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ORIGINAL ARTICLE

Effect of zinc-phosphate-solubilizing bacterial isolates on growth of *Vigna radiata*

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Abstract This study examined the effects of five bacterial isolates (U, 8M, 36, 102, and 111) on the growth of *Vigna radiata*. Bacterial isolates were applied alone, or together with zinc phosphate [$Zn_3(PO_4)_2 \cdot 4H_2O$]. The maximum increase in all plant growth parameters was seen when seedlings were inoculated with isolate 102. Isolate 36 with 1 mM zinc phosphate showed the maximum increase in seedling length (35.1 cm) as compared to controls. Isolate 111 was the best phosphate solubilizer, releasing 13.29 ppm phosphorous (P) in soil when used in combination with 1 mM salt, whereas isolate 36 showed maximum uptake of P, leaving only 4.63 ppm in soil.

Keywords Zinc solubilization · Plant growth promotion · Time induction assay

Introduction

In the rhizosphere, very important and intensive interactions take place between plants, soil, microorganisms and soil microfauna, influenced by compounds exuded by roots, and microorganisms feeding on these compounds (Antoun and Prevost 2006).

These plant-growth-promoting bacteria can enter into a symbiotic relationship with plants (i.e., *Rhizobium*-legume and *Frankia*-actinorhizal plant symbiosis), but non-symbiotic, free-living soil bacteria can also promote plant growth (Glick 1995). Beneficial rhizobacteria are termed either plant-growth-promoting rhizobacteria (PGPR) or plant-health-promoting rhizobacteria (PHPR) according to their mode of action (Sikora 1992). PGPR may induce plant growth promotion through different direct or indirect modes of action (Glick et al. 1999; Antoun and Prevost 2006).

Zinc—one of the eight essential trace elements or micronutrients required for the normal healthy growth and reproduction of crop plants—is required in relatively small concentrations in plant tissues (5–100 mg/kg). Root cell membrane permeability is increased under Zn deficiency, which might be related to the function of Zn in cell membranes (Parker et al. 1992). Zn deficiency is well reported in the soils of much of the world. Developing countries like Pakistan are facing problems in cereal cultivations. Cereals play role in satisfying daily calorie intake in the developing world, but the Zn concentration in the grain is inherently very low, particularly when grown on Zn-deficient soils. The major reason for the widespread occurrence of Zn deficiency problems in crop plants is the low solubility of Zn in soils rather than a low total amount of Zn (Cakmak 2008).

Chabot et al. (1993) demonstrated growth stimulation of maize and lettuce by several microorganisms capable of mineral phosphate solubilization. These microbes also exhibit other traits beneficial to plants, such as production of phytohormones, antibiotics, siderophores, vitamins, antifungal substances and hydrogen cyanide (Rodriguez and Fraga 1999). It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms (Salih et al. 1989). The nature and amount of organic acid generated by microorganisms depend mainly on medium pH, buffering capacity and carbon source (Mattey 1992).

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Table 1 Characterization of bacterial isolates

Isolate	Cell shape	Gram stain	Motility	Spore formation[a]	Oxidase	Catalase	Nitrate reduction	Glucose fermentation
U	Coccus (clusters)	+	+	NSF	+	+	−	+
8M	Coccus (chains)	+	+	NSF	−	−	+	+
36	Rods (scattered)	+	+	SF	+	+	+	+
102	Rods (single)	−	+	NSF	+	+	−	+
111	Rods (scattered)	+	+	SF	+	+	+	+

[a] *NSF* Non spore former, *SF* spore former

Gluconic acid is reported as the principal organic acid produced by phosphate-solubilizing bacteria such as *Pseudomonas* sp., *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia* (Goldstein et al. 1993). Other organic acids, such as glycolic, oxalic, malonic, and succinic acid, have also been identified among phosphate solubilizers (Hilda and Fraga 1999). A large number of bacteria (Rodriguez and Fraga 1999; Harris et al. 2006; Perez et al. 2007) have been isolated and characterized for their ability to solubilize unavailable reduced phosphorus (P) to available forms. *Vigna radiata* is one of the most important pulse crops of South Asia, and the major land area of this part of the world requires essential nutrients such as Zn and P for the proper growth of this plant. In this study, we report the isolation, characterization, and zinc phosphate solubilization of five bacterial isolates, and their ability to enhance the growth of *V. radiata* plants.

