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Characterization of Rhizobacteria for Multiple Plant Growth Promoting Traits from Mung Bean Rhizosphere

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A B S T R A C T

Soil microorganisms, that flourish in, on or around plant rhizosphere, have tremendous potential to stimulate plant growth by plethora of mechanisms. In this study, 40 rhizobacterial isolates from 15 mung bean rhizospheric soil samples were morphologically and biochemically analysed and tentatively assumed to belong to genera *Bacillus* (35%), *Pseudomonas* (55%) and *Rhizobium* (10%). Ten isolates were evaluated of plant growth promoting (PGP) traits at 35°C viz. production of IAA, GA (Gibberellic acid), flavonoid and siderophore as well as Zn and P solubilisation. Our study showed that all the 10 isolates produce IAA, GA and flavonoid in the range of 6.06-32.13 µg/ml, 109.8-126.3 µg/ml and 68.9-224.7 µg/ml respectively, whereas only 7 isolates were found to be efficient producers of both hydroxamate and catecholate siderophores in the range of 342.8-732.7 µg/ml. Again, all these isolates were potent solubiliser of Zn and P in plate assay. The solubilisation indices ranged from 2.1 to 4.4, 1.4 to 6.0 and 1.8 to 3.1 for ZnO, $ZnCO_3$ and tri-calcium phosphate respectively. From this study, 3 isolates; S1P1, S4P1 and S13P1 showing superior PGP traits can be executed as effective biofertilizer in field trials for mung bean production.

Introduction

Among the dozen of pulses, mung bean is the third important pulse crop cultivated in India and accounts for 65% of acreage and 54% of the world's production (Gowda *et al.,* 2015). It is consumed as whole grain or as dal and valued for its high quality protein that constitutes about 24-25% of total nutrients in seed (Singh, 2010). It is cultivated as sole or inter crop for grain or green manure. Being a leguminous crop, mung bean has the ability to establish a symbiotic relationship with its compatible microsymbiont *Rhizobium,* setting up of biological nitrogen fixation (BNF) in the root nodules and supply the plant's needs for nitrogen (Mandal *et al.,* 2009). This *Rhizobium*-legume symbiosis is the most important plant-bacterial interaction observed in legumes and can fix as much as 30-74 kg N/ha. This legume-*Rhizobium* interaction is of considerable agricultural importance; it leads to greater quantitative enhancement of combined N in soil thus, reduces the cost of fertilizer for farmers who grow legumes (Dudeja and Duhan, 2005). Besides

Rhizobium several other rhizospheric microbes play key role in ecosystem functioning by controlling bio-geochemical nutrient cycles, supplying available nitrogen and phosphorous to plants (Kirk *et al.,* 2004). These soil microbes are termed as plant growth promoting rhizobacteria (PGPRs). In recent years, PGPRs have received worldwide importance. Their ability for high rhizospheric competence, tolerance to biotic and abiotic stresses, mass multiplication, broad spectrum action for plant growth promotion, compatibility with other rhizobacteria, environmental safety and excellent control and reliability make them very useful in maintaining sustainability in agriculture (Jeyrajan and Nakkeeran, 2000).

Study and analysis of their potential for plant growth promotion is very crucial. In this study 10 rhizobacterial isolates were evaluated *in vitro* for their ability to synthesize IAA, Gibberllic acid (GA), flavonoids and siderophore, their potential for solubilisation of minerals; P and Zn was also tested.

Materials and Methods

Isolation of rhizobacteria

For isolation of rhizobacteria; the basic procedure of serial dilution and pour plate technique were used. Three different media; nutrient agar, king's B medium (King *et al.,* 1954) and YEMA medium were used to isolate *Bacillus*, *Pseudomonas* and *Rhizobium* respectively at 35°C.

Biochemical characterization of rhizobacteria

Biochemical characterization of the isolated rhizobacteria was done on the basis of catalase production, methyl red test, nitrate reduction, starch hydrolysis and gram reaction **(**Holt *et al.,* 1994).

IAA and GA production

Characterization of rhizobacterial isolates for IAA and GA production was done as per the methods given by Gordon and Weber (1951) and Borrow *et al.,* (1955) respectively.

Flavonoid production

Total flavonoid content was estimated by aluminium chloride method proposed by Zhishen *et al.,* (1999) using naringenin as standard. The absorbance was measured at 510 nm.

