Plant Growth Promoting Characteristics in Some *Flavobacterium* spp. Isolated from Soils of Iran

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Abstract

Plant growth promoting rhizobacteria (PGPR) is referred to a heterogeneous group of beneficial rhizosphere bacteria that could enhance plant yield through one or more mechanisms. *Flavobacterium* has been noted as PGPR in almost all review articles. However, there are a few studies regarding plant growth promotion imposed by them. Some of Plant growth promoting characteristics such as Phosphate solubilizing capacity, ability to use of 1-Amino Cyclopropan-1-Carboxylate (ACC) as sole nitrogen source and production of auxin, siderophore, salicylic acid, chitinase and hydrogen cyanide were evaluated in forty-four *flavobacteria* isolated from rhizosphere of wheat. Results showed that none of the isolates were able to produce siderophore, salicylic acid and chitinase and they were not able to use ACC as well. Determining the siderophore showed that none isolates did not grow on Chrome Azurol S (CAS) Agar medium. The results of this part were further analyzed using CAS Agar Diffusion (CASAD) method, but the results were also negative. HCN production was observed in all isolates, but in lowest limit. Thirty-four isolates were capable to solubilize insoluble inorganic Phosphate (P) sources. The average rate of P-solubilization was 3.54 μg Pml^{-1}, ranging from zero to 37.48 μgPml^{-1}. There was a significant negative correlation (r = -0.81**) between solubilized P and the final pH of the growth medium. In this study, all the isolates were able to produce auxin, ranging from 0.27 to 12.03 μgml^{-1} averaged by 2.03. Considering the ability of the isolates to produce auxin and for P-solubilization, it is necessary to evaluate their effect on growth and yield of different crop plants.

Keywords: Auxin, Chitinase, *Flavobacterium*, HCN, PGPR and Siderophore

1. Introduction

The rhizosphere as defined by Boven and Rovira (1999) is a tender zone (about 1-3mm) of soil surrounding a plant root where living organisms are influenced by root vital activities (like compounds exuded by the root and respiration) qualitatively and quantitatively. Wide range of microorganisms lives in this area that their variation and accumulation is influenced by plant type.

Rhizobacteria have been classified into beneficial, deleterious and neutral according to their effect on host (Benizri *et al.,* 2001). Classification of beneficial rhizobacteria to symbiotic and asymbiotic have been done on the base of their physical relationship with plant. The term PGPR was first used by Kloepper and Schroth (1978) and investigation on PGPR have been escalating at an ever increasing rate since then.

PGPR can stimulate plant growth directly as they can improve the supply of nutrients, such as nitrogen (Dobbelaere *et al*., 2003) and phosphorous (Rashid *et al*., 2004) or by production of phytohormones (Choong *et al.,* 2003; Stepanova *et al*., 2008) and ACC-deaminase synthesis (Arshad *et al*., 2007). Indirectly PGPR can also promote plant development by the suppression of pathogens mediated by different mechanisms such as antibiosis (Milner *et al*., 1996), iron sequestration by siderophores (Bar-ness *et al*., 1992), HCN (Keremer and Souissi, 2001), vitamin excretion (Streit *et al*., 1996), and cell wall degrading enzymes like chitinase (Ajit *et al*., 2006). So plant growth is promoted through reducing or neutralizing pathogen activity.

Azotobacter, Entrobacter, Bacillus, Burkholderia, Azospirillum, Pesudomonas, Acinetobacter, Arthrobacter, Alcaligenes, Serratia, Erwinia and *Flavobacterium* are some of the common PGPRs. (Sturz and Nowak, 2000; Bloemberg and Lugtenberg, 2001; Mayak *et al*., 2004). *Flavobacterium* is related to *chlorobia group* super phylum, *Bacteriodete* phylum, *Flavobacteria* class, *Flavobacterials* order and *Flavobacteriaceae* family. These bacteria are isomorphic, baciliform, aerobic and gram-negative. Lack of poly-β–hydroxybutyrate granules, endospore and fellagelin have been seen in these bacteria (Krieg and Holt, 1984). Plant growth promoting qualities of this bacterium is investigated despite it is plant growth stimulating bacteria. The most important growth promoting qualities like auxin production, the ability of using of ACC as nitrogen source, P-solubilization, siderophore, salicylic acid, chitinase and HCN production of *Flavobacterium* isolates were evaluated in microbial collection of soil biology department of soil and water institute.