Materials and methods

Isolation and characterization of microorganisms

Five bacterial isolates (Table 1) from the area of the University of the Punjab, Lahore, Pakistan, were allowed to grow in nutrient broth (pH 7) at 37°C for 72 h. During incubation, bacterial growth (in terms of optical density) was measured at equal time intervals, to determine the growth curve of each bacterium. Morphological and biochemical characterization of the bacteria was performed according to the Cappuccino and Sherman 2001.

Table 2 Zinc phosphate [$Zn_3(PO_4)_2{\cdot}4H_2O$ (5 mM)] solubilization activity of different bacterial isolates

Isolate	Zone of solubilization (mm)
U	28
8M	28
36	6
102	9
111	–

Antimicrobial activity of bacterial isolates

Four indicator groups, i.e., *Neisseria* as Gram negative cocci, *Staphylococcus* as Gram positive cocci, *Escherichia coli* as Gram negative rods and *Bacillus* sp. as Gram positive rods were used during this study. Antimicrobial activity of bacterial isolates was checked by the agar well diffusion method against the indicator strains (Parekh and Chanda 2006).

Growth conditions

Bacterial isolates were screened for zinc solubilization by plate assay. Tris minimal salt medium (TMSM) (Fasim et al. 2002) was used for the analysis of zinc phosphate solubilization activity at 5 mM concentration. The degree of solubilization by each isolate was determined by measuring the zone of solubilization. An induction time assay for metal solubilization was performed in an order to determine the time of initiation of metal solubilization while simultaneously monitoring growth of the source isolate.

Analysis of plant–microbe interaction

Soil samples were analyzed for the following physical properties: bulk density, soil moisture and texture, and pH. Seeds of *V. radiata* were sterilized by 0.1% $HgCl_2$ solution. Bacterial growth of each isolate was suspended

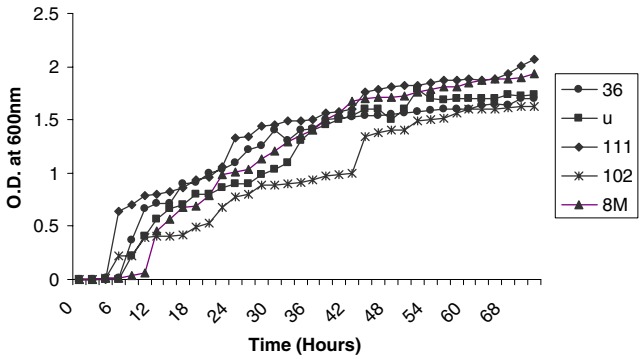

Fig. 1 Growth curves of five selected isolates

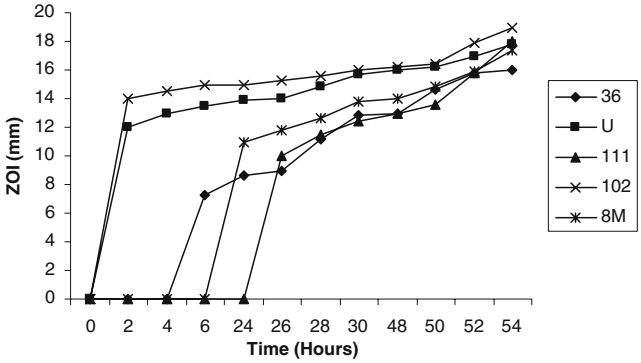

Fig. 2 Time induction assay for zinc phosphate solubilization of bacterial isolates

