



## Influence of plant growth promoting bacterial inoculants on soil nutrient status and health of the maize

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(Received: August 19, 2015; Revised received: March 02, 2016; Accepted: March 06, 2016)

**Abstract:** In this context first lab experiment was conducted in the year of 2013-14 at the Dept. of Agricultural Microbiology & bioenergy for efficient plant growth promoting bacterial screening and inoculants preparation. In this context sixteen bacterial isolates collected from different resource laboratories of Andhra Pradesh and screening for PGP properties such as P-solubilization IAA production, biocontrol activity. The efficient isolates of plant growth promoting properties shown (PGP-1(*Pseudomonas* spp.), PGP-9(*Azotobacter* spp), PGP-15(*Azospirillum* spp.) were selected for biofertilizer preparation and application in maize crop as carrier based bioinoculants with chemical fertilizers. Field experiment was conducted at college farm, college of agriculture for *Rabi* season of 2013-14 to find out most efficient and economic combination of different PGP bioinoculants and inorganic chemical fertilizer sources in increase the yield of hybrid maize without disturbing the soil properties. This experiment finally resulted the improved nutrient status of soil in respect of available N, available P and K compared to organic carbon compound to initial nutrient status. Maize yield was increased over control.

**Key words:** Plant Growth Promoting Rhizobacteria (PGPR), Inorganic fertilizers, Maize, Nutrient management

### Introduction

Maize or corn (*Zea mays*) is a plant belonging to the family of grasses (Poaceae). It is cultivated globally being one of the most important cereal crops worldwide. Maize is not only an important human nutrient, but also a basic element of animal feed and raw material for manufacture of many industrial products. The products include corn starch, maltodextrins, corn oil, corn syrup and products of fermentation and distillation industries. It is also being recently used as biofuel. *Zea mays* is a plant with food and energy value (Solomon *et al.*, 2007) and also with phytoremediation potential (Lin *et al.*, 2008; Meers *et al.*, 2010). Maize is the third most important food grain in India after wheat and rice. In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed.

PGPR have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized. PGPRs are the potential tools for sustainable agriculture and trend for the future. The enhancement of crop plant growth using PGPR is documented (Reed and Glick, 2004) and these organisms have been used to reduce plant stress associated with phytoremediation strategies for metal contaminated soils (Reed and Glick, 2005). Plant Growth Promoting Rhizobacteria (PGPR) is able to exert a positive effect leading plant growth. These beneficial effects of PGPR have direct or indirect performance on plants. Direct promotion of growth by PGPR including production of metabolites that enhances plant growth such as auxins (Ali *et al.*,

2010), cytokinins, gibberellins and through the solubilization of phosphate minerals. Indirect growth promotion occurs via the removal of pathogens by the production of secondary metabolites such as hydrogen cyanide and siderophores (Dastager *et al.*, 2011). In this regard, the most successful relationships studied are those between symbiotic Rhizobiaceae sp. with legumes, and those of free-living PGPR genera such as *Pseudomonas*, *Bacillus*, *Azotobacter*, and *Azospirillum*, which colonize the rhizosphere or root tissues of grasses or non legumes.

Pure cultures of Plant growth promoting rhizobacterial isolates collected from different resource laboratories of Andhra Pradesh. Attempts were also made to assess the screening and characterization of those isolates with multiple beneficial properties like biocontrol potential against plant pathogens, nitrogen fixation, mineral phosphate solubilization and production of plant growth promoting substances. The efficient PGPR isolates were selected for PGPR microbial inoculants and their influence on maize crop yield attributes. Among sixteen isolates, three efficient isolates selected based on multiple PGPR characters, biocontrol activity. For field experiment of maize crop, the selected isolates *Azotobacter* sp free-living nitrogen fixer, *Azospirillum* associative nitrogen fixer and phosphate solubilizing *Pseudomonas* spp.

### Materials and Methods

Pure cultures of Plant growth promoting rhizobacterial isolates collected from different resource laboratories of Andhra Pradesh and attempts were made to assess the screening and characterization of collected sixteen bacterial isolates with multiple

beneficial properties like nitrogen fixation, p-solubilization (Pikovskaya, 1948), production of plant growth promoting substances (Duby and Maheswari 2012) and biocontrol activity (Schwyn and Neilands, 1987) (Castric and Castric, 1983), (Skidmore and Dickinson, 1976). The efficient PGPR isolates were selected for PGPR microbial inoculants and their influence on maize crop yield attributes (table-1).

