



Research Article

Successful colonization of roots and Plant growth promotion of sorghum (*Sorghum bicolor* L.) by seed treatment with *Pseudomonas putida* and *Azotobacter chroococcum*

Uzma Sultana¹, Suseelendra Desai^{1*} and Gopal Reddy²

¹Research Scholar ICAR-Central Research Institute for Dryland Agriculture, Santoshnagar, Hyderabad 500059, India.

²Professor Emeritus, Department of Microbiology, Osmania University, Hyderabad 500007, India.

Pseudomonas putida (P29) and *Azotobacter chroococcum* (Azb19) are the efficient promising strains selected from *in vitro* plant growth promoting studies. These two strains were tested for their ability to promote growth of sorghum and colonize sorghum roots. Seed bacterization with P29 and Azb19 resulted in increased plant height, shoot height, root volume, leaf area and total plant dry mass. Further, bacterial inoculation also significantly increased macro-and micro-nutrient uptake by sorghum plants. Using electroporation method, pure cultures of P29 and Azb19 were transformed with pHC 60 plasmid containing *gfp* gene. Transformants detected by colony PCR were used to study the colonization pattern on roots of sorghum. Confocal fluorescence scanning microscope (CLSM) was used to locate the inoculants on or inside roots. Root colonization in sorghum by P29 was internal whereas Azb19 was detected on root surface. GFP-tagged *Pseudomonas* was predominantly detected at the root differentiation zone. In case of Azb19 small aggregates of micro-colonies were observed on the surface of the roots. The efficient sorghum root colonization by these inoculants clearly demonstrated that the introduced strains could successfully inhabit the rhizosphere and thus resulting in increased nutrient uptake. Inoculation with P29 resulted in increased uptake of P (288.5%), K (179.1%), Fe (242.7%), and Zn (168.1%) as compared to Azb19 where the uptake of P, K, Fe, Mn, and Zn increased by 142.6%, 161.6%, 199.5%, and 121.9%, respectively. On the other hand, inoculation with Azb19 could enhance better uptake of N (163.6%) as compared to P29 (133.3%). The strains also differed in their mode of root colonization.

Key words: Root colonization, *Pseudomonas*, *Azotobacter*, GFP, PGPR, Sorghum, Confocal, laser scanning microscope

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is an important rainfed crop, grown globally on 41.9 million ha. In India, sorghum is grown in a wide range of environments. It occupies around 7.7 million hectares especially grown in semi-arid regions of the country producing 7.0 million metric tons of grain (FAO). Plant growth promoting rhizobacteria (PGPR) have originally been defined as root-colonizing bacteria that cause either plant growth promotion or

biological control of plant diseases.

***Corresponding author:** Dr. Suseelendra Desai, Principal Scientist, Plant Pathology, Division of Crop Science, ICAR-Central Research Institute for Dryland Agriculture, Santoshnagar, Hyderabad, 500059, India. Email:desai1959@yahoo.com

selected by plating the serially-diluted suspension on LB plates amended with $10\mu\text{g mL}^{-1}$ tetracycline and incubated overnight at 37°C . Positive clones were then further confirmed by colony PCR. Individual colonies from the antibiotic amended plates were picked and amplified using *gfp* primer. PCR product was resolved on 1% agarose gel to detect the presence of amplified 730 bp *gfp* gene. For amplification, forward primer 5'-ATAGAATTCATGGCTAGCAAGGAGAA3' and reverse primer 5'AATCTCGAGGCAGCCGTTCTTTGTA-3' were used.

Colony PCR conditions

Colony PCR steps included i) denaturation for three min at 95°C ii) 30 cycles of denaturation for one min each at 94°C iii) annealing for 30 sec at 57°C iv) elongation for one min at 72°C and v) a final extension step of 2 min at 72°C . The PCR products were then held at 10°C .