Zn solubilisation

In vitro solubilisation of ZnO and $ZnCO_3$ by rhizobacteria was determined in plate assay method given by Fasim *et al.,* (2002) and Zinc solubilisation index (S.I.) was calculated as per the formula of Ramesh *et al.,* (2014).

Plate assay for phousphorous solubilisation

Cultures were spot inoculated on Pikovskaya's agar plates and incubated at 35ºC. The zone of clearance was recorded at 7th day after inoculation. P solubilisation index (S.I.) was calculated as per the formula of Ramesh *et al.,* (2014).

Production of siderophore

Detection of hydroxamate and cateholate type of siderophore was done as per the methods given by Snow (1954) and Neilands (1981) respectively.

Results and Discussion

Isolation and characterization of rhizobacteria

A total of 40 rhizobacterial isolates were obtained from 15 different mung bean rhizospheric soil samples collected from different parts of Punjab. Out of these, 35% isolates formed on nutrient agar medium, showed white to off-white coloration with round to spherical colony morphology, 55% isolates obtained from Kings B medium of which 9 isolates produced characteristic yellowish-green colouration and 10% isolates on Congo-red YEMA medium, showed characteristic round, gummy, white to translucent colony morphology. On the basis of cultural, morphological and biochemical characteristics these isolates were tentatively assumed to be *Bacillus*, *Pseudomonas* and *Rhizobium* (Table 1).

IAA production

Indole-acetic-acid (IAA) being the major and most abundant auxin in plants, plays a key role in plant growth regulation and development. Many PGPRs have the ability to produce IAA equivalents and remarkably affect the plant growth by altering the endogenous level of auxin synthesized in plants. In the present work, 10 rhizobacterial isolates were evaluated for their ability to synthesize IAA at 35ºC (Table 2).

A significant variation in IAA production by these isolates has been recorded that ranged from 1.94 -15.71 µg/ml and 6.06-32.13 µg/ml in Trp- and Trp+ medium respectively. The isolates S10B1 (15.71 µg/ml) and S1P1 (32.13 µg/ml) were the best IAA producers in Trp- and Trp+ medium respectively.

GA production

Gibberellins are the class of phytohormones that are commonly associated with modification of plant morphology. They are reported to stimulate stem elongation, flower and fruit development, breaking dormancy and seed germination. Kaur and khanna (2017) isolated 5 GA producer rhizobacteria that showed higher GA production at 30°C as well as 40°C. In the following study 10 rhizobacterial isolates were tested for their ability to produce GA at 35°C. Isolate S1P1 (126.3 µg/ml) was found to be most efficient GA producer followed by SR1 (121.1 µg/ml) and S4P1 (118.6 µg/ml). The GA production by these 10 isolate ranged from 109.8-126.3 µg/ml (Table 2).

Production of flavonoid

Flavonoids are documented to be the most important signalling molecules associated with plant-microbe interactions. Flavonoid production is an important plant growth promoting trait exhibited by many soil microbes that not only help in colonization in rhizosphere but also enhance nodulation by inducing nod gene. In the present study 10 rhizobacterial isolates were screened and their abilty for flavonoid production was evaluated *in vitro* (Table 2). The study revealed that isolate S13P1 (224.7 µg/ml) recorded with maximum flavonoid followed by S4P1 (197.5 µg/ml). Khan *et al.,* (2017) isolated 15 different strains of flavonoid and auxin producing rhizobacteria from the rhizosphere of *Medicago sativa* L.

Production of siderophore

Iron is an important micronutrient essential for bacterial metabolism. The bacteria inhabiting in soil acquire iron by secreting low-molecular weight iron chelators; siderophores which have high association constants for complexing iron. Rajkumar *et al.,* (2010) reported that siderophore producing bacteria directly enhance the plant growth by supplementing iron requirement of the host plant as well as inhibit the growth of phytopathogens in rhizosphere. In the following study, 7 rhizobacterial isolates exhibited the ability of siderphore production (Table 2).

Table.1 Cultural, morphological and biochemical characteristics of rhizobacterial isolates

Characteristics	*Bacillus* sp.	*Rhizobium* sp.	*Pseudomonas* sp.
Gram's reaction	+ve	-ve	-ve
Shape	Rods	Rods	Rods
Pigment	-	+	+
Pigment colour	White	Whitish pink	Fluorescent green
Starch hydrolysis	+	+	+
Catalase production	+	+	+
Methyl red test	-	-	-
Nitrate reduction	+	+	+
Characteristics	*Bacillus* sp.	*Rhizobium* sp.	*Pseudomonas* sp.