2. Material and Methods

2.1 Isolation of Flavobacterium

44 isolates of *Flavobacterium* were isolated from wheat rhizosphere. Fluorescent Pseudomonad, strain PA14 was investigated for auxin, salycilic acid, siderophore, chitinase and HCN production and P-solubilisation and PA25 was evaluated for ACC-deaminase production as control isolate.

2.2 Quantification of IAA

Production of IAA was assayed based on the method described by Patten and Glick (2002). Strains were grown at 28°C for 48h on a rotary shaker in 100ml flasks containing 50ml TSB medium. Cells were then collected by centrifugation at 10000g for 15min. Finally 2ml of salkowsky was added to the supernatant. The absorbance of the pink- auxin complex, was read at 535nm in a Spectrophotometer. A standard curve was prepared with auxin dissolved in TSB medium. The quantity of auxin in the culture was expressed as gml^{-1}.

2.3 ACC-deaminase assay

The ability of strains to utilize ACC as sole nitrogen source was assayed as described by Amico *et al*. (2005) with some modifications. The bacteria were cultured first in rich medium (TSB). Stock solution of ammonium sulphate (13.21 g of $(NH_4)_2SO_4$ in 1000 ml of distilled H_2O) and DF salts minimal medium (without nitrogen source) were prepared and sterilized by autoclaving and 3.0 mM solution of ACC (30.33 mg of ACC in 10 ml of distilled H_2O) as the source of nitrogen was also filter-sterilized (0.2 μm). 300μL of each bacterial culture were added to 50-ml flasks containing 15 ml of sterile DF salts medium (with out nitrogen source). Two hundreds micro liters of ACC and $(NH_4)_2SO_4$ were added to the flasks. Cultures were incubated at 30°C on a rotary shaker at 200 rpm for 48 h. After incubation for 48 h the density of the culture was measured at 405 nm and the ratio of density in the culture of DF +ACC medium and DF+ $(NH_4)_2SO_4$ medium was evaluated.

2.4 Phosphorous assay

Sterilized Pikovskaya media (PKV) was poured in to sterilized Petri plates after solidification of media, a pinpoint inoculation of bacterial strains was made on the plates under aseptic conditions. The plates were incubated at 28°C for 8 days and observed for colony diameter and diameter of solubilization zone regularly

during 8 days. Solubilization index was evaluated according to the ratio of the total diameter (colony + halo Zone) and the colony diameter (Rashid *et al*., 2004).

2.5 CAS agar assay

Production of siderophores was determined by the method of Alexander and Zuberer, (1991). Isolates were grown on CAS agar plates. The presence of orange halos was recorded up to 7 days after incubation.

2.6 CASDA assay

Siderophore production in CASAD method was determined by the method of Shin et al., (2001). Strains were grown at 28oc for 48h on a rotary shaker in 100mL flasks containing 50mL succinate medium (succinic acid, 4.0g; K_2HPO_4, 6.0g; KH_2PO, 3.0g; $(NH_4)SO_4$, 1.0g; $MgSO_4.7H_2O$, 0.2g; distilled water, 1000mL; pH: 7.0) Cells were then collected by centrifugation at 10000g for 10min. Punch the CAS medium with cork borer and pour 35 mL from supernatant to these sinks after absorbing. The equal amount of supernatant should be poured in sinks, read the halo diameter.

2.7 Production of Salicylic acid (SA)

Strains were grown at 28^{o}C for 48h on a rotary shaker in 100ml flasks containing 50ml succinate medium. Cells were then collected by centrifugation at 6000g for 5min and 4ml of cell free culture was acidified with 1N HCl to adjust the pH to 2.0 and SA was extracted in $CHCl_3$ (2×2ml). To the pooled $CHCl_3$ phases, 4ml of distilled water and 5µl of 2M $FeCl_3$ were added. The absorbance of the purple iron- SA complex, which was developed in the aqueous phase, was read at 527nm in a Spectrophotometer. A standard curve was prepared with SA dissolved in succinate medium. The quantity of SA in the culture was expressed as μgml^{-1} (Meyer *et al.,* 1992).

2.8 Agar plate assay for chitinase

Chitinase production was determined in a defined medium composed of (gL^{-1}) colloidal chitin (Berger and Reynolds, 1958). It was added to nutrient agar medium. 12 mL of bacterial suspension was cultured in this medium and incubated for 120 hours in 28^{o}C. The ability of chitinase production was shown by clear halo around colonies (Robert and Cabib, 1988).