Sorghum seedlings treatment with bacteria and plant growth conditions

Sorghum seeds were surface sterilized with 0.1% HgCl_2 for 3 min and then washed three times with sterile double-distilled water. After surface sterilization, seeds were placed on water agar and were incubated. After the emergence of primary roots, seedlings were uprooted carefully from the water agar. Excess agar was removed by carefully rinsing the roots in sterile double-distilled water. Bacterial cells from an overnight culture on LB agar plates, grown at 37°C were harvested. The final bacterial inoculum used for root dip inoculation of sorghum seedlings was 4×10^8 cfu mL^{-1} determined both spectrophotometrically (600nm) and by counting viable tetracycline resistant (TcR) colonies.

Sorghum (crop variety SPV 462) seedlings from water agar were dipped in the bacterial suspension and were transferred to 22cm diameter pots filled with sterile soil. Six replications for each treatment were maintained. Seedlings without bacteria were potted to serve as control samples. Plants were grown for three weeks with growth conditions as follows: temperature $26 \pm 1^\circ\text{C}$, humidity $70 \pm 2\%$, $200\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity and a photoperiod of 16 h light/8h dark. After three weeks, plants were uprooted carefully and microbial count on root surfaces was taken using dilution plating method.

Sample Preparation for Microscopy

Plants were uprooted carefully from the pots and excess soil was removed by shaking. Root samples (sections of root tip) were cut, soaked in sterilized ddH₂O and placed in sterile plastic petri dishes.

Evaluation of plant growth promoting ability

Agronomic parameters such as shoot length, shoot dry mass, root dry mass and root volume were recorded

following standard procedures. Chlorophyll content was estimated in terms of SPAD reading using a SPAD meter.

Nutrient Analysis

Nutrient analysis of the plant samples was done as described by (Tandon, 2001) which is briefly described here. Oven-dried plants were finely ground in a mortar and pestle to amorphous powder and 100 mg was taken in 150 mL conical flask containing 10 mL nitric acid (HNO_3) and perchloric acid (HClO_4) in 9:4 ratio. The flasks were placed on a hot plate and digested at 300°C until the entire plant material was digested and turned colorless. The extract was taken in 100 mL volumetric flask and the volume was made up to 100 mL with distilled water. These samples were used for estimation of sodium, potassium, and calcium by flame photometer. Phosphorus was quantified by sulphomolybdic acid method. Total nitrogen content of the plants was estimated by micro Kjeldahl method. Similarly, micronutrients such as iron, copper, manganese, zinc and magnesium were estimated by atomic absorption spectrophotometer.

Statistical analysis

The data were analyzed by using the appropriate statistical methods wherever needed. The pot culture studies data were analyzed by analysis of variance (ANOVA). The means were compared using LSD.

RESULTS

pHC 60 vector with *gfp* gene and tetracycline resistant marker was extracted from *E. coli* DH5 α cells. After successful extraction, *Pseudomonas*29 and *Azotobacter*19 were transformed with pHC 60 by electroporation technique. pHC 60 vector was isolated from the positive clones and the *gfp* gene was amplified (Fig 2).

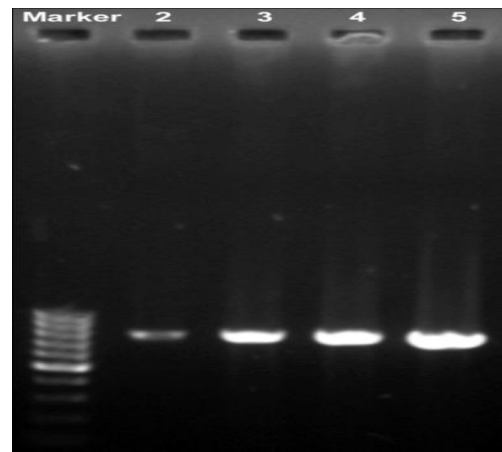


Fig2. PCR amplification of *gfp* gene from pHC 60 of transformed clones of Azb19 (lanes 2&3) and P29 (lanes 4&5)

