus*	*Bacillus cereus*	JX960426
NNB4	*Pseudomonas tolaasii*	*Pseudomonas fluorescens*	JX960423
NTB2	*Pseudomonas tolaasii*	*Pseudomonas simiae*	JX885487

produced similar effect on the vigor index as that of radian length. *P. simiae* gave higher vigor index in brinjal (408%) and chilli (235%) whereas in the case of okra *B. cereus* performed better (510%).

Six promising IAA producing isolates with multi-functional properties were tested for their influence on growth parameters on the brinjal, chilli and okra (Figures 2, 3 and 4). In brinjal, isolates NPB6 (75.04 and 58.15%), MKP3 (61.5 and 68.08%) and NTB2 (58.6 and 53.9%) have increased shoot and root length respectively, over control. In chilli, three isolates namely: *B. stratosphericus*-NFB3, *B. cereus*-MNB1 and *P. simiae*-NTB2 showed increased shoot (37.4, 32.2 and 39.4%) and root length (52.6, 42.8 and 38.4%) over untreated controls. In okra, *P. fluorescens*- NNB4, *B. cereus* -MKP3 and *B. cereus*- NPB6 showed significant plant growth promotion with respect to increase in root and shoot length. There was no clear trend in increasing secondary root number by all the isolates. It was found that seed bacterisation of *P. simiae*-NTB2 significantly increased the number of secondary root in chilli followed by NPB6 in brinjal and NNB4 in okra over control. Among theisolates, the overall performance of *B. cereus* - NPB6 for root length in brinjal, *P. fluorescens* - NNB4 for shoot length in okra and *P. simiae*-NTB2 for secondary root number in chilli were highly significant.

DISCUSSION

The PGPR isolates promote plant growth and induce resistance in different crops (Podile and Kishore, 2006). PGPR or potential biological control strains isolated from one region may not produce similar results in other soils

