Ann Microbiol (2012) 62:93–105
DOI 10.1007/s13213-011-0233-6

ORIGINAL ARTICLE

Characterization of phosphate-solubilizing microorganisms from salt-affected soils of India and their effect on growth of sorghum plants [*Sorghum bicolor* (L.) Moench]

Ramakrishnan Srinivasan · Ajjanna R. Alagawadi · Mahesh S. Yandigeri · Kamlesh Kumar Meena · Anil K. Saxena

Received: 22 July 2010 / Accepted: 9 February 2011 / Published online: 25 February 2011

Abstract Indigenous phosphate-solubilizing microorganisms (PSM) were studied in the rhizosphere and non-rhizosphere of salt-affected soils. A total of 23 phosphate-solubilizing bacteria (PSB) and 35 phosphate-solubilizing fungi (PSF) were isolated from 19 samples collected from different locations in Karnataka and Madhya Pradesh of India. The counts of PSB and PSF showed large variations. The bacterial isolates were identified using BIOLOG as belonging to *Pseudomonas*, *Xanthomonas*, *Bacillus*, *Aerococcus*, *Alteromonas*, *Erwinia* and *Enterobacter*. Fungal isolates were identified as *Aspergillus* and *Penicillium* based on colony morphology, microscopic observations and BIOLOG. All the PSB and PSF isolates were able to produce both indole acetic acid (IAA) and gibberellic acid (GA). Bacterial isolates produced IAA in the range of 0.74 to 9.53 μg 25 ml^{-1}, and GA ranged from 2.08 to 12.55 μg 25 ml^{-1}. The amount of IAA produced by the PSF isolates ranged from 2.33 to 8.69 μg 25 ml^{-1}, and GA ranged from 3.44 to 14.80 μg 25 ml^{-1}. Fungal isolates were superior to bacterial isolates in terms of P solubilization as measured by release of inorganic phosphate (Pi) from tricalcium phosphate, increase in stem girth, root length, root dry matter and total dry matter of sorghum plants.

Keywords Microorganisms · Sorghum · Phosphate solubilization · Plant growth · Salt-affected soils

R. Srinivasan
College of Dairy and Food Science Technology,
Maharana Pratap University of Agriculture and Technology,
Udaipur 313 001, Rajasthan, India

A. R. Alagawadi
Department of Agricultural Microbiology, College of Agriculture,
University of Agricultural Sciences,
Dharwad 580 005, Karnataka, India

M. S. Yandigeri (✉) · K. K. Meena
National Bureau of Agriculturally Important Microorganisms,
Kushmaur,
Mau Nath Bhanjan 275 101, Uttar Pradesh, India
e-mail: micromahesh@gmail.com

A. K. Saxena
Division of Microbiology, Indian Agricultural Research Institute,
Pusa Campus,
New Delhi 110 012, India

Introduction

Phosphorus (P) is second only to nitrogen as a mineral macronutrient required by both plants and microorganisms. It plays a major role in certain key processes, such as the accumulation and release of energy during cellular metabolism, photosynthesis, nutrient transport within the plant cell and the transfer of genetic characteristics from one generation to the next. Total P in the soil ranges from 0.01% to 0.2% but only a small amount of it is available to plants. The P in soil exists as soil solution P, insoluble inorganic P and insoluble organic P, of which only solution P is accessible to plants. Only a very small fraction of soil P exists in the soil solution because of its extreme reactivity. Consequently, P deficiency is a widespread problem and P fertilizers are almost universally required to maintain crop production. However, the efficiency of applied P rarely exceeds 30% due to its fixation either in the form of iron/aluminium phosphate in acidic soils or in the form of calcium phosphate in neutral-to-alkaline soils. Use of unprocessed rock phosphate (RP) is being given due importance in developing countries like India to meet the