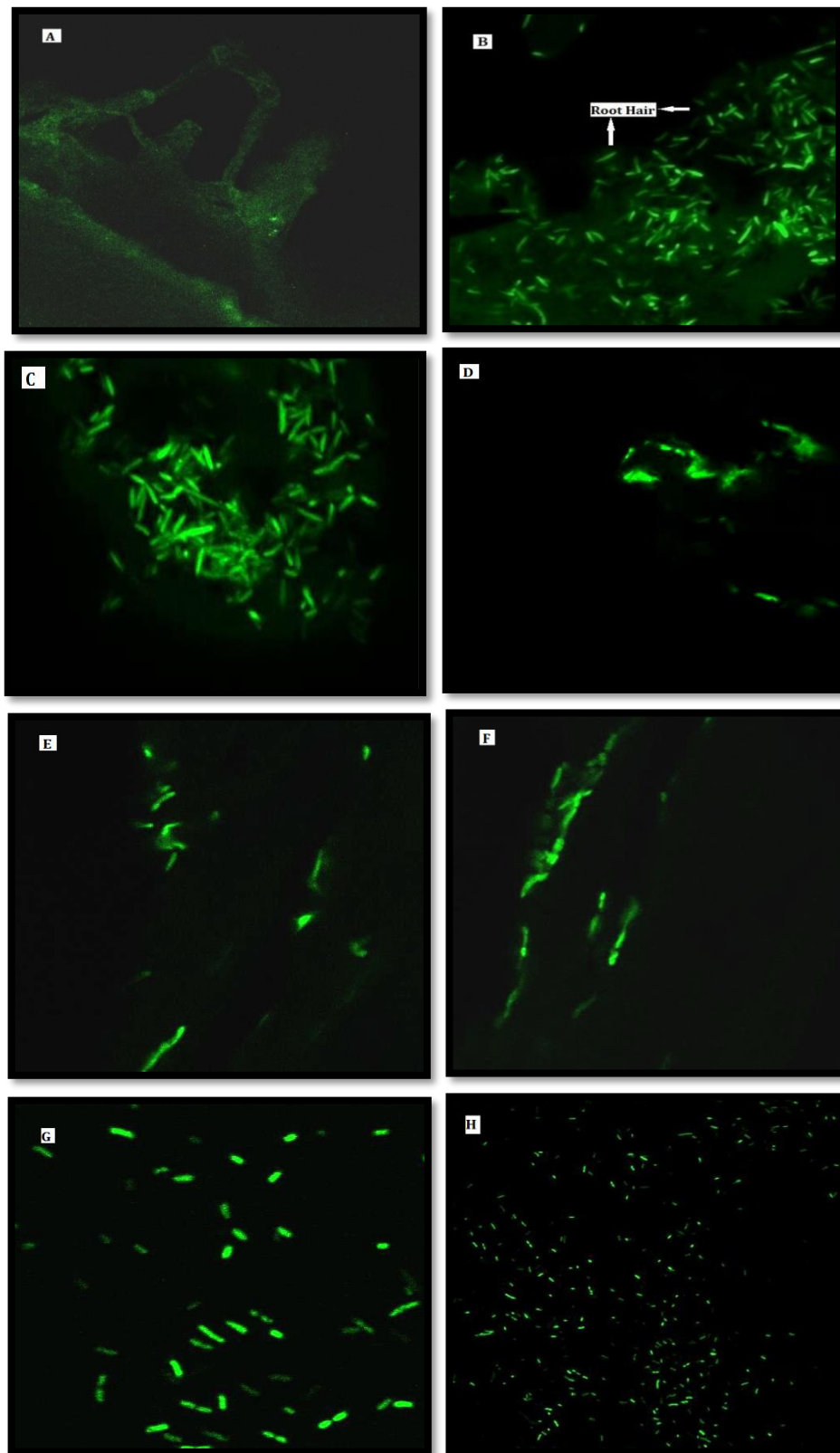


Figure 3. CLSM images of root colonization of Sorghum. Confocal analysis was performed on 1–2cm long root longitudinal sections to show internal colonization (A, B, C) by *Pseudomonas* and surface colonization (D, E, F) by *Azotobacter*. A) Confocal optical sections of root hairs, B) Detection of *gfp*-tagged *Pseudomonas* inside the root hairs, C) Cells of *Pseudomonas* near the boundary of a root hair, D) *gfp*-tagged *Azotobacter* attached to root surface of Sorghum, E) Cells of *Azotobacter* observed as single cells, F) as micro colonies (arrowed). Image G & H represents the pure culture of GFP tagged *Azotobacter* and *Pseudomonas*.

Successful colonization of roots and Plant growth promotion of sorghum (*Sorghum bicolor* L.) by seed treatment with *Pseudomonas putida* and *Azotobacter chroococcum*

Table 1. Effect of seed bacterization of sorghum with Azb19 and P29 on plant growth parameters

Treatments	RV (cc)	SL (cm)	RDM (gm)	SDM (gm)	SPAD reading
Azb19	23.39 ^a (±6.1)	24.35 ^{ab} (±6.2)	1.2 ^a (±0.0)	5.78 ^a (±1.96)	23.23 ^a (±0.25)
P29	19.8 ^b (±3.2)	26.3 ^a (±5.1)	0.8 ^{ab} (±0.0)	6.40 ^a (±2.03)	24.40 ^a (±1.5)
Control	16.01 ^c (±3.7)	20.46 ^b (±3.2)	0.1 ^b (±0.0)	4.09 ^b (±0.09)	23.10 ^a (±0.21)
CV%	20.39	18.27	24.15	16.89	15.57
LSD	1.83	4.90	0.36	0.8	4.13

Values in the brackets are standard errors

Treatments superscribed with same letter do not differ significantly (P<0.05)

Table 2. Nutrient analysis of sorghum plant samples inoculated with Azb19 and P29

Treatments	N%	P%	K%	Fe (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)
Azb19	4.81 ^a (±1.5)	0.87 ^{ab} (±0.4)	1.39 ^a (±0.4)	441.6 ^b (±15.5)	11.6 ^a (±0.0)	115 ^c (±5.4)	256 ^b (±14.5)