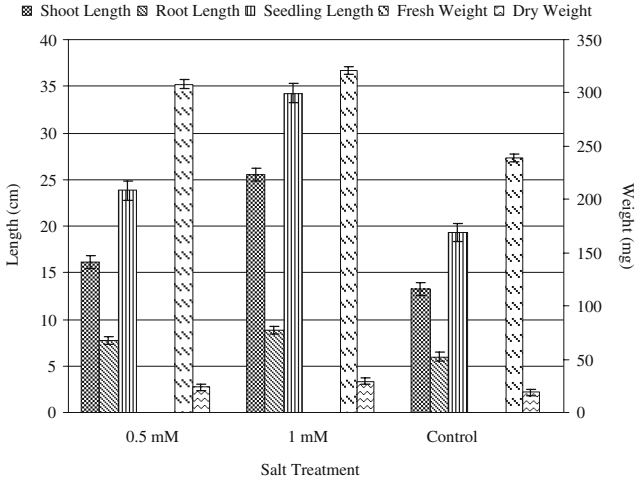

Fig. 3 Effect of zinc phosphate on plant growth promoting of *Vigna radiata*

in autoclaved distilled water and the suspension was prepared and adjusted to 10^8 cells/ml at 600 nm. Seeds treated with bacteria and salt were allowed to germinate and grow under controlled light (16 h light/8 h dark) and temperature (28°C) for 8 days along with non-treated seeds in various pots (120 g soil in each). The impact of $Zn_3(PO_4)_2{\cdot}4H_2O$ in two different concentrations (0.5 mM and 1 mM) on plant growth was assessed. The following different treatments were used to check their effect on plant growth promotion: (i) Seed + Bacterial isolates; (ii) Seed + Salt (0.5 mM and 1 mM); (iii) Seed + Water (Control); (iv) Bacterial isolates + Salt (0.5 mM and 1 mM) + Seed; (v) Salt + Water (Soil solubilization of salts).

Statistical analyses

After harvesting, the following parameters were measured: shoot length, root length, number of leaves, fresh weight and dry weight. Data obtained was analyzed statistically by following the method of Steel and Torrie (1981). Means, standard errors of the mean and least significant differences were calculated.

Table 3 Physicochemical characteristics of experimental soil. *SBP* Soil before planting, *EC* exchange capacity

Treatment	Texture class of soil	pH	EC	Organic matter (%)	Phosphorus (ppm)
Control	Silt Loam	8.35	1.428	3	8.22
Control+0.5 mM[a]	Silt Loam	8.25	1.394	2.8	9.12
Control+1 mM	Silt Loam	8.40	1.380	2.9	9.11
Isolate 36	Silt Loam	8.38	1.409	2.6	4.63
36+0.5 mM	Silt Loam	8.40	1.493	3.3	8.48
36+1 mM	Silt Loam	8.36	1.292	2.9	7.25
Isolate 111	Silt Loam	8.43	1.448	2.7	6.52
111+0.5 mM	Silt Loam	8.34	1.428	3.1	7.57
111+1 mM	Silt Loam	8.30	1.194	3.2	13.29
Isolate 102	Silt Loam	8.39	1.559	3	6.19
102+0.5 mM	Silt Loam	8.31	1.437	3.1	7.01
102+1 mM	Silt Loam	8.29	1.740	2.8	11.01
Isolate 8M	Silt Loam	8.40	1.412	3	7.35
8M+0.5 mM	Silt Loam	8.25	1.352	3.3	9.29
8M+1 mM	Silt Loam	8.22	1.646	3.2	10.04
Isolate U	Silt Loam	8.48	1.399	2.9	6.47
U+0.5 mM	Silt Loam	8.39	1.326	2.7	8.21
U+1 mM	Silt Loam	8.38	1.312	3.4	7.46
Soil+0.5 mM	Silt Loam	8.38	1.324	2.7	9.32
Soil+1 mM	Silt Loam	8.41	1.676	3	10.32
SBP	Sandy Loam	8.38	12.10	0.74	2.052