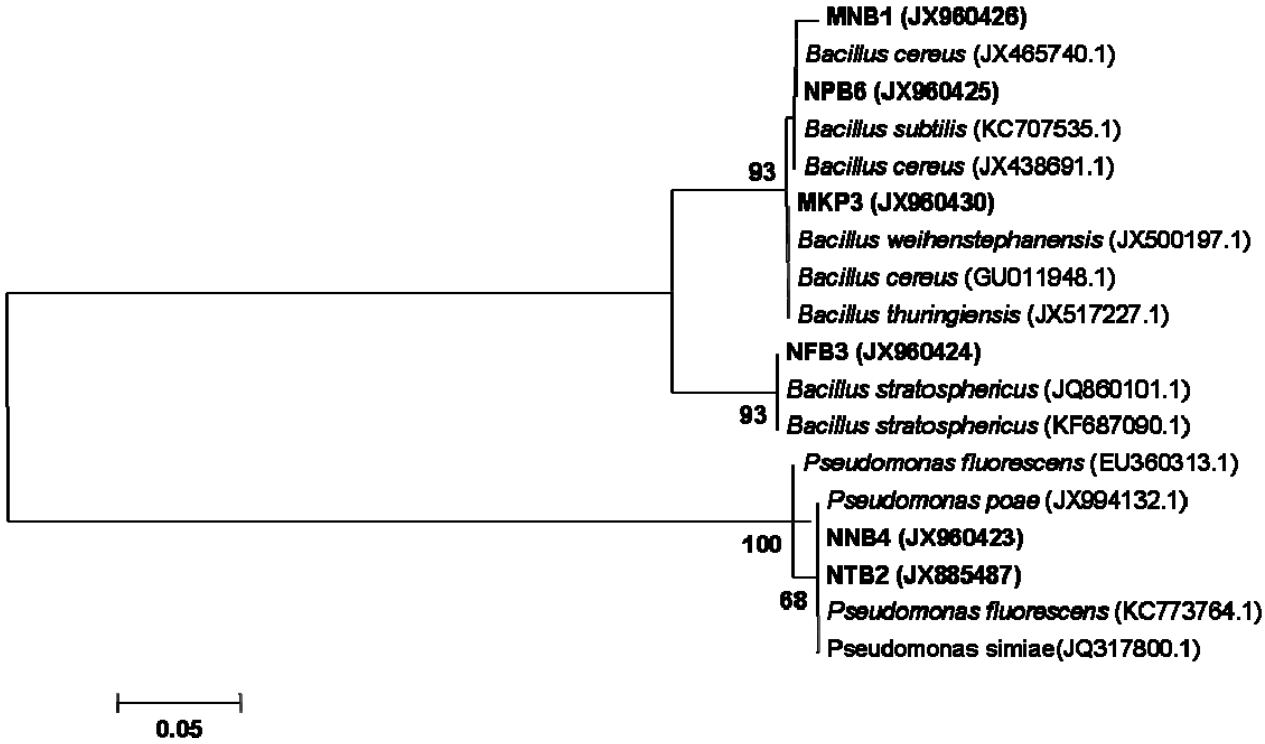

Figure 1. Neighbour-joining phylogenetic tree-based 16S rDNA sequences and their closest phylogenetic neighbours. Bootstrap values are indicated at nodes. Scale bar represents observed number of changes per nucleotide position.

Table 5. Effect of rhizobacteria on growth parameters of brinjal, chilli and okra seeds.

Isolate	Germination rate (%)			Radical length (cm)			Plumule length (cm)			Vigor Index (%)		
	B	C	O	B	C	O	B	C	O	B	C	O
B. stratosphericus- NFB3	100^{c}	80^{a}	100^{b}	1.05^{b}	0.65^{c}	3.51^{d}	1.30^{b}	0.63^{a}	1.04^{b}	234.0^{b}	102.4^{b}	456.0^{d}
B. cereus- NPB6	100^{c}	80^{a}	100^{b}	1.42^{bc}	0.96^{d}	2.62^{b}	2.01^{d}	0.97^{b}	1.15^{b}	308.7^{cd}	154.4^{c}	402.0^{c}
B. cereus- MKP3	90^{b}	90^{b}	100^{b}	1.43^{bc}	0.67^{c}	3.48^{d}	1.88^{cd}	1.44^{c}	1.24^{c}	298.8^{c}	192.6^{e}	482.0^{e}
B. cereus- MNB1	90^{b}	90^{b}	100^{b}	1.09^{b}	0.68^{c}	4.06^{e}	1.58^{bc}	1.32^{c}	1.04^{b}	240.3^{b}	180.0^{d}	510.0^{f}
*P. fluorescens-N*NB4	80^{a}	80^{a}	80^{a}	1.83^{cd}	0.49^{b}	3.69^{c}	2.17^{de}	0.83^{b}	1.22^{b}	320.0^{d}	106.4^{b}	392.8^{c}
P. simiae - NTB2	90^{b}	100^{c}	90^{ab}	2.11^{d}	1.03^{e}	3.09^{a}	2.43^{e}	1.32^{c}	1.03^{b}	408.6^{e}	235.0^{f}	367.6^{b}
Control	80^{a}	80^{a}	90^{ab}	0.73^{a}	0.44^{a}	2.09^{a}	0.80^{a}	0.50^{a}	0.74^{a}	122.4^{a}	76.00^{a}	254.1^{a}
CD ($p< 0.05$)	9.50	6.1	12.27	0.54	0.06	0.21	0.37	0.18	0.19	12.68	5.03	12.28

Results obtained were of mean of triplicates. Data were analyzed using one-way analysis of variance and treatment means were compared ($p \leq 0.05$). B = Brinjal; C = Chilli; O = Okra. Column with different letters are significant, with similar letter non significant at $p<0.05$

and climatic conditions as in the case of its original habitat (Duffy et al., 1997). Therefore, isolation of resident microbial strains and their utilization constitute the best alternate strategy. This is more pertinent to Andaman and Nicobar Islands, which is a repository of biodiversity by virtue of its nature and location. In the present study, six potential PGPR isolates were selected on the basis of their performance with multi-functional properties such as production of IAA, siderophore, ammonia, P-Solubilization, extracellular enzyme and antagonistic activity. Among the bacterial isolates, *P. simiae* (NTB2) showed significant concentration of IAA followed by *B. stratosphericus* (NFB3), *P. fluorescens* (NNB4), *B. cereus* (NPB6), *B. cereus* (MNB1) and *B. cereus* (MKP3) sugges-ting that these