P29	3.92 ^b (±0.9)	1.76 ^a (±0.6)	1.54 ^a (±0.3)	537.2 ^a (±23.7)	8.0 ^b (±0.0)	141 ^a (±8.0)	353 ^a (±19.5)
Control	2.94 ^b (±0.8)	0.61 ^b (±0.2)	0.86 ^a (±0.1)	221.3 ^c (±0.3)	13.0 ^a (±0.0)	125 ^b (±6.0)	210 ^{bc} (±12.5)
CV%	23.38	10.84	22.99	15.5	17.05	4.14	1.32
LSD	0.8	1	0.87	90	4.42	9.15	3.28

Values in the brackets are standard errors

Treatments superscribed with same letter do not differ significantly (P<0.05).

Colonization of sorghum rhizosphere by P29

A time-course colonization process of *gfp*-tagged plant growth promoting P29 including bacterial adhesion to root hair surface and the colonization of the intercellular spaces within the cortex of the differentiation zone was recorded using confocal laser scanning microscopy (Fig 3). It was observed that bacteria were attached along the entire root hair surface, mostly at random making it imperative that root hairs were required for the efficient root colonization of sorghum plants. Interestingly, often bacterial cells seemed to adapt themselves to the shape of root hairs orienting the bacterial cells in such a way that an intimate contact between bacterial cells and root hair surfaces was established. Further, often boundary regions of root surfaces were covered with significant portion of bacteria. After rapid colonization of sorghum rhizoplane, *gfp*-tagged P29 was predominantly detected at the root differentiation zone. Observation of primary roots revealed that the segments within one cm distance from plant basal sites were highly colonized by the inoculant. Few bacterial cells were observed within the range of two cm distance from the root tip. In general, the bacterial adhesion was very prominent on the lower root portions as compared to upper root portions.