2.9 Cyanide production

Hydrogen cyanide (HCN) production from glycine was tested growing the bacteria in 10% trypic soy agar (TSA) supplemented with glycine ($4.4gL^{-1}$) and cyanogenesis was revealed using picric acid and Na_2CO_3 (0.5 and 2% respectively). Impregnated filter paper fixed to the underside of the Petridis lids. Results were read after five days of culture at 28^{o}C. A change in filter paper colors from yellow to orange-brown indicated production of HCN.(Yellow (1) limit cyanide production, orange (2) moderate cyanide production, light brown (3) relatively high cyanide production and brown (4) high cyanide production) (Donate-correa *et al*., 2004).

3. Results

44 isolates of *Flavobacterium* were isolated from wheat rhizosphere were tested for their plant growth promoting qualities. All of the strains could produce auxin. The average amount of auxin was 2.03 μgml^{-1} and its extent was changeable between 0.27 to 12.03. The high auxin activity was recorded for F9 and F32 respectively (Table 1).

None of the isolates produced salicylic acid (Table 1) when, the control isolate, PA14, produced 9.05 μgml^{-1} salicylic acid. Any isolate couldn't generate chitinase while the culture media was suitable for responding against chitinase and some of bacteria which were isolated from soil, made a bright halo around themselves (Table 1). None of the isolates could grow on CAS- agar media. CASAD method indicated, none of the isolates could produce siderophore (Table 1).

Any isolate couldn't generate ACC-deaminase (Table 2). F1, F3, F5, F6, F8, F9, F10, F12, F13, F14, F15, F17, F18, F19, F20, F21, F22, F24, F26, F27, F29, F30, F31, F32, F33, F35, F36, F37, F38, F39, F40, F41, F42, and F44 couldn't grow on DF minimal medium.

The result of unsoluble mineral P-solubilisation indicated that 34 isolates had been able to solve unsoluble mineral phosphorous (F1, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13, F14, F15, F16, F17, F18, F19, F20, F22, F23, F24, F28, F29, F30, F33, F34, F36, F37, F38, F39, F40 and F41). The average of solubilisation was 3.54 μgml^{-1} and it was ranged from 0 to 37.48 μgml^{-1}. F11 achieved the highest solubilization activity (37.48 μgml^{-1}). 10 isolates (F2, F21, F26, F27, F31, F32, F35, F42, F43 and F44) weren't permitted to solve phosphorous. Some of powerful isolates reduced the pH of media (5.04–5.60) and some of them increased it (5.63–5.80) in comparison with untreated control (pH=5.62). PH alternations was significant (p=0.01). There

was a negative significant correlation (r=-0.81**) between $Ca_3(PO_4)_2$ solubilization and pH (Figure 1). The result of HCN investigation indicated that all of the isolates could produce little amount of this metabolite (Table 1).

4. Discussion

Auxin is the most investigated hormone between plant growth regulators. The most common, best characterized and physiologically most active auxin in plant is indole-3-acetic acid (IAA). IAA is known to stimulate both a rapid response (e.g. increased cell elongation) and a long-term response (e.g. cell division and differentiation) in plants (Ahmad et al., 2005). In this investigation, all *Flavobacterium* isolates have been able to produce IAA. Asghar *et al.* (2004) showed, S_{58} and S_{89} which were related to *Flavobacterium* genus could beget IAA. The amount of IAA was 24.03 μgml^{-1} & 2.27 μgml^{-1} for S58 and 24.6 & 4.6 μgml^{-1} for S89 in presence and absence of tryptophan, respectively. In the other research, Cattelan *et al,* (1999) reported, GW_{2103} and LC_{1118} isolates of *Flavobacterium indologenes* had the ability of IAA production. IAA production by PGPRs is different. Ahmad *et al*. (2005) indicated that the amplitude of IAA in different densities of tryptophan was changeable between 5.32- 5.34 μgml^{-1}. The extent of IAA in *Flavobacterium* was changeable and it was enranged from 0.27 to 12.03 μgml^{-1} in this research.

