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Effects of Rhizobacterial Bioinoculants on the Maximization of Plant Growth Promoting Characteristics in Maize (*Zea Mays* L.)

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A B S T R A C T

Among a total of 30 cultivable rhizobacteria isolated from Maize (*Zea mays* L.) grown in tropical areas. Two isolates were evaluated and screened by using different plant growth promoting (PGP) traits. Based on the above studied parameters, rhizobacterial isolates AUPF25 and AUBM29 were selected for investigation and identified as *Pseudomonas fluorescens* and *Bacillus megaterium* by 16S rRNA gene sequencing. With the ability of this isolates to assess the impact of bioinoculants application on Maize (*Zea mays* L.) plant productivity was studied under pot culture conditions. Understanding the growth performances of maize plants inoculated with bacterial biofertilizers under pot culture conditions is a key to improve crop production further in field (15-20%). Maize plants with 4 different combinations of bacterial bioinoculants were applied as single and dual combinations. The experimental plants were maintained based on completely randomized block design (CRBD). Both biotic and abiotic factors that influence the plant growth including soil nutrient status, plant hypertrophy, phytochemical composition, bacterial population density were taken into consideration and those were analyzed. Among the bioinoculants combinations applied, dual combinations worked out better than the single inoculation. The treatment such as T_4 (*P. fluorescens* and *B. megaterium*) was concluded as best combinations of bioinoculants and those were also found as compatible.

Introduction

The high usage of agrochemicals has made soil infertile, accumulation of toxic chemicals in the soil and food products and imbalanced nutrient cycling and ecosystem also occur. In order to maximize the agricultural productivity with minimum soil loss, a cheap, better and safest way is necessary. All these criteria can be achieved through application of microbial bioinoculants. Because, these micro-organisms are known to possess vast range of capabilities by producing growth promoting substances, enhancing the plant nutrients, biological N2 fixation and crop

protection against stress and diseased conditions. These PGPR have been shown to cause very real and positive effects when matched correctly to the right plant and the right environmental situation (Glick *et al*., 1999; Kloepper *et al*., 1989).

PGPR have been reported to be the key elements for plant establishment under nutrient imbalance conditions and their use in agriculture can favour a reduction in the use of synthetic chemical fertilizers and agrochemicals and support eco-friendly crop production (Glick, 1995).

Maize (*Zea mays* L.) is the third major crop of the world after wheat and rice which provides more nutrients for humans and animals than any other cereals and the same is grown in many countries, including Zambia. The positive effects of Maize-PGPR interaction have been reported by Gholami
et al., 2009. PGPR strains may be plant-specific, cultivar specific or non-specific for maize root colonization (Babalola *et al*., 2003) and after colonization, PGPR strains interact with host plant for the improvement of plant growth and induce defense mechanisms against phytopathogens.

The bacterial bioinoculants such as *P. fluorescens* AUPF25 and *B. megaterium* AUBM29 were chosen for the study was based on the metabolic activities they carry out. An important goal of nursery experiments is to produce consistence results of target morphometric, biochemical and microbiological characteristics with an input of microbial bioinoculants on maize growth and identification of nursery cultural treatments that promote these characteristics should help to improve field cultivation success (Jacobs *et al.,* 2003). If a positive effect of microbial biofertilizers is seen on a specific crop in nursery studies, there is a strong likelihood that those benefits will carry through to field conditions (Lusy *et al.,* 2004).

Hence, the present study has been undertaken with an aim to exploit the comparative performance of *P. fluorescens* AUPF25 and *B. megaterium* AUBM29 on the enhancement of plant growth promoting attributes and biocontrol of leaf blight disease in Maize.

Materials and Methods

Culture used

The plant growth promoting rhizobacteria such as *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were isolated from rhizosphere soil (Boominathan *et al*., 2012) and the Nucleotide Sequence (16S rRNA) of this isolates were deposited to NCBI Gene bank under the accession numbers.

Strain Name	**Accession No**	**Genus and species**
AUPF25	JQ 638587	*Pseudomonas fluorescens*
AUBM29	JN 990602	*Bacillus megaterium*

The *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) isolates were positive for their PGP traits, maintained in King's B medium (King *et al*., 1954) and Nutrient Glucose agar (Englesberg and Ingraham, 1957) slants respectively and incubated at 30 ± 2 °C with monthly transfer.

Comparative performance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) strains on growth promoting characteristics. Preparations of inoculum by using the above isolates were grown in King´s B broth and

nutrient glucose broth respectively in a shaking incubator at 30 ± 2 °C for 24 h. Then, the media were centrifuged separately at 5000 × g for 10 min to harvest the log phase cells of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) isolates. Then, the respective pellets were washed three times with 0.1 M phosphate buffer (pH 6.8) individually. Finally, the cells of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were resuspended separately in the same buffer at a cell concentration of 1×10^7 CFU ml^{-1} by measuring the OD at 420 nm for *P. fluorescens* and 540 nm for *B. megaterium***.**

The comparative performance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) on different PGPR characteristics *viz*., IAA, siderophore production, 'P' solubilization, adhesion to maize roots, EPS production, thermal and desiccation tolerance were studied under *in vitro* condition.