Colonization of sorghum rhizosphere by Azb19

Root colonization by Azb19 was restricted to surface of rhizoplane (Fig 3). The first site of colonization was the zone of cell elongation at the root tip and tip area of emerging lateral roots where bacteria started to accumulate. *gfp*-tagged cells of Azb19 were not evenly distributed along the root; instead they were found as

micro-colonies located either at the surface of the roots or on root hairs. On the highly colonized segments, a number of micro-colonies have been easily observed around root surfaces.

Plant growth promoting activity of bacterial strains

Seed bacterization of sorghum with P29 and Azb19 enhanced the plant growth significantly. Both the isolates showed plant growth promotion as compared to respective un-inoculated controls. Increase in root volume, shoot length and plant biomass were significantly higher in treated plants as compared to un-inoculated control. Inoculation of sorghum with Azb19 was superior than P29 in terms of increase in root volume (23.39 cm³) and root dry mass (1.2g) (Table 1).

Nutrient uptake by the Plants

There was a significant increase in the uptake of both major- and minor-nutrients when sorghum seeds were bacterized with P29 and Azb19 as compared to controls (Table 2). However, both strains had differentially influenced uptake of different nutrient elements which was reflected selective enhancement of uptake. Inoculation with P29 resulted in increased uptake of P (288.5%), K (179.1%), Fe (242.7%), and Zn (168.1%) as compared to Azb19 where the uptake of P, K, Fe, Mn, and Zn increased by 142.6%, 161.6%, 199.5%, and 121.9%, respectively. On the other hand, inoculation with Azb19 could enhance better uptake of N (163.6%) as compared to P29 (133.3%). Interestingly, uptake of both Mn and Cu appeared to be hindered in both treatments as compared to control (Table 2).

DISCUSSION

Microbe-mediated plant growth promotion has been proved successfully in many crop plants (Stephen 2009). Efficient root colonization and rhizosphere competence are two important parameters in determining the fate of introduced microorganism in the rhizosphere and thereby show its effects on plants through nutrient solubilization and plant growth promotion. For monitoring introduced microorganisms, it is a routine practice to tag these microorganisms with suitable marker gene so that they can be easily detected in the rhizosphere. Transformation of both P29 and Azb19 by electroporation using the plasmid pHc 60 resulted in stable transformants and showed satisfactory levels of *gfp* expression.

It is established that efficient colonization on plant roots is a critical step for PGPR for plant-microbe interactions (Chin-A-Woeng et al., 2000; Lugtenberg et al. 2001; Kamilova et al. 2005; Timmusk et al. 2005; Ongena et al. 2008). Our results have demonstrated surface and internal root hair colonization by Azb19 and P29 in sorghum. Similar observations were made by Pilar et al (2011) based on the investigations on olive roots inoculated with *Pseudomonas* spp. There is a strong aggregation pattern only at specific sites on the roots like root hairs, probably due to variations in root exudation at different places within the root system (Lugtenberg 2001). Number of investigations demonstrated a non-uniform distribution of *Pseudomonas* on plant root: heavily colonized areas are usually found at junctions between epidermal root cells, concave parts of the epidermal surface or sites where side roots appear (Bloemberg et al. 1997; Chin-A-Woeng et al. 1997), all assumed sites of exudation. There are increasing evidences that root exudates play a key role in plant-microbe interactions (Somers et al. 2004). Hiltner 1904 observed the abundance of microorganisms in the rhizosphere, which was later found to be related with root exudation (Hartmann et al. 2008). In agreement with these published results, mode of colonization of *Azotobacter* is small-aggregate, micro colonies and single-cell colonization. Plant growth conditions, including soil type and occurrence of stress factors, also play a part in determining the colonization of plant growth promoting microflora. Soil factors such as soil acidity, soil humidity and soil nutrients like aluminum and potassium levels, etc., influence root hair density, length, physiology and development (Hansen 2005 and R. Lanna Filho). Moreover, under soil drought conditions, some PGPR may stimulate cellular division in roots and increase root hairs (Mattos 2005). This further leads to enhanced water and nutrient uptake by plant roots, especially from deeper soil layers (Compant 2010). Accordingly, it is necessary to be aware of soil factors influencing root hairs that may affect bacteria-root hair interactions and subsequently, plant growth promotion and nutrient management abilities deployed by bacteria. Root colonization is successful if

