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EFFECT OF PHOSPHATE SOLUBILIZING BACTERIA ALONG WITH ACC-DEAMINASE ON WHEAT GROWTH UNDER AXENIC CONDITIONS

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ABSTRACT

Phosphorus is one of the major plant growth limiting nutrients and in Pakistan its deficiency found in 90% soils due to alkaline calcareous nature of soils. Plant growth promoting rhizobacteria are efficient in supplying nutrients to plants by solubilizing the unavailable/insoluble (P) in soil or by promoting extensive root growth through 1-aminocyclopropane-1-carboxylate (ACC-deaminase) activity. This study was conducted to assess the effects of phosphate-soluibilizing rhizobacteria and their impact on wheat growth under axenic conditions. Initially rhizobacteria (S1 to S10) were isolated through enrichment by rock phosphate [Ca_3 $(PO_4)_2$] and ACC as sole P and N source, respecitively. After isolation, identification and characterization of these strains was done for root colonization, P-solubilization, in vitro ACC-deminase ctivity, auxin production and chitinase activity. The results revealed that rhizobacterial isolate S8 was best strain and showed 2.6 fold higher fresh weight, 2.7 fold higher shoot length and 4 fold higher root length of wheat over uninoculated control. Similarly, S8 was best root colonizer in wheat rhizosphere (8.0×10^{-7} cfu g^{-1}) while isolate S9 showed the highest P- solubilization i.e. 14.36 ppm, S2 showed maximum ACC-deaminase activity (1.8 µmol α-ketobutyrate) and S4 showed highest auxin production (36.77 mgL^{-1}). This study concluded that S8 strain efficiently solubilize phosphate alongwith ACC-deaminase trait which improved the growth of wheat under axenic conditions.

Keywords: Rock phosphate, Phosphate Solubilizing bacteria (PSB), ACC-deaminase.

INTRODUCTION

Phosphours (P) is one of the major nutrients limiting plant growth. In contrast to other essential elements, P is required for growth and development of plants. It is involved in photosynthesis, energy transfer, singal transduction, macromolcular biosyntheis and respiration (Fernandez et al. 2007). However many agricultural soils worldwide are P-deficient (Arcand and Schneider, 2000) and therefore require P to replenish the P-demands by crop plants (Hamdali et al. 2012). Rock phosphate is a natural, cheap and clean compound but unfortunately it is a poor fertilizer since its solubilization is to slow to satisfy plant needs (Zapata and Zahara, 2002) Rock phosphate or phosphorite is a non-detrial sedimentary rock having phosphate in fluoroapatite Ca_5 $(PO_4)_3$ F (CFA) and hydroxyapatite Ca_5 $(PO_4)_3$ OH (Balte and Robert, 1996).

Plant growth promoting rhizobacteria (PGPR) encompass all the bacteria that inhabit plant roots and affect the promotion by mechanisms ranging from direct influence e.g. increased solubilization and uptake of nutrients or production of growth regulators (Steenhoudt and Vanderleyden, 2000) to direct influence e.g., suppression of pathogens by producing siderophores or antibiotics (Kloepper et al. 1989; Arshad and Frankenberger , 1998). Many PGPR are known as solubilizer of insoluble rock phosphate through the production of organic acids or root exudation that results in lowering soil pH (Gyneshwar et al. 2002; Puente et al. 2004). Organic acids mainly synthesized are citric, lactic,gluconic, oxalic etc (kucey 1983; Vazques et al. 2000) These acids act on insoluble phosphates and convert the same into soluble from, thus providing phosphorus to plants (Ponmurugan and Gopi, 2006; Nautiyal et al. 2000). Other strategie that lead to better uptake or acquisition of nutrients include increased root surface area through enhanced root growth and root hair development (Lynch and Brown, 1998; Gilory and Jones, 2000). Identification and characterization of soil PSB for the effective plant growth promotion broadens the spectrum of phosphate solubilizers available for field application (Chen et al. 2006).