P-solubilizing microorganisms (PSM) involve different character of microorganisms which turn insoluble organic compounds of phosphorous to soluble form (Raju and Reddy, 1999; Sundara *et al*., 2002). *Bacillus* and *pseudomonas* have been the most important P-solvent bacteria (Rashid *et al*., 2004; Bar-Yosef *et al*., 1999). Rashid *et al,* (2004) have shown that 10 isolates of different bacteria which were selected from rice rhizosphere, could solve $Ca_3(Po_4)_2$ in pikovskaya medium. The results indicated 10 isolates couldn't solve $Ca_3(Po_4)_2$ between 49 isolates. Cattelan *et al,* (1999) have shown that GW_{2103} and LC_{1118} isolates of *F. indologenes* couldn't solve it too. Remaining isolates had the low ability to solve it in this research. The highest solvability was 37.48 μgml^{-1} for F11.

In addition to the well-characterized mechanisms, such as auxin production and siderophore synthesis, that are employed by PGPR, it was recently proposed that many PGPR may stimulate plant growth through the activity of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Some bacteria degrade ACC to ammonia and α-ketobutyrate through ACC deaminase and finally it reduces ethylene (Penrose and Glick, 2003). Recent researches showed that some of *pseudomonas* could produce ACC deaminase (Safronova *et al*., 2006). Belimov *et al.* 2005 presented that different isolates of *Variovorax paradoxus* had the ability to make ACC deaminase but AY_{197006} and AY_{197009} isolates of *Flavobacterium* were unable to produce it. Cattelan *et al.* (1999) exhibited, GW_{2103} isolate of *F. indologenes* were unable to produce ACC deaminase and LC_{1118} isolate wasn't investigated due to its lack of growth. The result of our research demonstrated that no *Flavobacterium* isolate could make ACC deaminase too.

Siderophores are low-molecular-weight molecules that are secreted by many microorganisms in iron shortage condition. O'sullivan and O'Gara. (1992) revealed that many isolates of *P.fluorescens* have the ability of making siderophore. Belimov *et al.* (2005) reported, AY_{197010} isolate of *Pseudomonas* and AY_{197006} and AY_{197009} isolates of *Flavobacterium* could manufacture siderophore. In this research none of the isolates could grow on CAS-Agar medium. Probabely HDTMA (Hexadecyl trimethyl ammonium bromide) toxicity cause growth insufficiency (Sung *et al.,* 2001). The result of CASAD method have shown, any isolate couldn't make siderophore. Cattelan *et al*. (1999) reported, there was negligible production of siderophore by LC_{1118} and GW_{2103} of *F. indologenes*.

PGPR also activate plant defense resulting in systemic protection against plant pathogens, a phenomenon termed induced systemic resistance (ISR). Salicylic acid produced by PGPR in rhizosphere may be involved in ISR (Maurhofer *et al*., 1998). SA production by WC_{374}, WCS_{417r} (Leeman *et al*.,1996) and CHAO (Maurhofer *et al.*, 1994), *P.fluorescens* and also $7NSK_2$ isolate of *P.aeruginosa* (Demeyer and Hofte, 1997) was reported. Any *Flavobacterium* isolate could not produce SA in our study.

Chitinase activity is reported in broad-spectrum bacteria (Robert and Cabib, 1988; Neiendam-nielsen *et al*., 1998; Ajit *et al*., 2006). It has been reviwed by many researches that *P.fluorescens* could secrete chitinase as lytic enzyme (Nagarajkumar *et al*., 2004; Ajit *et al*., 2006; Saikiar e*t al*., 2005). Disability of chitinase production is reported in some bacteria. O'brien *et al*. 1987 reported *P.aeruginosa* and *P.putida* disabled to make chitinase. LC_{1118} and GW_{2103} isolates of *F. indologenes* couldn't generate chitinase too (Cattelan *et al.,* 1999). In this investigation, no *Flavobacterium* isolate produce chitinase.

Many different bacteria could produce HCN which is toxic for fungi (Blumer and Hass**,** 2000). HCN production by *P.fluoresens*, *P.aeruginosa* and *Chromobacterium uiolaceum* was reported by many researchers (Seddiqui *et al.,* 2003). Some of *Rhizobium* isolates were introduced as HCN producers by Antoun *et al*. (1998). Any isolate couldn't generate HCN in this study. Cattelan *et al*. 1999 indicated that LC_{1118} and GW_{2103} isolates of *F.*

indologenes weren't able to produce HCN. The investigation of these *Flavobacterium* isolates impression on plant growth factors is suggested according to their auxin production and mineral phosphorous solubilization ability.