the introduced bacteria are able to spread and propagate along the root in the presence of competitive indigenous rhizosphere microorganisms. Compared with *Pseudomonas*, however, so far only little was known about the colonization pattern of *Azotobacter*. In the current study, seed bacterization with plant growth promoting bacteria resulted in increased plant height (root volume and shoot height), leaf area and drymass. Similar increases in plant parameters were observed in different crops inoculated with *gfp*-tagged *Azospirillum* in wheat seedlings under salt stress conditions (Macario Bacilio 2004). This improved growth by PGPR is due to efficient root colonization and nutrient solubilization. The present study indicated that bacterial inoculation of sorghum with P29 and Azb19 significantly increased the N and P content in leaves of sorghum. Higher uptake of essential nutrients like phosphorous and zinc compared to un-inoculated control plants could be justified from the fact that the unavailable forms of these nutrients were solubilized and made available in the root region by applying PGPR. Plants which are inoculated with plant growth promoting rhizobacteria usually have higher N-P-K content than that of un-inoculated plants. Results showed that bacterial treated plants showed significant differences in Fe, Cu, Mn and Zn content in Sorghum leaves. The enhancement of macro and micronutrient uptake, except Cu and Mn by inoculation with PGPR may be due to efficient root colonization which has impact on initiation and development of lateral roots and increased root weight (Mattos 2008). The availability of Mn to plants is governed by oxidation and reduction processes (Marschner 1988). Decrease in Mn uptake in sorghum on inoculation with *Azotobacter* in this study could be attributed to their possible Mn oxidizing and reducing activities (Marschner 2003). Similarly lower uptake of Mn by mycorrhizal plants has been found by Pacovsky (1986) and Kothari et al (1991). The relative importance of root exudates and microorganisms in oxidation and reduction processes is still not clear.

CONCLUSION

In summary, genetically tagged strains of *Pseudomonas* and *Azotobacter* were capable of colonizing the roots of sorghum. Fluorescently tagged bacteria, CLSM and sorghum plants provided a powerful approach to study sorghum–bacteria interaction. The importance of efficient Sorghum root colonization by plant growth promoting *Azotobacter*19 and *Pseudomonas*29 has clearly demonstrated that colonization is the key factor for nutrient uptake and plant growth promotion.

REFERENCES

Behl RK, Sharma H, Kumar V, Narula N (2003). Interactions amongst mycorrhiza, *Azotobacter*