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Higher concentrations of ethylene are considered inhibitory for normal plant growth particularly for root growth. Phosporus deficiency could lead in production of higher levels of ethylene in roots and inhibit root and shoot growth (Sarquis et al. 1991; Morgan et al. 1993). ACC serves as precursor of ethylene. Inoculation of seeds or roots with specific inoculants containing ACC- deaminase enzyme could suppress the endogeneous ethylene synthesis which subsequently creates physiological responses (Glick et al. 1998). The enzyme which has been found in soil/some microoganism catalyses the hydrolysis of ACC to NH_3 and a-ketobutyrate (Mayak et al. 1999; Penrose and Glick, 2003).

Keeping in view the importance of P for plants this study was planned to isolate, characterize the phosphate solubilizing rhizobacteria with and/or without ACC- deaminase trait and to assess their effets on growth of wheat under P-deficient environment using rock phosphate (as sole P source) and ACC (as sole N source).

MATERIAL AND METHOD

This experiment was conducted under controlled P conditions to compare the effectiveness of phosphate solubilizing rhizobacteia and for this purpose initially isolation and characterization process was done.

Isolation of strains

For isolation of rhizobacteria different soils were collected from wheat plant rhizosphere. Rhizobacteria were isolated by dilution plate technique using salt minimal medium (Dworkin and Foster, 1958) containing ACC as sole nitrogen source or NBRI-P medium (Nautiyal, 1999) using rock phosphate as sole phosphorus source. S1, S2 strains had ACC- deaminicase activity, S3, S4 had phosphate solubilizing activity while S5-S10 strains had both the activites. The collected rhizobacterial isolates were purified by further streaking on fresh plates. All the isolates were stored in 20% glycerol at-20 ^oC for further studies.

Preparation of inocula

For preparation of inocula D.F salt minimal media (Dworkin and Foster, 1958) containing ACC as sole N source was used for strains containing ACC-deaminase activity. Modified NABRI-P media was used for preparation of inocula for strains containing phosphate solubilizing activity. Selected strains of bacteria were used for inoculation of broth and incubated for 48 hrs under shaking (100 rpm) conditions. After incubation, optical density of 0.5 at λ 550nm was achieved by dilution to maintain uniform cell density (10^7-10^8 cfu mL^{-1}). The suspension of selected rhizobacteria was used as inocula for wheat seed coating.

Seed Germination Assay

Wheat seed were surface sterilized by dipping in 95% ethanol solution for 5 min, 0.2% $HgC1_2$ solution for 3 min and washed thoroughly with distilled water. After sterilization these seeds were sown on sterilized filter paper sheets placed in Petri plates. These plates were incubated in a growth chamber at 28 ± 1 ^oC for uniform germination. After seed germination assay, different parameters were studied to characterize rhizobacterial strains e.g. gnotobiotic root colonization assay (Simon's *et al*., 1996) was done to calculate colony forming unit CFU/g of root biomass, solubilization of inorganic phosphate (Mehta and Nautiyal, 2001), ACC-deaminase activity (penrose and Glick, 2003), Chitinase activity (Chernin *et al*., 1998) and auxin production was measured as indole acetic acid (IAA) equivalent in the presence or absence of L-tryptophan (Khalid et al. 2004).

Jar Experiment

Ten PGPR strains containing phosphate solubilizing and/or ACC deaminase activity were used in sand jar and this sand was dipped in 5% HC1 solution and washed thoroughly with distilled water. Each glass jars contained 500g approx. sand and 1 g rock phosphate. Pregerminated seeds were sown in this sand at the rate of 3 seeds per jar and inoculated with respective strains. Two control treatments were also maintained. There were three replications for each treatment. In uninoculated rock phosphate control (So) only rock phosphate was added and P-deficient Hoagland solution was supplied. In uninoculated KH_2PO_4 control (N) only KH_2PO_4 was added and normal Hoagland solution was used. Appropriate temperature was maintained with 16 hours light and 8 hours dark period. After 14 days data regarding root length, shoot length, fresh and dry weights and number of tillers/plant were recorded. All the data were subjected to statistical analysis (Steel and Torrie, 2002).