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References

Ahmad, F., Ahmad, L. & Saghir, M. (2005). Indol acetic acid production by the indogenous isolate of *Azotobacter* and *Pseudomonas fluorescens* in the presence and absence of Tryptophan, *Turk. J. Biol*. 29:29-34.

Ajit, N.S., Verma, R. & Shanmugan, V. (2006). Extracellular chitinase of fluorescent pseudomonas antifungal to *Fusarium oxysporum* f.sp.*dianti* causing carnation wilt. *Curr. Microbiol*, 52:310-316.

Alexander, D.B. & Zuberer, D.A. (1991). Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol. Fertil. Soils*, 12: 39-45.

Amico, E.D., Cavalca, L. & Andreoni, V. (2005). Analysis of rhizobacterial communities in perennial *Graminaceae* from polluted water meadow soil, and screening of metal-resistant, potentially plant growth-promoting bacteria. *FEMS Microbiol. Ecol*, 52: 153-162.

Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R. & Lalande, R. (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as a plant growth promoting rhizobacteria on non legumes. *Plant Soil*, 204: 57-67.

Arshad, M., Saleem, M. & Hussain, S. (2007). Perspectives of bacterial ACC deaminase in phytoremediation. *Tren. Biotech*, 25(8): 356-361.

Asghar, H.N., Zaeir, Z.A. & Arshad, M. (2004). Screening rhizobacteria for improving the growth, yield and oil content of canola (*Brassica napus* L.). *Aust. J.Agric. Res,* 55:187-194.

Bar-ness, E., Hadar, Y., Chen, Y., Shanzer, A. & Libman, J. (1992). Iron uptake by plants form microbial siderophores. *Plant Physiol*, 99: 1329-1335.

Bar-Yosef, B., Rogers, R.D., Wolfram, J.H. & Richman, E. (1999). *Pseudomonas cepacia*-mediated rock phosphate solubilization in kaolinite and montmorillonit suspensions. *Soil Sci. Soc. Am. J,* 63: 1703-1708.

Belimov, A.A., Hontzeas, N., Safronova, V.I., Demchinskaya, S.V., Piluzza, G., Bulitta, S. & Glick, B.R. (2005). Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. czern.). *Soil Biol. Biochem*, 37: 241-250.

Benizri, E., Baudoin, E. and Guckert, A. (2001). Root colonization by inoculated plant growth-promoting rhizobacteria. *Biocon. Sci. Tech*, 11: 557-574.

Berger, L.R. & Reynolds, D.M. (1958). The chitinase system of a strain of *Streptomyces griseus*. *Biochem. Biophys. Acta*, 29: 522-534.

Bloemberg, G.V. & Lugtenberg, B.J.J. (2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria*., Curr. Opin. Plant Biol,* 4: 343-350.

Blumer, C. & Hass, D. (2000). Mechanism, regulation, and ecological role of bacterial cyanide biosenthesis. *Arch. Microbiol*, 173(3): 170-177.

Boven, G.D. & Rovira, A.D. (1999). The rhizosphere and its management to improve plant growth. *Adv. Agron*, 66: 1-102.

Cattelan, A.J., Hartel, P.G. & Fuhrmunn, J.J. (1999). Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil. Sci. Soc. Am. J*, 63: 1670-1680.

Choong, M.R., Mohamed, A.F., Chia-Hui, H., Munagala, S.R., Hun-Yun, W. & Puul, W.P. (2003). Bacterial volatiles promote growth in Arabidopsis. *Plant Biol*, 100:4927-4932.

De-Meyer, G. & Hofte, M. (1997). Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NKS2 induces resistance to leaf infection by *Botrytis conerea* on bean. *Phytopathology*, 87: 588-593.

Dobbelaere, S., Vanderleyden, J. & Okon, Y. (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci*, 22(2): 107-149.

Donate-Correa, J., Leon-Barrios, M. & Perez-Galdona, R. (2004). Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Island. *Plant Soil,* 266: 261-272.

Keremer, R.J. & Souissi, T. (2001). Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. *Curr. Microbiol,* 43(3): 182-186.

Kloepper, J.W. &Schroth, M.N. (1978). Plant growth-promoting rhizobacteria on radishes. *Proceeding of the International Conference on Plant Pathogenic Bacteria,* 2: 879-882.

Krieg, N.R., Holt, J.G. & Williams, H.J.H. (1984). *Bergeys Manual of Systematic Bacteriology*, Vol I, 9th ed.964p.