- chroococcum* and root characteristics of wheat varieties. *J Agron Crop Sci.* 198: 151–155.
- Bloemberg GV, Toole GA, Lugtenberg BJ, Kolter R, Chin-A-Woeng TF, Mulders IH, Dekkers LC, Lugtenberg BJ (2000). Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. *Mol. Plant-Microbe Interact.* 13: 1340–1345.
- Buddrus-schiemann K, Schmid M, Schreiner K, Welzl G, Hartmann A (2010). Root colonization by *Pseudomonas* sp. DSMZ 13134 and impact on the indigenous rhizosphere bacterial community of barley. *Plant-microbe interactions Microbial Ecol.* 60:381-393.
- Chin-A-Woeng TF, de Priester W, Van der Bij AJ, Lugtenberg BJ (1997). Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. *Mol.Plant-Microbe Interact.*10:79–86.
- Compant S, van der Heijden MGA, Sessitsch A (2010). Climate change effects on beneficial plant–microorganism interactions. *FEMS Microbiol Ecol.* 73:197–210.
- Egamberdiyeva D (2007). “The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils.” *Applied Soil Ecology*”. 36:184-189.
- de Weert S, Dekkers LC, Kuiper I, Bloemberg GV, Lugtenberg BJJ (2004). Generation of enhanced competitive root-tip colonizing *Pseudomonas* bacteria through accelerated evolution. *J Bacteriol.* 186:3153–3159.
- Desai S, Praveen Kumar G, Sultana U (2012). Potential microbial candidate strains for management of nutrient requirements of crops. *African Journal of Microbiology Research.* 6:3924–3931.
- El-Hawary MI, El-Hawary F, El-Ghamry AM, El-Naggar (2002). Effect of application of biofertilizer on the yield and NPK uptake of some wheat genotypes as affected by the biological properties of soil. *Pakistan Journal of Biological Sciences* 5:1181–1185.
- Fan B, Xiao Hua, Chena XH, Budiharjoa A, Bleiss W, Vaterc J, Borrissa R (2011). Efficient colonization of plant roots by the plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42, engineered to express green fluorescent protein. *Journal of Biotechnology.* 151:303–311.
- Haling RE, Richardson AE, Culvenor RA, Lambers H, Simpson RJ (2010). Root morphology root-hair development and rhizo sheath formation on perennial grass seedlings is influenced by soil acidity. *Plant Soil.* 335:457–468.
- Hansen M, Kragelund L, Nybroe O, Sørensen J(1997). Early colonization of barley roots by *Pseudomonas fluorescens* studied by immunofluorescence technique and confocal laser scanning microscopy. *FEMS Microbiol Ecol* 23:353–360.
- Hartmann AM, Rothballer, Schmid M (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *plant and soil* 312:7-14.
- Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environ. Microbiol.*7:1809–1817.
- Kloepper JW, Ryu CM, Zhang S (2004). Induced Systemic Resistance and Promotion of Plant Growth by *Bacillus* spp. *Phytopathology* 94:1259-66.
- Kothari Sk, Marschner H, Romheld V (1991a). Effect of a vesicular arbuscular mycorrhizal fungus and rhizosphere microorganisms on manganese reduction in the rhizosphere and manganese concentration in maize (*Zea mays* L.). *New Phytol* 117: 649-655.
- Krzyzanowska D, Obuchowski M, Bikowski M, Rychlowski M, Sylwia Jafra S (2012). Colonization of potato rhizosphere by GFP-tagged *Bacillus subtilis* MB73/2, *Pseudomonas* sp. P482 and *Ochrobactrum* sp. A44 shown on large sections of roots using enrichment sample preparation and confocal laser scanning microscopy *Sensors:* 1217608-17619.
- Lugtenberg BJJ, Kamilova F (2009). Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol* 63:541–556.
- Lugtenberg BJJ, Dekkers L, Bloemberg GV. (2001). Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol* 39:461–490.
- Macario Bacilio, Hilda Rodriguez, Manuel Moreno, Juan-Pablo Hernandez, Yoav Bashan (2004). Mitigation of salt stress in wheat seedlings by a gfp-tagged *Azospirillum lipoferum* *Biol Fertil Soils* 40:188–193.
- Marschner PQ, Fu, and Rengel Z (2003). Manganese availability and microbial populations in the rhizosphere of wheat genotypes differing in tolerance to Mn deficiency. *J. Plant Nutr. Soil Sci.* 166: 712-718.
- Mattos KA, Pádua VLM, Romeiro A, Hallack LF, Neve BC, Ulisses TMU, Barros CF, Todeschini AR, Previato JO, Mendonça-Previato L (2008). Endophytic colonization of rice (*Oryza sativa* L.) by the diazotrophic bacterium *Burkholderia kururiensis* and its ability to enhance plant growth. *Annais AcadBras. Ciencias.* 80: 477- 493.
- Michiels K, Vanderleyden J, Gool V (1989). *Azospirillum*–plant root associations: review. *Biol Fertil Soils.* 8:356–368.
- Mohd Mazid and Taqi Ahmed Khan Future of Biofertilizers in Indian Agriculture: An over view. *International journal of Agricultural and Food Research.* 3:10-23.
- Ongena M, Jacques P (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends Microbiol.* 16:115–125.
- Pacovsky RS (1986). Micronutrient uptake and distribution in mycorrhizal or phosphorus fertilized soybeans. *Plant and Soil.* 95:379-388.
- Pilar P, Elisabetta S, María M, Maldonado-González, Raquel