Charaterization of Rhizobacterial isolates

The results depicted in Table 1. revealed that ten rhizobacterial strains S1, S2, S3, S4, S5, S6, S7, S8, S9 and S10 were taken for the purpose of characterization. In case of ACC-deaminase activity, all rhizobacterial strains showed this activity except S3 and S4 but S2 has maximum ACC-deaminase activity (1.8 umol a-ketobutyrate g- biomass 1/2 h^{-1}) followed by S1 (1.5 umol a-ketobutyrate g- biomass 1/2 h^{-1}) giving an indication that these are AAC-deaminase containing bacteria only because these have negligible amount of phosphate solubilizing activity. Rhizobacterial strains like S7, S9 and S10 have maximum ability to solublize phosphorous i.e., 10.07, 14.36 and 10.57mg/kg. Further S3 and S4 have not shown ACC-deaminase activity giving an indication that these are phosphate solubilizing rhizobacteria only. All rhizobacteria strains have the ability to colonize roots as compared to uninoculated control but S8 (8×10^{7}cfug^{-1}) has more ability to colonize roots than other strains. Indole acetic acid (IAA) production was also maximum in case of S4 (36.15 mg L^{-1}) followed by S8 (30.92 mgL^{-1}) with L-TRP as compared to all other strains. While without L-TRP maximum IAA production was showed by S6 (1.23 mg L^{-1}) and S7 (1.0 mg L^{-1}) There was significant linear correlation between auxin produced by rhizobacteria in vitro and growths of wheat seedlings (particularly root and shoot parameters) under control condition. All the rhizobacterial isolates possessed the ability to produce auxins (IAA) in the presence of L-TRP while in the absence of L-TRP only S5, S6, S7, S8 and S9 have showed auxin activity. This IAA might have directly or indirectly affected the plant growth. High IAA stimulates more ACC which is converted into NH_3 and a-ketobutyrate, thus reducing inhibitory effect of ethylene (Glick et al. 1998). Okon and Vanderleyden (1997) suggested that the secretion of plant growth promoting substances by the bacteria could be responsible for the beneficial effects of PGPR (Neol et al. 1996). So the results showed that rhizobacterial isolate S8 was the best strain in relation to wheat seedling shoot/root growth because it has more ability to solubilizs phosphorus, colonize roots, produce more IAA and have optimum ACC-deaminase activity.

Effect of selected isolates on growth of wheat seedlings under axenic conditions

The screening of rhizobacterial isolates for wheat growth promotion under axenic condition was done under P-deficient environment. Phosphorus deficiency could lead in production of stress conditions i.e. higher levels of ethylene in roots and inhibit root and shoot growth. In this trial inoculation with phosphate solubilizing rhizobacteria containing ACC-deaminase activity S8, S7, S9 and S10 has increased wheat seedlings growth significantly than rhizobacteria containing phosphate solubilizing or ACC-deaminase activity alone i.e. S1, S2, S3 and S4. The results depicted in Fig.1 shows that S8 (20 cm) produced the more shoot length where as S0, S1 and S2 had minimum shoot lengths. These results clearly indicated that inoculated strains produced 64% more shoot length than uninoculated control. Similarly in case of root length, the roots of strain S8 proliferated 75% more area than all other strains (Fig.2). This result implies that rhizobacertia grown in rock phosphate, under P-deficient environment, utilized it as a sole phosphorus source through solubilization of insoluble compounds by decreasing pH through excretion of organic acids and by the activity of phosphate enzyme (Nautiyal et al. 2000; Puente et al. 2004; Marschner et al. 1986).