Table.2 IAA, Gibberellic acid, flavonoid and siderophore (hydroxamate and chatechol type) production at 35°C

Rhizobacterial isolates	IAA-equivalent (µg/ml)		GA production (µg/ml)	Flavonoid production (µg/ml)	hydroxymate type	Catechol type (µg/ml)
	L-TRP (-)	L-TRP(+)				
S1P1	11.88	32.13	126.3	170.1	++	677.0
S4P1	5.97	10.38	118.6	197.5	+	441.9
S4P5	9.11	15.43	113.5	114.9	+	430.4
S10B1	15.71	22.05	116.8	135.2	-	-
S12P4	8.31	12.13	111.3	101.9	+	465.1
S12P6	5.93	7.42	110.2	116.7	++	493.7
S13P1	10.65	16.93	117.6	224.7	+	732.7
S13P4	1.94	6.06	109.8	166.3	+	342.8
SR1	9.92	28.77	121.1	147.7	-	-
SR3	13.40	21.24	114.4	68.9	-	-

Table.3 ZnO, $ZnCO_3$ and phosphate solubilisation index by rhizobacterial isolates at 35°C

Isolates	Solubilisation Index (S.I.)		
	ZnO	$ZnCO_3$	P
S1P1	3.8	2.6	3.1
S4P1	4.4	6.0	2.6
S4P5	2.6	2.1	1.8
S10B1	2.8	2.0	2.1
S12P4	3.1	2.7	1.9
S12P6	2.8	2.0	1.8
S13P1	3.5	2.4	3.0
S13P4	2.4	1.4	2.4
SR1	2.1	2.0	2.3
SR3	2.2	2.2	2.2

These isolates showed positive test for production of both hydroxamate and chatecholate type siderophores of which S13P1 (732.7 µg/ml) followed by S1P1 (677.0 µg/ml) found to be the best catecholate siderophore producers whereas higher hydroxamate production was observed in S1P1 and S12P6.

Solubilisation of Zn

Zinc is an essential nutrient for plant growth and mainly found in the form of ZnS (sphalerite), zincite (ZnO), smithsonite ($ZnCO_3$) zinkosite ($ZnSO_4$), franklinite ($ZnFe_2O_4$) etc. (Hafeez *et al.*, 2013). It is also evident that, inoculation of potent strains of Zn solubiliser rhizobacteria increases the yield of field crops such as wheat, rice, maize and barley.

In this study 10 rhizobacterial isolates were screened for their ability to solubilise ZnO and $ZnCO_3$ in the plate assay. Among all the rhizobacterial isolates; S4P1 showed maximum solubilisation index of 4.4 and 6.0 for ZnO and $ZnCO_3$ respectively. However, the range of 2.1 to 4.4 for ZnO and 2.0 to 6.6 for $ZnCO_3$ solubilisation was recorded from the isolates (Table 3).

Phosphate solubilisation

Microorganisms enhance the P availability to plants (Chen *et al.*, 2006). It seems probable that plant growth substances produced by PSBs (phosphate solubilising bacteria) improve plant growth by their direct effects on metabolic processes. However, they induce proliferation of lateral roots and root hairs resulting in increased nutrient absorbing surfaces leading to greater nutrient absorption (Shahab *et al.*, 2009). Among the 40 rhizobacterial isolates only 10 isolates were able to solubilise tri-calcium phosphate *in vitro* with maximum solubilisation index of 3.1 recorded from isolate S1P1. Whereas the range for P solubilisation was recorded to be 1.9 to 3.1 (Table 3).

The present study revealed that soil micro-organisms exhibit several plant growth promoting traits that could directly influence plant growth and development affecting its yield. In our study the isolate S1P1 showed maximum production of IAA (32.13 µg/ml), GA (126.3 µg/ml) and showed maximum P solubilization index of 3.1 whereas, isolate S13P1 was found to be the best flavonoid (224.7 µg/ml) and siderophore (732.7 µg/ml) producer again isolate S4P1 was maximally recorded with ZnO (4.4) and $ZnCO_3$ (6.0) solubilization indices. Considering all the above parameters the isolates S1P1, S4P1 and S13P1 were found to be most feasible for use as bio-inoculants alone or in combination with *Rhizobium* in mung bean cultivation.

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