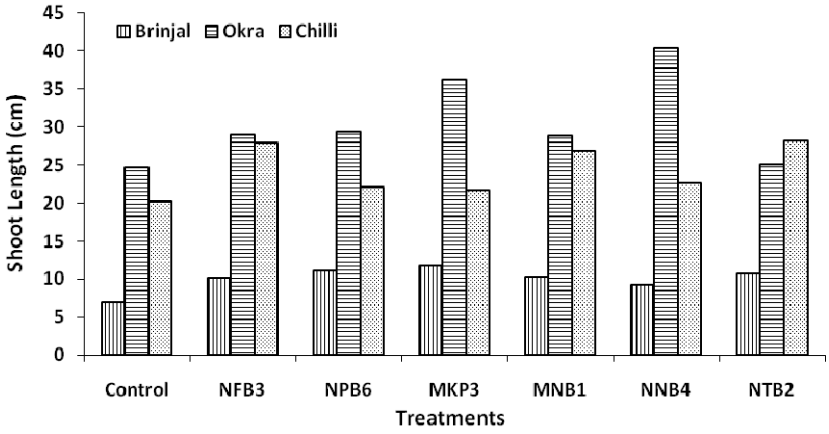

Figure 2. Inoculation effect of PGPR strains on shoot length of seedlings after one month.

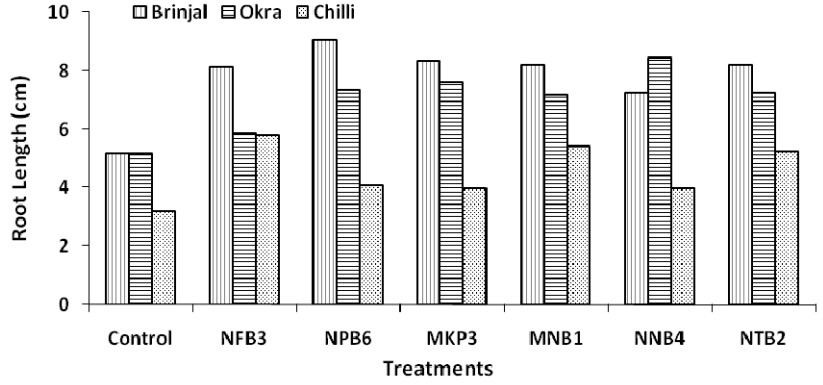

Figure 3. Inoculation effect of PGPR strains on root length of seedlings after one month.

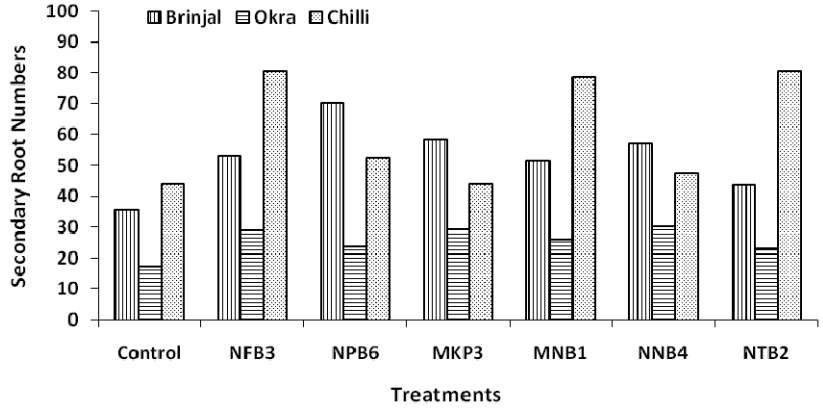

Figure 4. Inoculation effect of PGPR strains on secondary root numbers seedlings after one month.

isolates could be used for plant growth promotion. A higher amount of IAA was produced in the presence of L-tryptophan by *P. simiae* (NTB2) because L-tryptophan acts as suitable precursor for IAA biosyn-thesis. Similarly, higher level of IAA production in the presence of L-tryptophan by *Pseudomonas* and *Bacillus* sp. was reported by Xie et al. (1996) and Srinivasan et al. (1996).

All isolates showed solubilization zone on Pikovskaya medium, indicating its potential role as a P-solubilizer. The phosphate solubilization by the isolate could increase the availability of phosphorous in the rhizospheric region. It is a well established fact that improved phosphorous nutrition results in the overall plant growth and root development (Jones and Darrah, 1994). All the selected isolates utilized a significant amount of iron by siderophore production, which is indicative of their ability to suppress fungal pathogens in the rhizosphere by chelating iron. It also exhibited production of ammonia, which could be taken up by plants as a source of nitrogen for their growth (Ahmad et al., 2008).