In case of fresh shoot weight (Fig.3) 49% increase in weight was found in case of S8 followed by S7 which cased 46% over uninoculated control (So). While the maximum dry weight of shoot (Fig. 4) was found in S8 i.e. 83% and minimum in S1 (20%) over uninoculated control. Similarly other scientists also relate that PSM inoculants with lettuce and maize give higher biomass increase up to 20% (Chabbot et al. 1993). Bacterial strains isolated from alkalin soil have been able to solubilize phosphate in higher salt, pH and temperature stress condition (Nautiyal et al. 2000). Both rock phosphate addition and microbial inoculantion improved biomass production and P accumulation in test plants (Barea et al. 2005) Phosphate solubilizing microorganisms can promote plant growth directly by solubilizing this unavailable/insoluble P in soil through plant microbe interaction and indirectly enhance P acquisition by plant through promoting extensive root growth because of their ACC-deaminase activity. The results of fresh weight of root (Fig.5) showed that

rhizobacterial isolate S8, S7 and S10 increased weights up to 48%, 43% and 42% over uninoulated control. While the loose in weight in case of root (Fig.6) was also found maximum in S8 i.e. 94% followed by S9 and S10 which caused 93% decrease in weight of root than fresh weight over uninoculated control. This implies that rhizobacteria grown on ACC utilized it as a nitrogen source via deaminase trait i.e., ACC is coverted into ammonia and a-ketobutyrate instead of ethylene. Thus it is highly like that the ability of ACC enriched rhizobacteria isolated to deaminate ACC was the responsible mechanism of action for promoted root shoot growth because lowering of ACC levels results in decreased endogenous ethylene. This contention is strongly supported by the work reported by several other researchers (Glick et al. 1998; Hall et al. 1996; Mayak et al. 1999; Penrose and Glick, 2003). Further, maximum number of tillers (Fig.7) was also found in S8 (4.2) followed by S9, S7 and S10 which produced 4.0, 3.4 tillers, respectively.

Table 1. Characterization of rhizobacterial isolates

PGPR strains	**ACC – deaminase activity (µmol α-ketobutyrate)**	**IAA production (mgL^{-1})**		**Phosphorus solubility (mg/kg)**	**Root colonization (cfu g^{-1} root)**	**Chitinase activity**
		Without L-TRP	**With L-TRP**			
Uninoculted control (S_0)	0	0	0	0	0	0
S1	1.50	0	1.62	0	2×10^{-7}	+ve
S2	1.80	0	8.15	0	4×10^{-7}	+ve
S3	0	0	19.54	8.98	1×10^{-7}	+ve
S4	0	0	36.77	8.13	3×10^{-7}	+ve
S5	1.40	0.15	22.00	7.72	4×10^{-7}	-ve
S6	1.40	1.23	18.92	7.45	3×10^{-7}	-ve
S7	0.83	1.00	6.92	10.07	6×10^{-7}	+ve
S8	0.77	0.92	30.92	9.98	8×10^{-7}	+ve
S9	0.25	0.77	5.54	14.36	7×10^{-7}	+ve
S10	0.24	0	18.08	10.57	5×10^{-7}	+ve

Effect of phosphate solubilizing rhizobacteria on wheat growth under axenic conditions

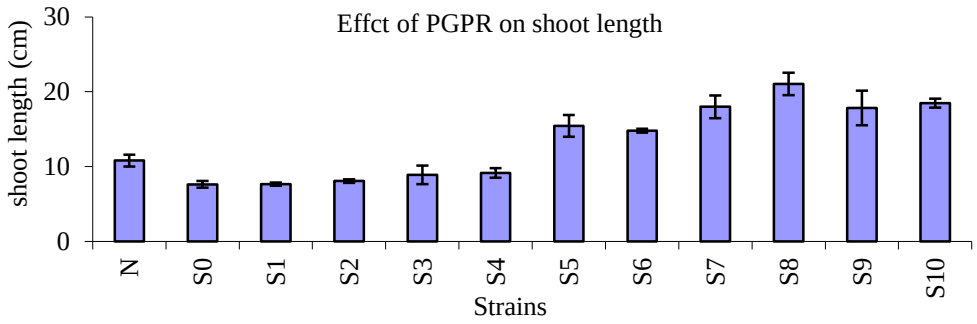

Fig.1: Effect of phosphate solubilizing rhizobacteria on shoot length of wheat seedlings

