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CHARACTERIZATION OF PLANT GROWTH PROMOTING TRAITS IN *BACILLUS SPP.* ASSOCIATED WITH WHEAT RHIZOSPHERE

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ABSTRACT

In search of efficient PGPR strains with multiple activities, a total of 21 isolates belonging to Bacillus spp. were isolated from different rhizospheric soils collected from various districts of Uttar Pradesh. These test isolates were biochemically characterized and screened for their plant growth promoting traits like production of indole acetic acid (IAA), ammonia production, siderophore production, phosphate solubilization, salt tolerance and antibiotic sensitivity test. These isolates evaluated for their quantitative IAA production, the isolates ABN-I and ABN-3 produced IAA in highest amount (120 mg/l). Out of these, sixteen and twelve isolates produced ammonia and siderophore, respectively while five isolates solubilized phosphate on the pikovskaya's agar medium. Only five isolates showed tolerance against 7% NaCl concentration. Isolate ABN-1 showed resistance against tetracycline at the concentration of 20 µg /ml in the medium. This study has pointed out 9 isolates could exhibit six PGP traits, which may promote plant growth directly and indirectly. Thus, these isolates may be applicable as bio-inoculants according to each supporting characters as plant growth promoter rhizobacteria.

Key words: PGPR, wheat, IAA, ammonia, siderophore, P solubilization, salt tolerance, antibacterial activity.

INTRODUCTION

Rhizosphere is a rich niche of microbes and should be explored for obtaining potential plant growth of growth and yield of crop plants. In last few decades, a large assay of bacteria including species of *Pseudomonas, Bacillus, Azotobacter, Azospirillum*, *Klebsiella, Alcaligens, Burkholderia, Serratia, Arthrobacter* and *Enterobacter* have reported to enhance plant growth (Kloepper , 1993). It has been well established that PGPR enhance plant growth by direct or indirect means. The direct means may includes production of NH_3 (Cappuccino and Sherman, 1992), production of siderophore (Schwyn and Neilands, 1987), solubilization of minerals like phosphorus (Tilak *et al.,* 2005) and synthesis of phyto-hormones like indole acetic acid (IAA) (Huddedar *et al.,* 2002). On the other hand, in indirect promotion, bacteria protect the plant against soil borne fungal pathogens or deleterious bacteria (Kloepper, 1993; Glick, 1995). Wheat is one of the major crops cultivated in India and all over the world. Wheat grows in temperate climates and it is the staple for 35% of the world's population. On the other hand, it provides more calories and proteins in the diet than any other crop. The different stages of the life cycle of wheat consists of elongation (30 days), flowering stage (45 days), fruiting stage (60 days) and ripened fruiting stage (75 days). The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effect on the plants. The environmental factors include climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil.

Therefore, it is necessary to develop efficient strains in the field conditions. One possible approach is to explore soil microbial diversity for PGPR having combination of PGP activities and well adopted to particular soil environment. So keeping in view the above constraints, the present study was designed to isolate and characterize *Bacillus* from the rhizosphere of wheat and to screen their abilities for plant growth promotion.

MATERIAL AND METHODS

The rhizosphere soil samples of the wheat were collected from Faizabad (FZB), Kanpur (KNP), Barabanki (BRK), Muzzaffarnagar (MUZ), Ambedkarnagar (ABN), Jaunpur (JAN) and Lucknow (LKO) district of Uttar Pradesh. The wheat plants were uprooted from the agricultural fields and the rhizosphere soil was pooled together and immediately microbiological processing was carried out. Serial dilutions were made up to 10^{-4} for all seven soil samples and 10^{-3} and 10^{-4} dilutions were taken for spread plating on nutrient agar plate containing per liter of distilled water:5.0 g peptone, 3.0g beef extract, 5.0 g NaCl, 20.0 g Agar and pH 7.0. The plates were incubated at 30^0C for 24-48 hours. After incubation, plates were observed for different isolates based on morphological traits. Morphologically,