It was also shown that rhizobacteria inhibits phytopathogens by producing chitinase, β-1, 3-glucanase and pectinase, which degrade the fungal cell wall (Friedlander et al., 1993; Bloemberg and Lugtenberg, 2001; Persello-Cartieaux et al., 2003). In the present study five isolates were found positive for protease production followed by four isolates for cellulase, three each for chitinase and amylase and only one isolate produced pectinase whereas, none of the isolates produced lipase. This could be a principal reason for the observed antagonistic activity against *S. rolfsii* and *Macrophomina* sp. Characterization of the isolates on the basis of carbon source utilization in Biolog system revealed that they are gram-positive *Bacillus* and gram-negative *Pseudomonas* sp. Compared to other bacterial groups, *Bacillus* group were found to have the ability to survive in hot humid climate of Andaman and Nicobar Islands because of its ubiquitous nature like multilayered cell wall, stress resistant, endo-spore formation, secretion of peptide antibiotics, peptide signal molecules and extracellular enzyme productions. Among the identified isolates, three isolates of *Bacillus* sp. followed by two isolates of *Pseudomonas* sp. were found to exhibit multi-trait properties. There were large differences in the C-utilizations among the *Bacillus* sp., which are arranged according to the order of best C sources utilized. The data obtained showed a greater abundance of Gram-positive bacteria in the tropical soils of Andaman and Nicobar Islands. This was in agreement with the previous studies (Garbeva et al., 2003; Rau et al., 2009; Kumar et al., 2011; Amaresan et al., 2012) that showed a higher level of Gram-positive *Bacillus* and *Paenibacillus* species in the rhizosphere soils of cultivated vegetable crops and wild grass. GEN III micro-plates contain some substrates that commonly occur in root exudates, which represent a primary source of carbon and energy and are likely to favor fast-growing microbes in the rhizosphere (Alisi et al., 2005). Thus, the ability to metabolize root exudates at high rates has been often related to the colonization of roots by bacteria (Baudoin et al., 2003).

Bacteria are known to produce different metabolites like IAA, gibberellins and cytokinin like substances, which can exert positive effect on seed germination and radicle length (Tien et al., 1979). In the present study, under laboratory conditions, seed treatment with the test strain improved seed germination and seedling emergence of brinjal, okra and chilli over the control, which might be due to the production of IAA by the rhizobacteria (Mirza et al., 2001). Similar improvement of seed germination parameters by rhizobacteria was reported in rice seedlings, cowpea seedlings (Minaxi et al., 2011) and sorghum (Raju et al., 1999) due to increased synthesis of gibberellins, which triggered the activity of specific enzymes such as α-amylase that promoted early germination by increasing the availability of starch assimilation.

IAA-producing bacteria are known to promote root elongation and plant growth (Patten and Glick, 2002). The significant increase on the root and shoot length of brinjal, chilli and okra crops could be attributed to the production of growth promoting substances by the inoculated bacteria that carry out an important role in the stem expansion process (Burd et al., 2000). In a similar study, Adesemoye et al. (2008) reported that inoculation of tomato, okra and African spinach with *Bacillus subtilis* and *P. aeruginosa* enhanced percent emergence and growth of seedlings. In addition, the isolates significantly increased the secondary roots production prominently. *B. stratosphericus* (NFB3) showed increase in secondary roots in all the crops.

As these isolates were observed to produce significant amount of IAA, which positively influenced the root growth and development thereby enhancing nutrient uptake (Khalid et al., 2004). In addition, healthy plants may exhibit greater resistance to pathogens than that which are under stress. Among the isolates, *B. cereus* (NPB6) in brinjal, *B. stratosphericus* (NFB3) in chilli and *P. fluorescens* (NNB4) in okra significantly improved the growth parameters.

These IAA producing multi-functional *Bacillus* and *Pseudomonas* sp. with broad range of carbon sources has the potential to be used as bio-inoculants to attain the desired plant growth promotion in brinjal, okra and chilli seedling. Also, it showed the usefulness of PGPR-inoculated treatment for improving crop productivity of tropical crops growing in hot humid climate of Andaman and Nicobar Islands, which help to minimize the uptake of fertilizers, reduce environmental pollution and promote sustainable agriculture. These isolates having the property to tolerate 8% NaCl stress and pH 5, can also

be used as a bio-inoculants in alkaline and acidic agriculture soil conditions of Andaman and Nicobar Islands.

ACKNOWLEDGEMENTS

The authors are grateful to ICAR, New Delhi for funding this study under network project on Application of Microorganisms in Agriculture and Allied Sectors.

REFERENCES

Adesemoye AO, Torbert HA, Kloepper JW (2008). Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54:876-886.

Ahmad F, Ahmad I, Khan MS (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities. Microbiol. Res. 163:173-181.