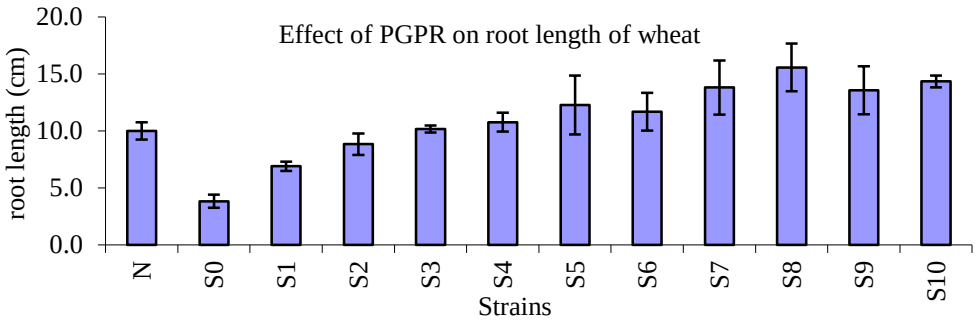

Fig.2. Effect of phosphate solubilizing rhizobateria on root length of wheat seedlings

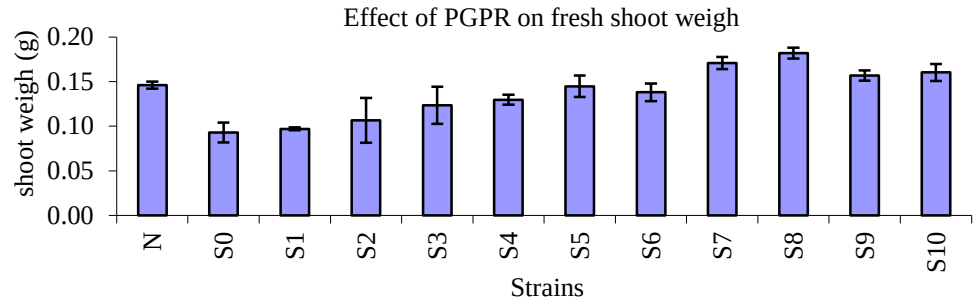

Fig.3. Effect of phoaphate solubilizing rhizobacteria on fresh weigh of shoot

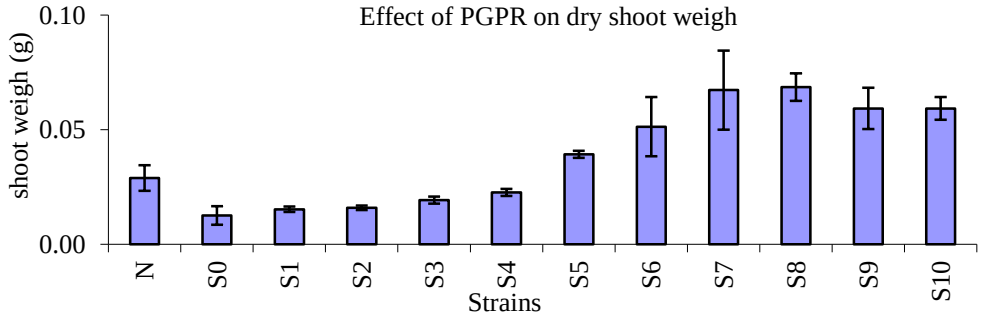

Fig.4. Effect of phosphate solubilizing rhizobacteria on dry weigh of shoot of wheat seedlings