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variable colonics picked up and purified on nutrient agar plates. Pure cultures were maintained on the respective status. The bacterial isolates were characterized by their morphological and biochemical characteristics (Gram's reaction, carbohydrate fermentation, oxidase test, H_2s production, starch and gelatin hydrolysis, IMVic test, NO_2 reduction, citrate and catalase reactions) using standard methods (Cappuccino and Sherman, 1992). The indole acetic was detected as described by Brick *et al.* (1991). The bacterial cultures were grown in Luria- Bertani broth at 30 for 48 hours. After incubation fully grown cultures were centrifuged at 10000 rpm 10 min. at 4^0C. The supernatant 2 ml was mixed with two-tree drops of orthophosphoric acid and 4ml of the salkouski reagent (50 ml, 35% of perchloric acid 1ml 0.5 $FeCl_2$ solution).The development of pink color indicates IAA production. Absorbance was taken at 530 nm with help of UV visible spectrophotometer. Concentration of IAA produced by culture was measured with the help of standard graph of IAA obtained in the range of 20-200 µg/ml. Similarly all the bacterial isolates were tested for the production of ammonia in the peptone water. Freshly grown culture were inoculated in 10 ml peptone water in each test tube and incubated for 4 days at 30^0 C. Nessler reagent 1ml was added in each test tube. Development of brown to yellow color was a positive test for ammonia production. (Cappuccino and Sherman 1992). Bacterial isolates were assayed for siderophore production on the chome azurol S agar (CAS) medium described by Schwyn and Neilands (1987). Bacterial isolates were spot inoculated on the chrome azurol S (CAS) ager plates and incubated at 30 for 72 h. Development of orange halo around the growth was considered as positive for siderophore. Solubilization of phosphate was detected on pikovskaya's agar plate (Wahyudi *et al.* 2011). All the bacterial isolates streaked on the surface of pikovskaya's agar plate and phosphate solubilizing activity was estimated after 4 days of incubation at 30 h. Phosphate solubilization activity was determined by the development of the clear zone around the bacterial colonies. All the twenty one isolates were tested against the tetracycline by agar dilution method as described by Ahmad *et al.* (2004). The stock solution (5 mg/ml) of antibiotic i.e. tetracycline has prepared and used four different concentration 1µg/ml, 5 µg/ml, 10 µg/ml, and 20 µg/ml for antibiotic sensitivity test. The tetracycline was dissolved in 70% ethanol and sterilized with membrane filler (Axiva Schema biotech). The nutrient agar medium was prepared in four 500 ml flask and allowed to cool to 50 ^{0}C. The diluted tetracycline concentration were mixed in cool molten agar medium and poured in petriplates. The *Bacillus i*solates spot inoculated on solidified agar plate and incubated at 30 ^{0}C for 48 hours. After incubation, the plates were examined for the presence or absence of growth on the spotted area. The *Bacillus* strains which were sensitive against tetracycline did not grow on the plate and resistant strains shows the growth on the plates against tetracycline. The pure cultures of all *Bacillus* isolates were streaked on nutrient agar medium, containing 2% to 7% NaCl concentration. Control plates with NaCl amendment were also kept for observation for all strains. All plates were incubated at 30 ^{0}C for 48 hours and observed for the presence or absence of the growth.

RESULTS AND DISCUSSION

On the basis of cultural, morphological and biochemical characteristics a total of 21 bacterial strains were isolated and identified as *Bacillus* as described in Bergeys manual of determinative bacteriology (Holt *et al.,* 1994). The *Bacillus* strains from rhizosphere of different crops were isolated and extensively studied by Joseph *et al* (2007), Fisher *et al. (*2007), Wahyudi *et al.* (2011). The general characteristics of the isolates were illustrated (Table 1). These isolates were tested for quantitative estimation of IAA and the entire test *Bacillus* strains showed IAA production in the range of 5-120 µg/ml (Table 2). The strain ABN-3 and JAN-1 produced