Alisi C, Lasinio GJ, Dalmastri C, Sprocati A, Tabacchioni S, Bevivino A, Chiarini L (2005). Metabolic profiling of *Burkholderia cenocepacia, Burkholderia ambifaria* and *Burkholderia pyrrocinia* isolates from maize rhizosphere. Microb. Ecol. 50:385-395.

Amara MAT, Dahdoh MSA (1997). Effect of inoculation with plant growth promoting rhizobacteria (PGPR) on yield and uptake of nutrients by wheat grown on sandy soil. Egypt. J. Soil Sci. 37:467-484.

Amaresan N, Jayakumar V, Kumar K, Thajuddin N (2012). Isolation and characterization of plant growth promoting endophytic bacteria and their effect on tomato (*Lycopersicon esculentum*) and chilli (*Capsicum annuum*) seedling growth. Ann. Microbiol. 62:805-810.

Antoun H, Kloepper JW (2001). Plant growth promoting rhizobacteria. In: Brenner S, Miller JH Eds, Encyclopedia of genetics. Academic, New York, pp. 1477-1480.

Asghar HN, Zahir ZA, Arshad M, Khaliq A (2002). Relationship between production of auxins by rhizobacteria and their growth promoting activities in *Brassica juncea.* L. Biol. Fertil. Soil 35:231-237.

Babalola OO, Sanni AI, Odhiambo GD, Torto B (2006). Plant growth-promoting rhizobacteria do not pose any deleterious effect on cowpea and detectable amounts of ethylene are produced. World J. Microbiol. Biotechnol. 23:747-752.

Baudoin E, Benizri E, Guckert A (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biol. Biochem. 35:1183-1192.

Benizri E, Courtade A, Picard C, Guckert A (1998). Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens*. Soil Biol. Biochem. 30:1481-1484.

Bloemberg GV, Lugtenberg BJJ (2001). Molecular basis of plant growth promotion and Biocontrol by rhizobacteria. Curr. Opin. Plant Biol. 4:343-350.

Burd GI, Dixon DG, Glick BR (2000). Plant growth promoting bacteria that decrease heavy metal toxicity in plants. Can. J. Microbiol. 46:237-245.

Cakmakci R (2005). Bitkigelifliminde fosfat cozucu bakterilerin onemi. Selcuk Univ Ziraat. Fakultesi. Dergisi. 35:93-108.

Chen WP, Kuo TT (1993). A simple and rapid method for the preparation of gram negative bacterial genomic DNA. Nucleic Acids Res. 21:2260.

Collins CH, Lyne PM (1980). Microbiological Methods. Butterworth, London, 4:215-217.

DES (2011). Directorate of Economics and Statistics. Andaman and Nicobar Administration, Port Blair, India. p. 21.

Duffy BK, Ownley BH, Weller DM (1997). Soil chemical and physical properties associated with suppression of take-all of wheat by *Trichoderma koningii*. Phytopath. 87:1118-1124.

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S r DNA. Nucleic Acids Res. 17:7843-7853.

Friedlander AM, Welkos SL, Pitt MLM, Ezzell JW, Worsham PL, Rose KJ (1993). Postexposure prophylaxis against experimental inhalation anthrax. J. Infect. Dis. 167:1239-1242.

Garbeva P, Van veen JA, Van Elas ID (2003). Predominant *Bacillus* sp. in agricultural soil under different management regimes detected via PCR-DGGE. Microbiol. Ecol. 45:302-316.

Gutierrez-Manero FJ, Ramos B, Probanza A, Mehouachi J, Talon M (2001). The plant growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active Gibberelins. Physiol. Plant 111:206-211.

Hurek T, Reinhold-Hurek B (2003). *Azoarcus* sp. strain BH72 as a model for nitrogen fixing grass endophytes. J. Biotechnol. 106:169.

Idris EES, Iglesias DJ, Talon M, Borriss R (2007). Tryptophan-dependent production of Indole-3- Acetic Acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. Mol. Plant Microbe Interact. 20:619-626.

Jackson ML (1973). Soil Chemical Analysis. Pentice Hall of India Pvt. Ltd, New Delhi. pp. 38-204.

Jones DL, Darrah PR (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil 166:247-257.

Jukes TH, Cantor CR (1969). Evolution of protein molecules. In: Munro HN Eds. Mammalian protein 242 metabolism. Academic Press, New York, pp. 21-132.