IAA Production and Estimation

The IAA production by the *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were carried out in King's B and Nutrient glucose broth respectively together amended with 100 mg/L of DL-Tryptophan and the extraction and estimation of IAA were done according to the Tien *et al*. (1979).

Siderophore production and estimation

The 2,3 dihydroxy benzoic acid and Salicylic acid of siderophore production by the *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were estimated according to Modi *et al*. (1985) and Gibson and Magrath (1969) respectively.

Phosphate Solubilizing Efficiency

The phosphate solubilizing efficiency of the *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were carried out in Pikovskaya (PVK) broth according to the procedure of Olsen and Sommers (1982).

Exopolysaccharide (EPS) Production

The EPS productions of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were estimated as per the procedure of Englesberg and Ingraham (1957).

Adsorption Assay

Adsorption Assay of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were estimated as per the procedure of Gafni *et al*. (1986).

Thermal and Desiccation Tolerance

Thermal and desiccation tolerance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) were described by Gafni *et al*. (1986).

Results and Disucssion

Totally 30 rhizobacterial isolates were obtained from maize rhizosphere soil. But for the present study, only two effective isolates were selected based on their plant growth promoting traits. The comparative performance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) isolates on the enhancement of different PGPR characteristics *viz*., IAA, siderophore production, 'P' solubilization, adhesion to maize roots and thermal and desiccation tolerance was investigated (Table 1).

Table.1 Comparative performance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) on different plant growth promoting characteristics

Isolate*	IAA production (µg/ml)+	Siderophore production (µg/ml)++		'P' solubilisation efficiency (µg/ml)**	EPS production (g/ml)		Thermal tolerance (No. of viable cells/ ml)	Desiccation tolerance (No. of viable cells/ after l week incubation) b,c
		2,3-DHBA	Salicylic acid		Water soluble polysaccharide	Alkali stable polysaccharide		
AUPF25	5.2±0.46[a]	4.3± 0.30[a]	3.8±0.27[a]	-	0.21±0.56[b]	6.80±0.42[b]	6.84±0.18[b]	7.04±0.16[a]
AUBM29	4.8±0.16[b]	4.1± 0.22[b]	3.4±0.27[b]	186±0.86[a]	0.24±0.56[a]	7.12±0.42[a]	6.94±0.18[a]	6.20±0.16[b]

a,b - Values are mean of three replication ± SD.
* - at 1x 10^7 CFU/ml inoculum level.
+ - Estimation of Indole acetic acid according to Tien *et al*. (1979).
+ + - Estimation of Siderophore production according to Modi *et al.* (1985) and Gibson and Magrath (1969).
** - Estimation of Phosphate solubilization according to Olsen and Sommers (1982).
2,3 DHBA - 2,3 dihydroxy benzoic acid.

Table.2 Impact of rhizobacterial bioinoculants on growth and yield of maize under pot trials

Treatment	Plant height (cm)	Root dry weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Grain yield (t ha^{-1})	Stalk yield (t ha^{-1})	Cob yield (g)
T_1	57.42[d]	0.26[c]	1.48[c]	2.38[bc]	2.43[d]	42.76[d]
T_2	63.20[b]	0.32[b]	1.52[b]	2.62[c]	2.88[b]	56.22[c]
T_3	62.48[c]	0.33[b]	1.50[c]	2.68[b]	2.76[c]	57.10[b]
T_4	66.86[a]	0.35[a]	1.63[a]	2.72[a]	3.26[a]	60.29[a]

Note: T_1- Control; T_2- *P. fluorescens*; T_3- *B. megaterium*; T_4- *P. fluorescens* + *B. megaterium*;
The values bearing the same letters are not significantly different at 5% level according to DMRT

It was observed that the wild strains of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) could augment the above mentioned PGPR characteristics. The improved PGPR characteristics of *P. fluorescens* regarding the IAA and siderophore production, adhesion to maize roots and thermal and desiccation tolerance have been reported by Hayat *et al.*, (2010).

In the same manner, the *B. megaterium* (AUBM29) strain exhibited a higher performance for 'P' solubilization and EPS production. Moreover, the biocontrol of maize leaf blight disease due to *P. fluorescens* and *P. polymyxa* bioinoculation has already been reported by Belimov *et al*., (2007) and Haggag (2007).

The treatment T_4 showing increased plant height (66.86 cm), root dry weight (0.35 g plant^{-1}), shoot dry weight (1.63 g plant^{-1}) and grain yield (2.72 t ha^{-1}) than compare to single inoculation treatment T_1, T_2 and T_3 (Table 2). The concentrations of these nutrients in improving the crop yield (Renal *et al.*, 1999). However, there were no earlier reports on the comparative performance of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) on the enhancement of PGPR characteristics in maize, available for discussion. This is the first comprehensive report regarding the beneficial effect of *P. fluorescens* (AUPF25) and *B. megaterium* (AUBM29) on the enhancement of plant growth promoting characteristics and biocontrol against *H. turcicum* in maize.

In conclusion, among the bioinoculant treatments, dual combination worked out better than single inoculation. Of all the Biotic and Abiotic factors analyzed, the treatments such as T_4 (*P. fluorescens* and *B. megaterium*) was concluded as best combination of biofertilizers and those were also found as compatible. So, these combinations of biofertilizers can be recommended for application in maize fields.

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