[a] mM $Zn_3(PO_4)_2{\cdot}4H_2O$

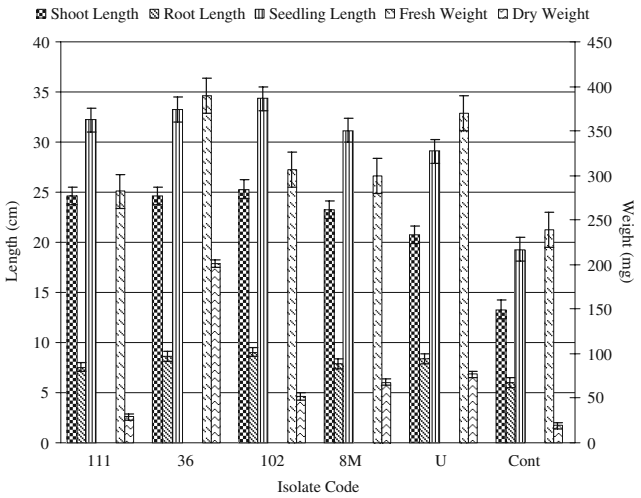

Fig. 4 Effect of bacterial isolates on promoting plant growth of *V. radiata*

Results and discussion

Time induction assay for zinc phosphate solubilization

The selected isolates were characterized morphologically and physically as shown in Table 1. For the majority of isolates, the lag phase lasted for 2 h, after which an increase in growth was recorded. Only in isolate U, was a prolonged lag phase of 6 h observed. In all isolates, growth increased gradually with time and in no case was a decline phase observed (Fig. 1). All isolates showed sizeable antimicrobial activity against the tested bacterial species, confirming their capability to produce antibiotics (data not shown). In the plate assay, formation of clear halos in close vicinity to bacterial growth in the presence of the insoluble salt of $Zn_3(PO_4)_2{\cdot}4H_2O$ indicated metal solubilization (Table 2). Isolates 102, U, 111, 8M and 36 began solubilization of $Zn_3(PO_4)_2{\cdot}4H_2O$ after 2, 2, 10, 8 and 6 h of incubation, respectively (Fig. 2).

Effect of zinc phosphate on plant growth

Some physicochemical characteristics of the experimental soil are given in Table 3. There was an increase in root length, shoot length and fresh weight of seedlings when salt was mixed in soil. With 1 mM $Zn_3(PO_4)_2{\cdot}4H_2O$, the increase in seedling length was 34.25 cm as compared to 23.8 cm and 19.3 cm seedling length with 0.5 mM $Zn_3(PO_4)_2{\cdot}4H_2O$ and control seedlings, respectively (Fig. 3).