- V, Juan B, Barroso-Albarracín, Jesús M (2011). Root Hairs Play a Key Role in the Endophytic Colonization of Olive Roots by *Pseudomonas* spp. with Biocontrol Activity *Microb Ecol.* 62:435–445.
- Lanna Filho R, de Souza RM, Ferreira A, Quecine MC, Eduardo Alves J.L, de Azevedo Biocontrol activity of *Bacillus* against a GFP-marked *Pseudomonas syringae* pv. tomato on tomato phylloplane. Australian Plant Pathology Society Inc. DOI 10.1007/s13313-013-0233-z.
- Riccardo Tombolini, Dirk Jan van der Gaag, Berndt Gerhardson, Janet K. Jansson (1999). Colonization Pattern of the Biocontrol Fluorescent Protein on Barley Seeds Visualized by Using Green Strain *Pseudomonas chlororaphis* MA 342. *Appl. Environ. Microbiol.* 65:3674.
- Rodríguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances.* 17:319–339.
- Römheld V, Kirkby EA (2010). Research on potassium in agriculture: needs and prospects. *Plant Soil.* 335:155–180.
- Olsen SR, Cole C.V, Watanabe FS, Dean LA. (1954). "Estimation of available phosphorus in soils by extraction with sodium bicarbonate." *United States Department of Agriculture Circular.*93:1–19.
- Shaukat K, Affrasayab S, Hasnain S (2010). Growth responses of *Triticum aestivum* to plant growth promoting rhizobacteria used as a bio fertilizer. *Research Journal of Microbiology.* 5:1022–1030.
- Singh B, Natesan SKA, Singh BK, Usha K (2005). Improving zinc efficiency of cereals under zinc deficiency. *Current Science.*88:36–44.
- Somers E, Vanderleyden J, et al (2004). "Rhizosphere bacterial signalling: a love parade beneath our feet." *Crit Rev Microbiol.* 30: 20-40.
- Stephen Cummings P (2009). The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. *Environmental Biotechnology.* 2:43-50.
- Tandon HLS (2001). Methods of analysis of soils, plants, water and fertilizers. Fertilizer development and consultation organisation. New Delhi. India. 144.
- Tanimoto E, Fujii S, Yamamoto R, Inanaga S (2000). Measurement of visco elastic properties of root cell walls affected by low pH in lateral roots of *Pisum sativum* L. *Plant and Soil* vol. 226:21–28.
- Timmusk S, Grantcharova N, Wagner GH (2005). *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Appl. Environ. Microbiol.* 71:7292–7300.
- Vaculik M, Lux A, Luxova M, Tanimoto E, Lichtscheidl I (2009). Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany* vol 67:52–58.
- Xue GP, Johnson JS, Dalrymple BP (1999). High osmolarity improves the electro transformation efficiency of the gram-positive bacteria *Bacillus subtilis* and *Bacillus licheniformis*. *J. Microbiol. Methods* 34: 183–191.

Accepted 02 August, 2016.

Citation: Sultana U, Desai S, Reddy G (2016). Successful colonization of roots and Plant growth promotion of sorghum (*Sorghum bicolor* L.) by seed treatment with *Pseudomonas putida* and *Azotobacter chroococcum*. *World Journal of Microbiology*, 3(1): 043-049.



Copyright: © 2016 Sultana et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are cited.