**Field experiment:** A field experiment was conducted to investigate the effect of efficient free living nitrogen fixers and phosphate solubilizing biofertilizers on yield attributes of maize crop. The field experiment was carried out in the year of 2013-14 *rabi* season at College Farm, College of Agriculture, Rajendranagar, Hyderabad. The soil of the experimental site was red clay loam with soil reaction (pH) about 7.9, electrical conductivity about 0.34 ds m<sup>-1</sup>, organic carbon 0.39 %, available nitrogen about 232.7 kg Nha<sup>-1</sup>, available phosphorus about 25.9 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Available potassium about 272.5 kg K<sub>2</sub>O ha<sup>-1</sup>. Viable population of bacteria, fungi, actinomycetes, *Rhizobium* and *Pseudomonas* was analyzed by the standard serial dilution plate count method, microbial group bacteria: 7.50 × 10<sup>3</sup> CFU g<sup>-1</sup> of soil, Fungi: 4.33 × 10<sup>3</sup> CFU g<sup>-1</sup> of soil, *Azotobacter*: 3.51 × 10<sup>3</sup> CFU g<sup>-1</sup> of soil, *Rhizobium*: 3.85 × 10<sup>3</sup> CFU g<sup>-1</sup> of soil, phosphate solubilizers :4.71 × 10<sup>4</sup> CFU g<sup>-1</sup> of soil). Treatments with efficient PGPR inoculants (PGP-1, PGP-9, PGP-15) were T1: 100 % RDF (Irrigated crop - N2: P<sub>2</sub>O<sub>5</sub>: K<sub>2</sub>O ratio: 180kg: 50kg :60kg ha<sup>-1</sup>) T2: 75 % RDN + Nitrogen fixer *Azospirillum*, T3: 75 % RDN+ Nitrogen fixer *Azotobacter*, T4: 75 % RDP + Phosphate solubilizers, T5: 75 % RDN and P (Recommended dose of Nitrogen and Phosphorus) + Nitrogen fixer *Azospirillum* + Phosphate solubilizers, T6: 75 % RDN and P + Nitrogen fixer *Azotobacter* + Phosphate solubilizers, T7: 75 % RDN and P+ *Azotobacter* + *Azospirillum* + Phosphate solubilizers. When applying *Azotobacter*, *Azospirillum* inoculum, applied 75% RDF of N and P, K is 100% RDF. When applying phosphate solubilizers inoculum, apply 75% RDF of P was applied and N and K were applied 100% RDF. Plot size: 4.5 m×4.3 m, Spacing: 60 cm x 20 cm. Experimental design with randomized block design.

The fertilizers were applied as per the treatments combinations. The crop was supplied with recommended dose of fertilizers. Nitrogen @180 Kg ha<sup>-1</sup>, phosphorus @ 50 Kg ha<sup>-1</sup> and potassium @60 Kg ha<sup>-1</sup> in the form of single super Phosphate, urea and muriate of potash, respectively were adopted. Chemical fertilizers were applied in and 75% reduced amount of nitrogen and phosphatic fertilizers. Entire dose of P and K was applied as basal at the time of sowing. Nitrogen was applied through urea in three split

applications and free living *Azotobacter* sp, *Azospirillum* sp, Phosphate solubilizing bacteria (PSB) of efficient PGPR carrier based microbial inoculants along with FYM were incorporated in soil. After harvesting of the crop, check the nutrient status such as available N, P, K, organic carbon and pH of the soil.

### Results and Discussion

Among sixteen bacterial isolates PGP-1(*Pseudomonas* spp.), PGP-9(*Azotobacter* spp), PGP-15 (*Azospirillum* spp.) were shown best multiple beneficial plant growth promoting activities (Table-2). These were selected for field experiment of maize crop as carrier based PGPR inoculants along with chemical fertilizers. Different treatments found to be effective significantly pH of the soil observed in the application of PGPR Biofertilizers. The decrease in the pH over initial value is ore in treatments with chemical and biofertilizer than control plots. Application of *Azotobacter*, *Azospirillum* and PSB as biofertilizers are responsible for decreasing soil pH with producing organic acids. The organic carbon content also improved in all treatments over control plots.