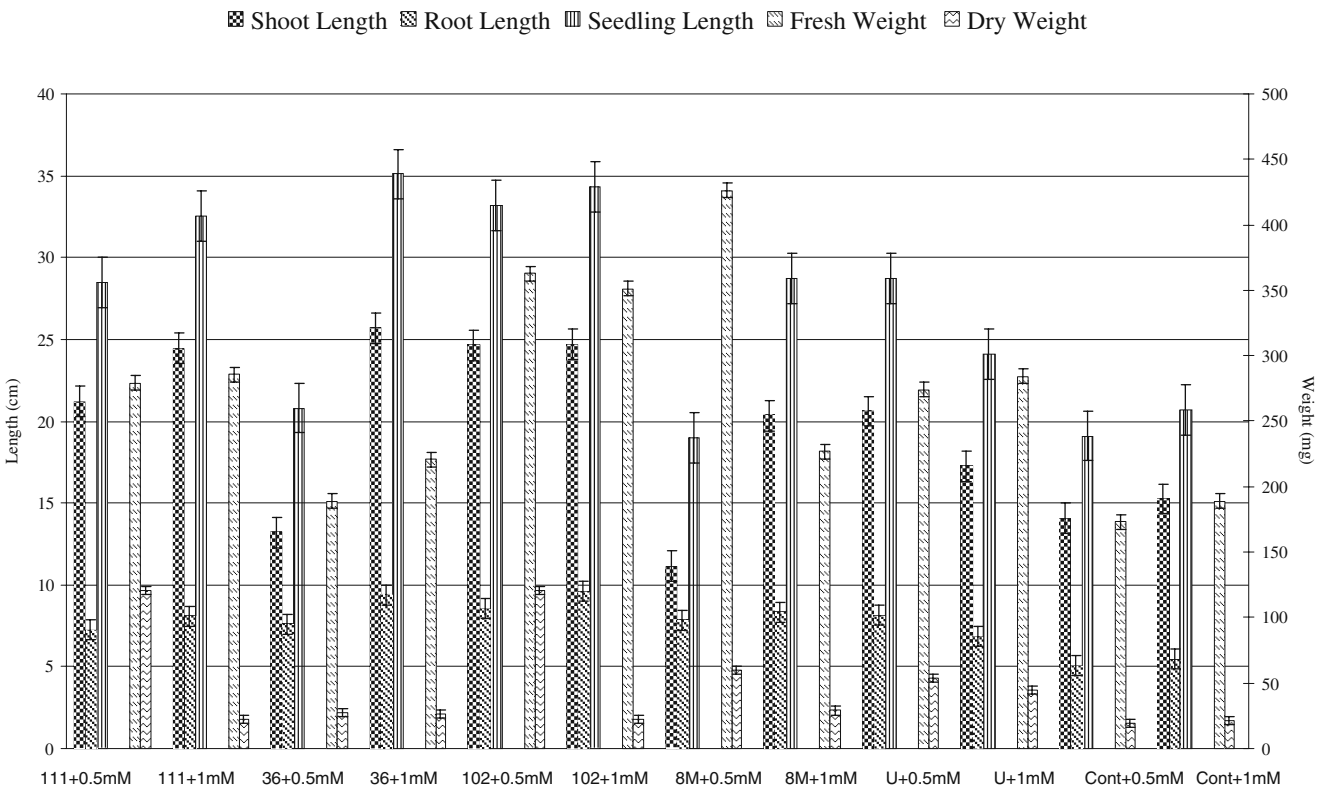

Fig. 5 Effect of bacterial isolates supplemented with zinc phosphate on promoting plant growth of *V. radiata*

Effect of bacteria on plant growth

Significant increases in root length and shoot length were observed in seedlings inoculated with isolates 111, 36, 8 M, U over non-inoculated controls, with maximum increase in the case of isolate 102 (Fig. 4). Similar results were noted with both fresh weight and dry weight of inoculated seedlings as compared to controls.

Effect of bacteria supplemented with zinc phosphate on plant growth

The increase in plant growth was observed not only with supplementation of bacterial isolates but also with increased salt concentration. The maximum rise in seedling length was seen in the case of isolate 36 with 1 mM salt concentration, with an increase of almost 1.7 times compared to salt alone. However, with isolate 102, the difference in seedling length between salt concentrations 1 mM and 0.5 mM was not significant (Fig. 5).

Statistical analyses

To check the extent of $Zn_3(PO_4)_2{\cdot}4H_2O$ solubilization in soil, 0.5 mM and 1 mM of $Zn_3(PO_4)_2{\cdot}4H_2O$ salt was mixed with soil, and subjected to the same incubation period and conditions as the other treatments. Increased phosphorus content was observed in soil: 9.32 ppm in the case of 0.5 mM salt and 10.32 ppm in the presence of 1 mM salt as compared to 2.05 ppm in unused soil and 8.22 ppm in the control. However, this increase did not reach the level of the P contents of isolate 111 + 1 mM salt and isolate 102 + 1 mM salt, with 13.29 ppm and 11.0 ppm P, respectively (Table 3).