Table 1: Morphological and cultural characteristics of test isolates

Biochemical characters	***Bacillus* spp.**
Number of isolates	21
Grams reaction	+ve
Shape	rod
Pigment	Cream
Colony morphology	Circular, lobate to serrated margin
Sucrose	+
Dextrose	+
Mannitol	+
H2S production	-
Indole	-
Methyl red	-
Vogues Prokauer	-
Citrate Utilization	+
Starch	+
Gelatin hydrolysis	-
Catalase test	+
Nitrate reduction	-
Lipid hydrolysis	+
Casein hydrolysis	+

IAA (110µg/ml) in the broth culture medium. Wahyudi *et al.* (2011) reported that *Bacillus* spp. Cr4 produced 86.82 mg/l IAA in culture medium supplemented with L Tryptophan, while 32.80 µg/ml IAA productions was reported by Ahmad *et al.* (2004) and Shobha and Kumudini (2012) reported IAA 35-127 µg/ml in *Bacillus* isolates. Out of 21 *Bacillus* strains, 16 strains produced NH_3 and few isolates like ABN-1, ABN-3, MUZ-2, MUZ-4, BRK-1, BRK-2 and BRK-3 produced deep brown color indicating higher production of ammonia, whereas strain KNP-1, KNP-3, MUZ-1, MUZ-3, ABN-2, JAN-1, JAN-3, LKO-1 and LKO-2 produced deep yellow color showing medium production of ammonia. The *Bacillus* isolate FZB-1, FZB-2, KNP-2, KNP-4 and JAN-2 did not produce ammonia (Table-2). Only six strains solubilized phosphate on pikovskaya's agar plates at 30 ^{0}C and remaining strains did not show phosphate solubilization. Several other workers like Agrawal *et al.* (2011), Ahmad (2008), Joseph *et al.,* (2007), Sachdev *et al.* (2009) observed phosphate solubilization and ammonia production by *Bacillus* spp. isolated from cereal vegetable and other crops.

Table 2: Plant growth promoting characteristics of rhizobacteria test isolates

Sl. No.	Isolate	Plant growth promoting characteristics			
		IAA Production mg/ml	Ammonia production[a]	Phosphate solubilization[c]	Siderophore production[b]
1	FZB-1	42.00	-	+	-
2	FZB-2	20.00	-	+	-
3	KNP-1	20.00	+	_	-
4	KNP-2	33.00	-	_	-
5	KNP-3	20.00	+	-	-
6	KNP-4	42.00	_	-	-
7	BRK-1	90.00	+++	-	+
8	BRK-2	63.00	+++	-	+
9	BRK-3	05.00	+++	-	+
10	MUZ-1	80.00	++	-	+
11	MUZ-2	25.00	+++	-	+
12	MUZ-3	05.00	+	-	-
13	MUZ-4	05.00	+++	-	+
14	ABN-1	20.00	+++	-	+
15	ABN-2	110.00	+	-	-
16	ABN-3	39.00	+++	+	+
17	JAN-1	110.00	+	+	+
18	JAN-2	25.00	-	+	-
19	JAN-3	30.00	+	+	-
20	LKO-1	65.00	+	_	+
21	LKO-2	10.00	++	_	+

Note: *[a]; + = low production of NH_3, ++ = medium production of NH_3, +++ = strong production of NH_3, [b] ; + = siderophore production, - = no siderophore production [c]; + = able to solublize P in pikovskaya's medium, - = not able to solublize P,*

Only 11 *Bacillus* isolates produced siderophore and siderophore producing bacterial isolates formed on orange halo surrounding bacterial colonies on CAS-agar plate (Table 3). Wahyudi et *al.* (2011), Rawat *et al.* (2011), Joshi and Bhatt, (2011), Ahmad, 2008, Joseph *et al.* (2007) found that the *Bacillus* strain isolated from different plant rhizosphere could produce siderophore which in turn suppressed the phytopathogenic fungal infection. Rangarajan *et al.,* (2002) screened the bacterial strains for salt tolerance, out of 256 strains, only 36 strains could grow at 4.5% NaCl concentration and no strain was able to grow at 6% NaCl concentration. But in the present study out of 21 strains, six strains tolerated even 7% NaCl concentration while isolate JAN-2 could not tolerate even 2% NaCl concentration (Table 3).