Leeman, M., Den-ouden, F.M., Van-Pelt, J.A., Dirkx, F.P.M., Steijl, H., Bakker, P.A.H.M. & Schippers, B. (1996). Iron availibility affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology*, 86: 149-155.

Maurhofer, M., Hase, C., Meuwly, P., Metraux, J.P. & Defago, G. (1994). Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizeing *Pseudomonas fluorescens* strain CHAO: influence of the gacA gene and of pyoverdine production. *Phytopathology,* 88: 139-146.

Maurhofer, M., Reimmann, C., Schmidli-sacherer, P., Heeb, S., Haas, D. & Defago, G. (1998). Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of system resistance in tobacco against tobacco necrosis virus. *Phytopathology*, 88: 678-684.

Mayak, S., Tirosh, T. &Glick, B.R. (2004). Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem*, 42:565–572.

Meyer, J.M., Azelvander, P. & Georges, C. (1992). Iron metabolism in *Pseudomonas*. Salicylic acid, a siderophore of *Pseudomonas fluorescens* CHAO. *Biofactors*, 4: 23-27.

Milner, J.L., SiloSuh, L., Lee, J.C., He, H., Clardy, J. & Handelsman, L. (1996). Produiction of kanosamine by *Bacillus cereus* UW85. *Appl. Environ. Microbiol*, 62:3061-3065.

Nagarajkumar, M., Bhaskaran, R. & Velazhahan, R. (2004). Involvement of secondary metabolities and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice, sheath blight pathogen. *Microbiol. Res*, 159: 73-81.

Neiendam-Nielsen, M., Sørensen, J., Fels, J. & Pedersen, H.C. (1998). Secondary metaboliteand endochitinase-dependent antagonism toward plant-pathogenic microfungi of *Pseudomonas fluorescens* isolates from sugar beet rhizosphere. *Appl. Environ. Microbial*, 64:3563-3569.

O'Brien, M. & Colwell, R. (1987). A Rapid Test for Chitinase activity that uses 4-Methylumbelliferyl-N-Acetyl-β-D-Glucosaminide. *Appl. Environ. Microbiol*, 53:1718-1720.

O'Sullivan, D.J. & O'Gara, F. (1992). Traits of *Pseudomonas fluorescens* spp. involved in suppression of plant root pathogens. *Microbiol. Rev*, 56: 662-676.

Patten, C. & Glick, B. (2002). Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Appl. Environ. Microbiol*, 3795-3801.

Penrose, M. & Glick, R. (2003). Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant*, 118: 10-15.

Raju, R.A. & Reddy, M.N. (1999). Effect of rock phosphate amended with phosphate solubilizing bacteria and farmyard manure in wetland (*Oryza sativa*). *Ind.J. Agri. Sci,* 69: 451-453.

Rashid, M., Khalil, S., Ayub, N., Alam, S. & Latif, F. (2004). Organic Acids productions solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pak. J. Biol. Sci,* 7: 187-196.

Roberts, W.K. & Selitrennikoff, C.P. (1988). plant and bacterial chitinases differ in antifungal activity. *J. Gen. Microbiol,* 134: 169-176.

Safronova, V.I., Stepanok, V.V., Engqvist, G.L., Alekseyev, Y.V. & Belimov, A.A. (2006). Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fret. Soils*, 42:267–272.

Saikiar, R., Singh, B., Kumar, R. & Arora, D. (2005). Detection of pathogenesis-related proteins-chitinase and β-1,3-glucanse in induced chickpea. *Curr. Sci*, 89: 659-663.

Shin, S.H., Yong, L., Lee, S.E., Yang, W. & Rhee, J.H.N. (2001). CAS agar diffusion assay for the measurement of siderophores in biological fluids. *J. Microbiol. Meth*, 44:89-95.

Siddiqui, I.A., Shaukat, S.S., Khan, G.H. & Ali, N.I. (2003). Supperssion of *Meloidogyne javanica* by *Pseudomonas aeruginosa* IE-6S+ in tomato: the influence of NaCl, oxygen and iron level. *Soil.Biol.Biochem*, 35:1625-1634.

Stepanova, A.N, Robertson-Hoyt, J., Yun, J., Benavente, L.M., Xie, D.Y., Dolezal, K., Jurgens, S.G. & Alonso, J.M. (2008). TAA1-mediated auxin biosenthesis is essential for hormone crosstalk and plant development. *Cell*. 133:177-191.