Gould (1991) and Podolak et al. (1996) suggested that the antimicrobial effect of organic acids could be due to the fact that they cause a reduction in pH. Thus, organic acids can be said to be acting as antimicrobial agents on the one hand because they act as agents causing zinc solubilization, and on the other because, as mentioned above, acid production is an important phenomenon in metal solubilization. The bacterial species used in this study show promise for use in soils that are deficient in Zn or where insoluble zinc phosphate is abundant. Overall, the isolates in this study showed beneficial traits that can qualify them as potential biofertilizers. As the bacteria present in the rhizosphere interact with *V. radiata*, affecting plant growth by enhancing mineral and water uptake, providing antibiotics to inhibit soil pathogens and producing the plant growth regulators. In this regard, our results parallel those of Kapulnik (1996). Some other factors, such as production of hormones and siderophores, and enhancement of the uptake of other essential plant nutrients, might also be involved in the improvement of seedling growth. The significance and impact of this study is that these bacteria have the ability to enhance mungbean plant growth in the presence of water-insoluble zinc phosphate, and could be utilized to improve the growth of economically important cash crops. More research using consortia of different PGPR working together is needed in order to reveal some of the mechanisms involved in the synergistic interactions that lead to increased plant growth promotion.

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References

Antoun H, Prevost D (2006) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302:1–17

Cappuccino J, Sherman N (2001) Microbiology: a laboratory manual, 6th edn. Benjamin/Cummings, New York

Chabot R, Antoun H, Cescas MP (1993) Stimulation of maize growth and lettuce with Cos inorganic phosphorus solubilizing microorganisms. Can J Microbiol 39:941–947

Fasim F, Ahmed N, Parsons R, Gadd GM (2002) Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery. FEMS Microbiol Lett 213:1–6

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London

Goldstein AH, Rogers RD, Mead G (1993) Mining by microbe. Biotechnology 11:1250–1254

Gould GW (1991) Antimicrobial compound. In: Goldberg I, Williams R (eds) Biotechnology and food ingredients. Van Nostrand Reinhold, New York, pp 461–483

Harris JN, New PB, Martin PM (2006) Laboratory tests can predict beneficial effects of phosphate-solubilizing bacteria on plants. Soil Biol Biochem 38:1521–1526

Hilda R, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–359

Kapulnik Y (1996) Plant growth promotion by rhizobacteria. In: Waisel Y, Eshel A, Kafkazi U (eds) Plant roots, the hidden half. Dekker, New York, pp 769–781

Mattey M (1992) The production of organic acids. Crit Rev Biotechnol 12:87–132

Parekh J, Chanda S (2006) In-vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr J Biomed Res 9:89–93

Parker DR, Aguilera JJ, Thomason DN (1992) Zinc-phosphorus interactions in two cultivars of tomato (*Lycopersicon esculentum* L.) grown in chelator-buffered nutrient solutions. Plant Soil 143:163–177

Perez E, Sulbaran M, Ball MM, Yarzabal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria

naturally colonizing a limonitic crust in the southeastern Venezuelan region. Soil Biol Biochem 39:2905–2914

Podolak PK, Zayas JF, Kastner CL, Fung DYC (1996) Inhibition of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on beef by application of organic acids. J Food Prot 59:370–373

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Salih HM, Yahya AY, Abdul-Rahem AM, Munam BH (1989) Availability of phosphorus in a calcareus soil treated with rock phosphate or superphosphate as affected by phosphate dissolving fungi. Plant Soil 5:120–181

Sikora RA (1992) Management of the antagonistic potential in agricultural ecosystems for the biological control of plant parasitic nematodes. Annu Rev Phytopathol 30:245–270

Steel RGD, Torrie JH (1981) Principles and procedures of statistics, a biometric approach, 2nd edn. McGraw Hill, New York