existing total demand for P fertilizers because of high costs involved in processing. The direct use of RP is restricted to acidic soils as the benefit from the addition of RP to neutral calcareous soils is low in comparison to the benefits obtained with more acidic soils. However, the use of RP in combination with phosphate-solubilizing biofertilizers could enhance P availability to crop plants. Diverse groups of microorganisms, such as bacteria, fungi, actinomycetes and yeasts, are known to solubilize insoluble forms of inorganic P (Kucey et al. 1989) by bringing about favorable changes in soil reactions in the soil microenvironment leading to solubilization of inorganic phosphate sources. Many microorganisms in the soil are able to solubilize insoluble forms of Ca-bound P by excreting organic acids that either dissolve RP directly or chelate Ca^{2+} ions to bring the P_i into solution (Katznelson and Bose 1959), or by producing various siderophores, mineral acids, protons, humic substances, CO_2 and H_2S. This phenomenon of solubilizing insoluble inorganic phosphates by microorganisms is known as 'mineral phosphate solubilization' (Goldstein 1986). The important genera of phosphate-solubilizing bacteria (PSB) include *Achromobacter*, *Aerobacter*, *Alkaligenes*, *Bacillus*, *Pseudomonas*, *Serratia* and *Xanthomonas*, *Aeromonas*, *Klebsiella*, *Enterobacter* (Chen et al. 2006; Kundu et al. 2009). In addition, certain fungi known as phosphate solubilizing fungi (PSF) have also been shown to solubilize insoluble phosphate (Rajankar et al. 2007). Several workers have studied mineral phosphate solubilizers of different locality, soils, crops, etc., but little is known about the phosphate solubilizers of problem soils in general and salt-affected soils in particular.

Salt-affected soils contain various ions that can interfere with uptake of water and may be toxic to a large number of organisms. El-Gibali et al. (1977) reported higher populations of PSB in the rhizosphere zones of alkaline soils, whereas Raj (1980) found lower populations of PSB in alkaline soil as compared to red sandy loam, medium black clay and laterite soils. Phosphobacteria capable of dissolving insoluble P compounds have been reported from marine environments, indicating their presence in habitats containing high salt (Promod and Dhevendaran 1987). El-Din and Saber (1983) investigated the effect of inoculation of a salt-affected calcareous soil with phosphate-dissolving bacteria on P-uptake by barley plants and found a significant increase in P-uptake as a result of inoculation; this increase was negatively correlated with increasing salinity levels. A continued exploration of the natural biodiversity of soil microorganisms, and the optimization and manipulation of microbial interactions in the rhizosphere of crops represents a pre-requisite step towards developing more efficient microbial inoculants. The establishment and performance of the introduced microbes are affected severely under stress such as high salt, acidic pH and alkalinity that are prevalent in degraded ecosystems (Surange et al. 1997). This may result in poor growth and survival of inoculated organisms. However, locally isolated stress-tolerant microorganisms are likely to survive and perform better under such conditions. The present study was therefore aimed at isolation of superior phosphate-solubilizing microorganisms (PSMs) from salt-affected soils of different regions of India and investigating their effect on the growth of sorghum plants.

Materials and methods

Collection and characterization of soil samples

A total of 19 rhizosphere and non-rhizosphere soil samples (0–15 cm depth) from the salt-affected areas of Koppal (Gangavathi, Marali, Herur, Vaddarahatti), Belgaum (Hooli) and Dharwad (Alagawadi and Benakoppa villages) districts of Karnataka were collected, and four saline soil samples from the College of Agriculture, Indore were also used for isolation of PSMs. After collection, samples were stored in plastic bags at low (4°C) temperature until further processing. The soil samples were analyzed for pH, electrical conductivity (EC), exchangeable sodium percentage (ESP), Ca and Mg contents following standard procedures (Jackson 1973; Baruah and Barthakur 1997).