All the twenty one *Bacillus* strain were tested against the tetracycline. The most of the isolates were inhibited at the concentration of 10 µg/ml of antibiotic. The isolate FZB-2, BRK-3, MUZ-1, MUZ-4, JAN-1 and LKO-2 showed inhibition at the concentration of 5 µg/ml, while ABN-1 found resistant at 20 µg/ml concentration of tetracycline. More interesting finding is that JAN-2 showed very high sensitivity against the test antibiotic and could not tolerate even 1 µg/ml concentration. Thakuria *et. al., (*2004) observed that PSB isolates showed higher

sensitivity towards all the antibiotics and isolates Psd5 and Psd6 showed exceptionally higher resistance (1200 ppm) towards antibiotics ampicillin and chloramphenicol while Joshi and Bhatt (2011) showed that all isolates were resistant against Chloramphenicol (30 µg mL^{-1}), Streptomycin (10 µg mL^{-1}), Kanamycin (5 and 30 µg mL^{-1}), Penicillin (10 µg mL^{-1}) and Tetracyclin (30 µg mL^{-1}). Our results suggests that Plant growth promoting rhizobacteria are able to enhance the production of IAA, ammonia production, siderophore production, solubilization of phosphorus, resistance to antibiotic and NaCl tolerance.

Table 3: Antibiotic sensitivity and salt tolerance of rhizobacterial isolates

Sl. No.	Isolate	Tetracycline concentration (µg/ml)				NaCl concentration (%)					
		1	5	10	20	2	3	4	5	6	7
1	FZB-1	++	++	-	-	+++	+++	++	++	++	++
2	FZB-2	++	+	-	-	++	++	-	-	-	-
3	KNP-1	++	+	-	-	++	++	-	-	-	-
4	KNP-2	+	-	-	-	++	++	++	-	-	-
5	KNP-3	++	++	-	-	++	++	++	++	++	++
6	KNP-4	+++	+	-	-	++	++	-	-	-	-
7	BRK-1	+++	++	-	-	++	+	-	-	-	-
8	BRK-2	+++	++	-	-	+++	+++	++	++	++	++
9	BRK-3	+	-	-	-	+++	+++	+	-	-	-
10	MUZ-1	+	-	-	-	+++	+++	+++	++	+	+
11	MUZ-2	++	++	-	-	++	+	-	-	-	-
12	MUZ-3	++	++	-	-	++	++	++	-	-	-
13	MUZ-4	+	-	-	-	++	-	-	-	-	-
14	ABN-1	+++	+++	+++	+++	+++	+++	++	++	+	+
15	ABN-2	++	++	+	-	+++	+	-	-	-	-
16	ABN-3	+++	+++	++	-	+++	+++	-	-	-	-
17	JAN-1	++	-	-	-	++	+	-	-	-	-
18	JAN-2	-	-	-	-	-	-	-	-	-	-
19	JAN-3	++	++	-	-	+++	+	-	-	-	-
20	LKO-1	+++	++	-	-	++	++	++	++	++	++
21	LKO-2	++	-	-	-	++	-	-	-	-	-

+++=shown maximum growth ++= shown medium growth + shown poor growth - = Shown no growth

The present investigation highlighted that IAA producing bacteria from local soil could be easily isolated and may be exploited after strain improvement for local use. However, further studies using IAA mutant strains of these isolates are needed to explore the exact contribution of IAA production in the promotion of plant growth as well as the contribution of other PGP traits. The use of PGPR as inoculants is an efficient approach to replace chemical fertilizers and pesticides for sustainable cultivation of wheat in India. Further investigations such as green house and field studies are needed to clarify the role of PGPR as bio-fertilizers.

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