**Effect on soil nutrient status after harvest:** The available major nutrients viz., nitrogen, phosphorus and potassium contents in soil as influenced by application of different combinations of microbial inoculants and chemical fertilizers were presented in the table-2. Lowest available nitrogen at the time of harvest which was recorded in T1 (228.63 kg ha<sup>-1</sup>) control plot (100% recommended dose of chemical fertilizer). The available nitrogen is highest in T7, which is combinational application of *Azospirillum*, *Azotobacter*, PSB with 75% chemical fertilizers dose. There is a progressive increase in the balance with decrease in yield as registered in various treatments. Nitrogen fixers were enhancing the available nitrogen content in the experiment field plots.

Similarly phosphorus available in the soil at the time of harvest varied from 23.9 kg ha<sup>-1</sup>) control and the highest (36.33 kg ha<sup>-1</sup>) in T<sub>4</sub> (T4: 75 % Recommended Dose of Phosphatic chemical fertilizer + Phosphate solubilizers). The available phosphorus increased highest in T4 with PSB microbial inoculants application. Lowest available phosphorus recorded in control T1 (100% recommended dose of chemical fertilizer) (23.9kg ha<sup>-1</sup>) compared to all treatments. Similar views were expressed by (Yazdan *et al.*, 2009), who demonstrated that use of growth stimulating bacteria and phosphate solute in combination with chemical fertilizer was able to reduce phosphorus fertilizer application by 50% without of reduction in corn yield. Higher phosphorus content may be due to inoculation and availability of phosphorus in soil by microbes. Biofertilizer enhances soil fertility by solubilizing unavailable sources

**Table-1:** Multiple PGP characteristics of efficient PGP isolates

Efficient isolates	Plant growth promoting characters				Biocontrol activity			
	p-solubi- lization (PSE%)	Ammonia production	Nitrogen fixation (nmol C <sub>2</sub> H <sub>4</sub> /mg protein/h)	IAA production (µg/ml)	HCN production	Siderophore production	Antifungal activity <i>S.rolfsi</i> <i>R.solani</i>	
PGP-1( <i>Psudimonas</i> spp)	500	+++	-	7.41	+++	+++	4.00	1.00
PGP-9( <i>Azotobacter</i> spp)	190.9	++	8.55	5.98	+	+	3.00	1.00
PGP-15( <i>Azospirillum</i> spp)	138.4	++	9.25	7.76	+	++	1.00	2.00

+ Weak production; ++ Moderate production; +++ Strong production; IAA indole acetic acid production; HCN hydrogen cyanide production

**Table-2:** Influence of PGPR microbial inoculants and chemical fertilizers on the available soil N, P and K after harvesting of Maize crop

Treat-ments	Available Nitrogen (Kg ha <sup>-1</sup> )	Available Phosphorus (Kg ha <sup>-1</sup> )	Available Potassium (Kg ha <sup>-1</sup> )	Organic carbon	pH of the soil
T1	228.63	23.9	276.36	0.37	8.2
T2	239.63	24.03	276.244	0.55	7.6
T3	242.20	25.50	264.30	0.61	7.4
T4	240.90	36.33	266.05	0.57	7.4
T5	247.60	28.366	264.04	0.61	7.4
T6	236.93	28.30	261.60	0.62	7.4
T7	244.13	31.06	275.28	0.64	7.3
SE(m)	1.887	1.034	11.89	0.031	0.058
CD(0.05%)	5.814	3.186	37.06	0.097	0.180

**Table-3:** Influence of PGPR inoculants on kernel yield of Maize

Treatments	Grain yield (kg.ha <sup>-1</sup> )	Stover yield(kg.ha <sup>-1</sup> )
T <sub>1</sub>	2653.33	4100.00
T <sub>2</sub>	2750.00	4133.33
T <sub>3</sub>	2676.66	3540.00
T <sub>4</sub>	2683.33	4183.33
T <sub>5</sub>	2820.00	4276.66
T <sub>6</sub>	2866.66	4272.00
T <sub>7</sub>	3366.66	4530.00
CD (P=0.05%)	6.723	4.424
SED	155.422	203.071
SE(m)	109.901	143.59