Isolation, characterization and identification of PSMs from salt-affected soils

The mineral PSMs were isolated from all soil samples by dilution plating on Pikovskaya's agar medium (Pikovskaya 1948) containing tricalcium phosphate (TCP). The plates were incubated at 28±2°C for 2–7 days and colonies with clear zones around them were counted. Representative colonies of each type of bacteria and fungi with a clear halo around were purified, subcultured and maintained on Pikovskaya's agar slants containing TCP. PSBs isolated from salt-affected soils were identified to genus level based on morphological and biochemical tests as specified in *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994) and carbon substrate utilization using BIOLOG. The fungal isolates were identified to genus level based on their colony morphology and microscopic examination as outlined in the manual of Gilman (1957). Molecular identification of bacterial isolates was carried out using 16S rRNA gene amplification using universal primer pair pA (5′AGAGTTT GATCCTGGCTCAG 3′) and pH (5′AAGGAGGTGATC CAGCCGCA 3′) (Edwards et al. 1989). Similarly, fungal 18S rRNA was amplified using universal primer pair NS1 (5′GTAGTCATATGCTTGTCTC3′) and NS8 (5′TCCG CAGGTTCACCTACGGA3′) (White et al. 1990). The

amplified rRNA gene was sequenced and subjected to identification using BLAST homology search. The reference culture *Pseudomonas striata* was obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India and *Aspergillus awamori* MTCC 6486 was procured from the culture collection of the Institute of Microbial Technology, Chandigarh, India (Table 1).

Estimation of mineral phosphate solubilization by the isolates

Isolates of PSMs (1.0 ml overnight grown culture of PSBs and 3-day-old homogenized culture of fungi) were inoculated in 100 ml Pikovskaya's broth in six (PSB) or eight (PSF) replicates with an equal number of uninoculated controls. The flasks were incubated on an orbital shaker at 150 rpm at 28±2°C for 15 (PSB) or 9 (PSF) days. The amount of Pi released in the broth in duplicate flasks was estimated at 5, 10 and 15 days after inoculation (DAI) for PSBs and 3, 5, 7, and 9 DAI for PSF in comparison with a set of uninoculated controls. The broth cultures of PSB were centrifuged at 9,000 rpm for 20 min in a Sigma 3K30 centrifuge (Sigma, St. Louis, MO) to separate the supernatant from the cell debris and insoluble phosphate. In the case of fungal isolates, the cultures were filtered through Whatman No. 1 filter paper and the filtrate was used for estimation of Pi released. The available P content in the supernatant/filtrate was estimated using the phosphomolybdic blue color method (Jackson 1973). The pH of the supernatant was also recorded using a digital pH meter.

Estimation of indole acetic acid and gibberellic acid produced by the isolates

The PSB and PSF isolates were further examined for production of indole acetic acid (IAA) and gibberellic acid

Table 1 Microorganisms used in the study with their GenBank accession numbers and source of isolation

Microorganism	Isolate name	GenBank accession number	Source
Bacteria	*Aerococcus* sp. PSBCRG$_1$-1	HQ393855	Rhizospheric soil
Bacteria	*Alteromonas* sp. PSBCRG$_2$-1	HQ393856	Rhizospheric soil
Bacteria	*Alteromonas* sp. PSBI$_2$-1	HQ393857	Rhizospheric soil
Bacteria	*Bacillus subtilis* PSBCRG$_3$-1	HQ393858	Rhizospheric soil
Bacteria	*Enterobacter* sp. PSBWRB-1	HQ393859	Rhizospheric soil
Bacteria	*Pseudomonas mendocina* PSBW$_1$RH-1	HQ393860	Rhizospheric soil
Bacteria	*Pseudomonas aeruginosa* PSBCRG$_4$-1	HQ393861	Rhizospheric soil
Bacteria	*Pseudomonas* sp. PSBNRG$_5$-1	HQ393862	Rhizospheric soil
Bacteria	*Pseudomonas* sp. PSBORB-1	HQ393863	Rhizospheric soil
Bacteria	*Pseudomonas fluorescens* PSBNRM-1	HQ393864	Rhizospheric soil
Bacteria	*Pseudomonas aeruginosa* PSBI$_3$-1	HQ393865	Rhizospheric soil
Bacteria	*Xanthomonas axonopodis* PSBNRA-1	HQ393866	Rhizospheric soil
Fungi	*Aspergillus terreus* PSFCRG$_2$-1	HQ393867	Rhizospheric soil
Fungi	*Aspergillus awamori* PSFCRG$_4$-1	HQ393868	Rhizospheric soil
Fungi	*Aspergillus awamori* PSFCRG$_3$-4	HQ393869	Rhizospheric soil
Fungi	*Aspergillus awamori* PSFW$_1$RH-1	HQ393870	Rhizospheric soil
Fungi	*Aspergillus versicolor* PSFW$_1$RH-2	HQ393871	Rhizospheric soil
Fungi	*Aspergillus flavus* PSFW$_1$RH-4	HQ393872	Rhizospheric soil
Fungi	*Aspergillus* sp. PSBORB-4	HQ393873	Rhizospheric soil
Fungi	*Aspergillus flavipes* PSFNRM-1	HQ393874	Rhizospheric soil
Fungi	*Aspergillus versicolor* PSFNRO-2	HQ393875	Rhizospheric soil
Fungi	*Aspergillus* sp. PSFNRH-2	HQ393876	Rhizospheric soil
Fungi	*Penicillium* sp. PSFNRG$_5$-3	HQ393877	Rhizospheric soil
Fungi	*Penicillium* sp. PSFWRB-2	HQ393878	Rhizospheric soil
Fungi	*A. awamori* MTCC 6486 (reference strain)	-	Microbial Type Culture Collection, Chandigarh, India
Bacteria	*Pseudomonas striata* (reference strain)	-	Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