Streit, W.R., Joseph, C.M. & Phillips, D.A. (1996). Biotin and other water-soluble vitamins are key growth factors for alfalfa root colonization by *Rhizobium meliloti* 1021. Mol. *Plant-Microbe Interac,* 5: 330-338.

Sturz, A.V. & Nowak, J. (2000). Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. *Appl. Soil Ecol,* 15: 183-190.

Sundara, B., Natarajan, V. & Hari, K. (2002). Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crop. Res*, 77:43-49.

Sung, H.S., Yong, L., Shee, E. & Nam, W.Y. (2001). CAS agar diffusion assay for the measurement of siderophores in biological fluids. *J. Microbial. Methods,* 44:89-95.

Table 1. PGP qualities in studied isolates

isolate	chitinase	siderophore CASAD (mM)	siderophore CAS (halo colony^{-1})	SA (μgmL^{-1})	DF+ACC DF+ A.S.	Phosphate solubilization (μgmL^{-1}) and pH	auxin (μgmL^{-1})	HCN
F1	-	-	-	-	-	1/24, 5/64*NOPQ	1/10KLMNOP	1
F2	-	-	-	-	0/10	0, 5/60T	0/50OPQR	1
F3	-	-	-	-	-	4/26, 5/58FGH	0/57NOPQR	1
F4	-	-	-	-	0/35	3/85, 5/64HI	0/53NOPQR	1
F5	-	-	-	-	-	./62, 5/65RST	0/43PQR	1
F6	-	-	-	-	-	3/64, 5/59I	1/17KLMNO	1
F7	-	-	-	-	0/24	6/28, 5/36E	5/17E	1
F8	-	-	-	-	-	4/67, 5/67F	0/80LMNOPQR	1
F9	-	-	-	-	-	0/83, 5/62PQRS	12/03A	1
F10	-	-	-	-	-	7/15, 5/45D	3/10G	1
F11	-	-	-	-	0/26	37/48, 5/04A	0/50OPQR	1
F12	-	-	-	-	-	0/76, 5/63QRS	1/17KLMNO	1
F13	-	-	-	-	-	1/79, 5/63KLMN	0/83LMNOPQR	1
F14	-	-	-	-	-	1/11, 5/71OPQR	1/10KLMNOP	1
F15	-	-	-	-	-	3/78, 5/63HI	3/93F	1
F16	-	-	-	-	0/18	1/17, 5/60OPQR	2/47H	1
F17	-	-	-	-	-	4/54, 5/59FG	0/63MNOPQR	1
F18	-	-	-	-	-	9/62, 5/52C	2/33HI	1
F19	-	-	-	-	-	3/03, 5/54J	3/13G	1
F20	-	-	-	-	-	16/75, 5/19B	6/97C	1
F21	-	-	-	-	-	0, 5/60T	8/83B	1
F22	-	-	-	-	-	6/46, 5/42E	3/20G	1
F23	-	-	-	-	0/27	1/86, 5/63KLM	0/40QR	1
F24	-	-	-	-	-	3/71, 5/55HI	1/20KLMN	1
F25	-	-	-	-	0/07	1/86, 5/72KLM	1/87IJ	1
F26	-	-	-	-	-	0, 5/60T	1/40JKL	1
F27	-	-	-	-	-	0, 5/60T	0/37QR	1
F28	-	-	-	-	0/19	1/38, 5/57MNOP	0/47PQR	1
F29	-	-	-	-	-	2/13, 5/80K	6/10D	1
F30	-	-	-	-	-	1/99, 5/63KL	0/47PQR	1
F31	-	-	-	-	-	0, 5/60T	0/67MNOPQR	1
F32	-	-	-	-	-	0, 5/60T	0/27R	1
F33	-	-	-	-	-	0/07, 5/63T	0/57NOPQR	1
F34	-	-	-	-	0/44	4/46, 5/47E	4/33F	1
F35	-	-	-	-	-	0, 5/60T	0/37QR	1
F36	-	-	-	-	-	2/34, 5/78K	0/33QR	1
F37	-	-	-	-	-	3/99, 5/82GHI	0/60MNOPQR	1
F38	-	-	-	-	-	1/45, 5/70LMNO	0/33QR	1
F39	-	-	-	-	-	0/62, 5/70RST	0/60MNOPQR	1
F40	-	-	-	-	-	0/48, 5/59ST	1/27KLM	1
F41	-	-	-	-	-	1/45, 5/75LMNO	1/03KLMNOPQ	1
F42	-	-	-	-	-	0, 5/60T	0/70MNOPQR	1
F43	-	-	-	-	-	0, 5/60T	4/00F	1
F44	-	-	-	-	0/15	0, 5/60T	1/63JK	1
PA14	-	0/92	0/17	9/05	0/10	362/34, 3/26	2/46	5

- No production of metabolite

*From left direction, the first number is related to phosphorous solubilization and the second number is related to pH.