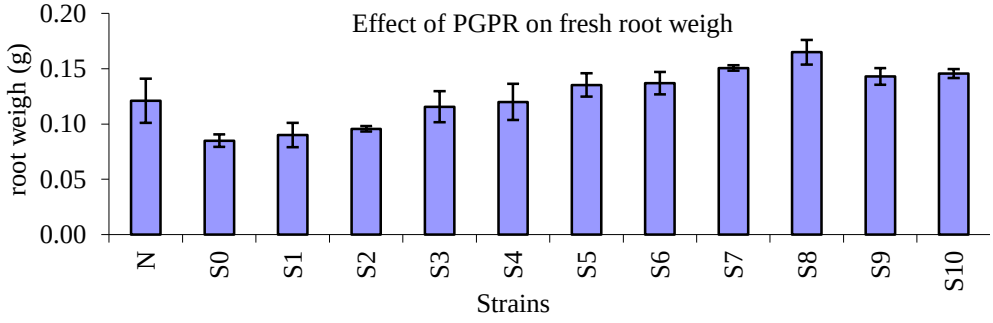

Fig.5. Effect of phosphate solubilizing rhizobacteria on fresh weigh of root of wheat seedlings

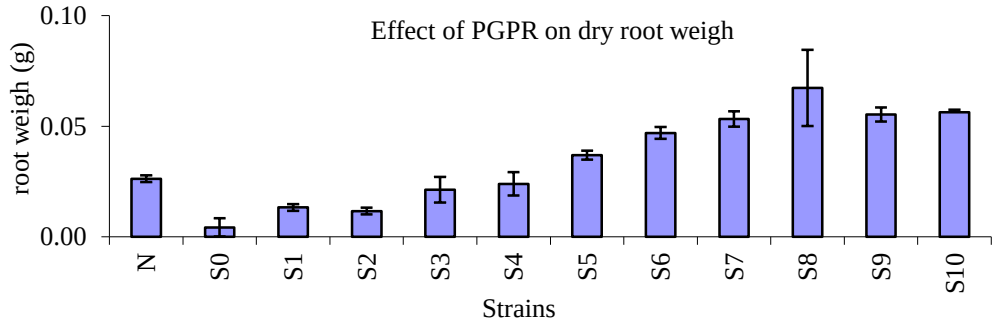

Fig.6. Effect of phosphate solubilizing rhizobacteria on dry root weigh of wheat seedlings

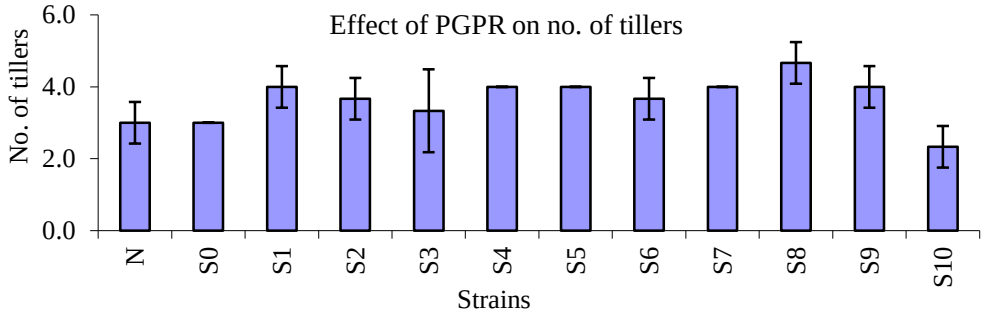

Fig.7. Effect of phosphate solubilizing rhizobacteria on number of tillers of wheat seedlings

CONCLUSION

This is concluded that inoculation with PGPR containing phosphate solubilizing activity alone could increase P availability to plants as it caused decreased endogeneous ethylene levels. Also inoculation with rhizobacteria containing ACC-deaminase activity alone enhances plant growth and could decrease endogenous ethylene levels. As this study showed that S8 strain increased 64%, 75%, 49%, 83%, 48% and 94% shoot length, root length, shoot fresh and dry weigh, fresh root and dry weighs respectively. Any factor/stimulus, with causes a change in the endogenous ethylene in a plant results in modified growth and development. While utilization of ACC-deaminase and phosphate solubilizing (organic acids and phosphatases) biotechnology could be useful tool for sustainable agriculture.

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