Kasama T, Shiozawa F, Isozaki T, Matsunawa M, Wakabayashi K, Odai T, Yajima N, Miwa Y, Negishi M, Ide H (2008). Effect of the H2 receptor antagonist nizatidine on xerostomia in patients with primary Sjögren's syndrome. Mod. Rheumatol. 18:455-459.

Khalid A, Arshad M, Zahir ZA (2004). Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J. Appl. Microbiol. 46:473-480.

Kloepper JW (1993). Plant growth-promoting rhizobacteria as biological control agents. In: Metting FB Jr Eds. Soil microbial ecology-applications in agricultural and environmental management. Marcel Dekker, Inc, New York, pp. 255-274.

Kumar K, Amaresan N, Bhagat S, Madhuri K, Srivastava RC (2011). Isolation and characterization of rhizobacteria associated with coastal agricultural ecosystem of rhizosphere soils of cultivated vegetable crops. World J. Microbiol. Biotechnol. 27:1625-1632.

Kumar S, Tamura K, Nei M (2004). MEGA: molecular evolutionary genetics analysis software for microcomputers. Comput. Appl. Biosci. 10:189-191.

Lucy M, Reed ER, Glick B (2004). Application of free living plant growth promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1-25.

Mantelin S, Touraine B (2004). Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. J. Exp. Bot. 55(394):27-34.

Minaxi NL, Yadav RC, Saxena J (2012). Characterization of multifaceted *Bacillus* sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semi arid deserts. Appl. Soil. Ecol. 59:124-135.

Mirza MMQ, Warrick RA, Ericksen NJ, Kenny GJ (2001). Are floods getting worse in the Ganges, Brahmaputra and Meghna basins. Environ. Hazards 3:37-48.

Naik PR, Raman G, Narayanan KB, Sakthivel N (2008). Assessment of genetic and functional diversity of phosphate solubilizing fluorescent *Pseudomonads* isolated from rhizospheric soil. BMC Microbiol. 8:230-244.

Patten CL, Glick BR (1996). Bacterial biosynthesis of indole-3-acetic acid. Can. J. Microbiol. 42:207-220.

Patten C, Glick CR (2002). Role of *Pseudomonas putida* indole acetic acid in development of host plant root system. Appl. Environ. Microbiol. 68:3795-3801.

Persello-cartieaux F, Nussaume L, Robaglia C (2003). Tales from the underground molecular plant-rhizobacteria interactions. Plant Cell

Environ. 26:189-199.
Podile AR, Kishore GK (2006). Plant growth promoting rhizobacteria. In: Gnanamanickam SS Eds. Plant-associated bacteria. Springer, Amsterdam, pp. 195-230.
Raju NS, Niranjana SR, Janardhana GR, Prakash HS, Shetty HS, Mathur SB (1999). Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents. J. Sci. Food Agric. 79:206-212.
Rau N, Mishra V, Sharma M, Das MK, Ahaluwalia K, Sharma RS (2009). Evaluation of functional diversity in rhizobacterial taxa of a wild grass (*Saccharum ravennae*) colonizing abandoned fly ash dumps in Delhi urban ecosystem. Soil Biol. Biochem. 41:813-821.
Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305-339.
Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophore. Anal Biochem. 160:47-56.
Silva WA Jr, Bonatto SL, Holanda AJ, Ribeiro-dos-Santos AK, Paixão BM, Goldman GH, Abe-Sandes K, Rodriguez-Delfin L, Barbosa M, Paçó-Larson ML, Petzl-Erler ML, Valente V, Santos SEB, Zago MA (2003). Correction: mitochondrial DNA variation in Amerindians. Am. J. Hum. Genet. 72:1346-1348.
Srinivasan MV, Zhang SW, Lehrer M, Collett TS (1996). Honeybee navigation en route to the goal: Visual flight control and odometry. J. Exp. Biol. 199:237-244.
Tien TM, Gaskins MH, Hubbett DH (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of Pearl Millet. Appl. Environ. Microbiol. 37:1016-1024.
Verma SC, Ladha JK, Tripathi AK (2001). Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J. Biotechnol. 91:127-141.
Whipps JM (2001). Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. 52:487-512.
Xie X, Kokubo T, Cohen SL, Mirza UA, Hoffmann A, Chait BT, Roeder RG, Nakatani Y, Burley SK (1996). Structural similarity between TAFs and the heterotetrameric core of the histone octamer. Nature 380 (6572):316-322.
Zucconi FA, Pera M, Forte M, de Bertoldi (1981). Evaluating toxicity of immature compost. Biocycle 2:54-57.