The averages containing same letter don't have significant difference in each column.

Table 2. Growth comparison of isolates in DF, DF containing ACC and DF containing Ammonium sulfate

isolate	Different medium absorbance in 405 nm			
	DF+A.S.	DF+ACC	DF	DF+ACC
F_1	*	*	*	*
F_2	0.16	0.02	0	0.10
F_3	*	*	*	*
F_4	0.15	0.05	0.02	0.35
F_5	*	*	*	*
F_6	*	*	*	*
F_7	0.16	0.04	0.02	0.24
F_8	*	*	*	*
F_9	*	*	*	*
F_{10}	*	*	*	*
F_{11}	0.23	0.06	0.06	0.26
F_{12}	*	*	*	*
F_{13}	*	*	*	*
F_{14}	*	*	*	*
F_{15}	*	*	*	*
F_{16}	0.17	0.03	0.03	0.18
F_{17}	*	*	*	*
F_{18}	*	*	*	*
F_{19}	*	*	*	*
F_{20}	*	*	*	*
F_{21}	*	*	*	*
F_{22}	*	*	*	*
F_{23}	0.15	0.04	0.01	0.27
F_{24}	*	*	*	*
F_{25}	0.34	0.03	0.16	0.07
F_{26}	*	*	*	*
F_{27}	*	*	*	*
F_{28}	0.16	0.03	0.01	0.19
F_{29}	*	*	*	*
F_{30}	*	*	*	*
F_{31}	*	*	*	*
F_{32}	*	*	*	*
F_{33}	*	*	*	*
F_{34}	0.20	0.09	0.01	0.44
F_{35}	*	*	*	*
F_{36}	*	*	*	*
F_{37}	*	*	*	*
F_{38}	*	*	*	*
F_{39}	*	*	*	*
F_{40}	*	*	*	*
F_{41}	*	*	*	*
F_{42}	*	*	*	*
F_{43}	0.17	0.03	0.01	0.15
F_{44}	*	*	*	*
PA_{25}	2.72	2.23	0.28	0.82

*: No growth A.S: Ammonium Sulphat

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Table 3. Analysis of variance of different isolate of Flavobacterium in Auxin production and phosphorous solubilization

Alternation sources	Freedom degree	Mean square	
		Auxin production	phosphorous solubilization
isolate	43	18/717**	4/536**
error	88	0/115	0/004

**- significant ($p<0.01$)

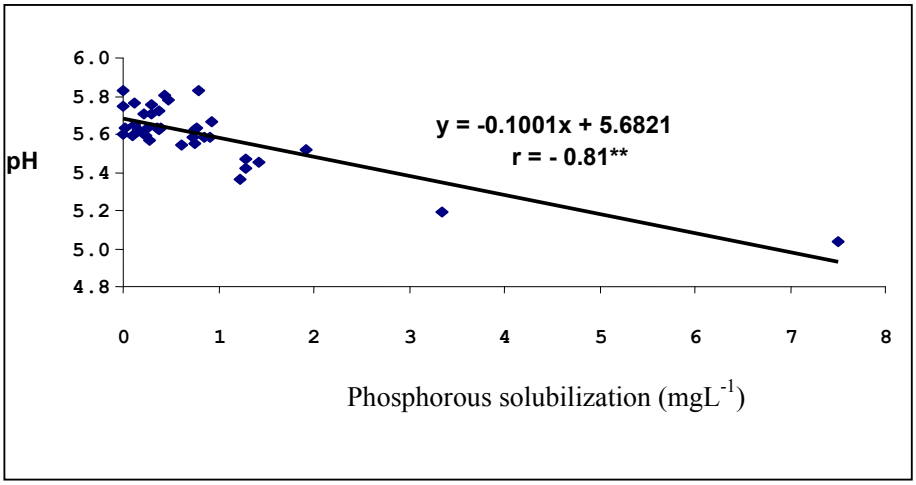

Figure 1. Correlation between Phosphorous solubilization and pH