**Table-4:** Influence of microbial inoculants and chemical fertilizers on the economics of maize

Treatments	Gross returns Rs ha <sup>-1</sup>	NET returns Rs ha <sup>-1</sup>	Cost of cultivation Rs	B:C Ratio
T1	34493.29	17,993.29	17,000	1.02
T2	35750.00	20750.00	15000	1.38
T3	34796.58	19796.58	15000	1.31
T4	34883.29	18883.29	16000	1.20
T5	36660.00	20660.00	16000	1.30
T6	37266.58	21266.58	16000	1.32
T7	43766.58	27266.58	16500	1.65

of bound phosphate into available forms in order to facilitate the plant to absorb them. The root system of legumes has capacity to solubilize soil phosphorous through the excretion of amino acids and encourage the growth and multiplication of phosphate solubilising bacteria. Soil available potassium differed significantly as influenced by application of microbial inoculants and chemical fertilizers with 75% RDF application in different combinations after harvest of Maize crop. The basal potassium level in the soil was 235 kg ha<sup>-1</sup>. Lowest potassium available was (274.1 kg ha<sup>-1</sup>) in control T1 (100% RDF) without microbial inoculants application. Highest available potassium was recorded in treatment T7 (75% RDN& P + *Azospirillum* + *Azotobacter* + PSB+ FYM) by (Nagaraju *et al.*, 2009), reported similar results.

Han and Lee (2005) also observed increase in N, P and K concentration under salinity stress due to inoculation with PGPR. Similarly, Vivas *et al.* (2003) reported that N, P and K concentration in lettuce inoculated by *Bacillus sp.* under stress conditions were increased by about 5, 70 and 50 %, respectively, over control.

Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPR were reported by other workers (Gravel *et al.*, 2007), (Kozdroja *et al.*, 2004), (Shaharoon *et al.*, 2006). Inoculation of maize seeds with *Azospirillum* strains compared with *Pseudomonas* strains under experiment conditions resulted in a more visible increase in shoot development, especially during the establishment of the plant. Khalid *et al.* (2004) showed that responses of wheat growth to inoculation with rhizobacteria depend on plant genotype and PGPR strains as well as environmental conditions.

**Microbial population dynamics (log CFU g<sup>-1</sup> of dry soil):** Fluctuations in the microbial community in the maize rhizosphere, due to bacterial inoculations was recorded. Data interpreted with two factorial RBD.

**Bacterial population at different growth stages of crop (log10<sup>6</sup> CFU g<sup>-1</sup> of dry soil):** An increasing trend in bacterial population was observed till 70 DAS of crop growth. Highest population was observed in the treatment of 75% RDN and P + *Azospirillum* + *Azotobacter* + PSB (T7) at 70 DAS. Bacterial population was increased at flowering stage due to release of root exudates in the rhizosphere of the plant. Total bacterial count was significantly more in all inoculated treatments over T1 (100% RDF). A decline in population was observed at harvesting stage in all the treatments.

*Azotobacter* population increased upto 70DAS of crop growth in all the treatments and maximum population was observed in T6 at 70 DAS followed by T7 (75 % RDN and P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers), T3 (75 % RDN+ Nitrogen fixer *Azotobacter*) and T5 (75 % RDN & P + Nitrogen fixer *Azospirillum* + Phosphate solubilizers) were on par. *Azotobacter* population was increased in T1 (100% RDF) also because of the nature of the host plant, it being cereal crop. In the *Azospirillum* microbial population gradually increased in all treatments till 70DAS irrespective of treatments, but maximum population was recorded in T7 (75 % RDN and P + *Azotobacter* + *Azospirillum* + Phosphate solubilizers) and T2 (% RDN + Nitrogen fixer *Azospirillum*) where these two received inoculation of *Azospirillum*. Similarly the retention of population at harvesting stage was also higher in T2 and T7. These results indicate that inoculation with *Azospirillum brasilense* may contribute to maize yield increase, particularly under the most difficult cultivation conditions, when plant access to mineral nitrogen in soil is restricted.

PSB population recorded an increasing trend in all the treatments upto 70DAS of crop growth. Population showed inclination towards harvest in all treatments except in T4 (75 % RDP + Phosphate solubilizers). In case of T4, phosphate solubilizing bacteria alone inoculated with 25% reduced chemical phosphatic fertilizer could support the significantly high population of PSB up to harvest. The current finding concerning earlier response of soil microbial population to mixed treatment was also reported by (Balakrishnan *et al.*, 2007). Available phosphorus was highest in available phosphorus in T4 with 36.66 kg.ha<sup>-1</sup>. Similar kinds of results were also reported by Ponnuragan and Gopi (2006) they found that phosphate solubilizing bacteria (PSB) isolated from the rhizosphere of different field crops including maize, were capable of producing auxin under *in vitro* conditions.

The rhizobial population data reveals that at 0 DAS, across all the treatments, in the rhizosphere of maize *Rhizobium* population has been increased upto 70DAS and same was maintained till harvest. Increase may be due to root exudates and soil nutrients in the rhizosphere of maize. Retained population at harvesting stage, maximum in T4 followed by T3 (75 % RDN+ Nitrogen fixer *Azotobacter*). The T3 and T4 treatments were supplemented with respective *Azotobacter* and phosphate solubilizing bacteria where in these PGPR isolates were known to produce various vitamins, siderophores, IAA. All these cofactors might have supported the saprophytic growth of *Rhizobium*. The fungal populations results show that, the application of PGPR inoculants suppress the soil fungal population from 0DAS to 70 DAS. At harvesting stage where the decrease in population of PGPR isolates with raise in the fungal population was observed. This indicates that the PGPR microbial inoculants were suppressing the fungal population.

**Grain yield (kg ha<sup>-1</sup>):** The grain yield significantly influenced by treatments of combination of chemical and microbial inoculants. The grain yield varied from 2653.33kg ha<sup>-1</sup> to 3366 kg ha<sup>-1</sup> depicted in table .3 The highest grain yield (3366 kg ha<sup>-1</sup>) was recorded in treatment T7 (75 % RDN and P + *Azospirillum* + *Azotobacter* + PSB), followed by T6, T5. In contrast lowest grain yield (2653.00kg ha<sup>-1</sup>) was in treatment T1 (100%RDF). The enhancing effect of seed inoculation with rhizobacteria on shoot dry weight and yield of maize were reported by many researchers (Pandy et al., 1998), (Shaharoon et al., 2006). Such an improvement might be attributed to N<sub>2</sub>-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances (Salantur et al., 2006).

**Economics:** The data pertaining to the economics of maize crop as influenced by application of PGPR microbial inoculants and 75% reduced chemical fertilizers in different combinations were presented in the table-4. The highest net return (27,266.58 Rs. ha<sup>-1</sup>) with high B:C ratio (1.65) was recorded in the treatment supplied with 75 % RDN & P+ *Azospirillum* + *Azotobacter* + PSB. Further it was followed by treatment supplied with 75 % RDN and P + *Azospirillum* + *Azotobacter* + PSB (T7) (21,266.58 Rs ha<sup>-1</sup>) and with 75 % RDN+ *Azospirillum* (20,750 Rs. ha<sup>-1</sup>) and lowest net returns in treatment control T1 with 100% RDF (17,993.29 Rs ha<sup>-1</sup>). The results show that the combination of PGPR inoculants and 75% reduced chemical fertilizers will increase the net returns over 100% RDF without inoculation of microbial inoculants. Among all treatments T7 (75 % RDN and P+ *Azospirillum* + *Azotobacter* + PSB) improved yield better than all other treatments because of co inoculation of three microbial inoculants which could have improved the soil fertility and nutrient status, inhibition of the growth of phytopathogens, Production of growth promoting substances etc.

In the present study application of microbial inoculants with different combinations were improved the soil nutrient status of the maize and microbial populations leading to improved soil health and also crop growth and yield were improved than without microbial inoculants treated plots. So that using the microbial inoculants in crop improvement methods leading to reduce the use of chemical fertilizers and cost of cultivation also.

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