(GA) in order to identify multibeneficial isolates for future applications. Each isolate was inoculated into 50 ml Czapek's medium supplemented with 0.005 M L-tryptophan and incubated at 30°C for 10 days in the dark. After 10 days of incubation, the bacterial cultures were centrifuged at 6,000 rpm for 15 min to remove the cells, and the supernatant was used for quantitative estimation of IAA and GA. Fungal cultures were filtered through Whatman No. 1 filter paper and filtrate was used for quantitative estimation of IAA and GA. The supernatants in both cases were analyzed for IAA and GA content using the methods described by Gordon and Paleg (1957) and Paleg (1965), respectively.

Effect of selected PSMs on the growth of sorghum plants

Six efficient strains each of bacteria and fungi were further tested for their efficiency to promote growth of sorghum plants in a sterile salt-affected soil under pot culture conditions.

Soil

The salt-affected black soil collected from Agricultural Research Station, Gangavathi, Karnataka State, India was mixed thoroughly, sieved, filled in gunny bags and autoclaved at 15 psi for 20 min three times at intervals of 24 h. The sterilized soil was filled in earthenware pots at a rate of 2.0 kg pot^{-1}. The soil had a pH of 8.37, EC 13.0 dS m^{-1}, ESP 17.81 %, organic carbon 0.26%, available N 273 kg ha^{-1} and available P 15.06 kg ha^{-1}.

Seeds

Sorghum [*Sorghum bicolor* (L.) Moench] seeds of variety CSH-16 used as millet and fodder crop were obtained from Sorghum Breeder, Main Research Station, University of Agricultural Sciences, Dharwad, India.

Fertilizers

Calculated quantities of N in the form of urea, P in the form of Mussoorie rock phosphate (MRP) or single super phosphate (SSP) as per treatment schedule, and K in the form of muriate of potash were applied at recommended levels (100: 75: 37.5 kg NPK ha^{-1}) entirely as a basal dose at the time of sowing.

Treatments

The experiment consisted of 16 treatments that included six efficient isolates each of bacteria and fungi, one of each reference strain of bacteria (*P. striata*) and fungi (*A. awamori*) and two uninoculated controls (one with MRP and the other with SSP as P-source at recommended level).

Statistical analysis

The data recorded on different parameters were subjected to statistical analyses using M-STAT-C version 1.4 package using factorial CRD. Data on means of strains, standard error of means (SEM±) and critical difference at 1% probability level were calculated.

Results and discussion

Characteristics of soils used for isolation of P-solubilizers

The soil samples used for isolation of PSMs were also analysed for pH, EC, ESP, and calcium and magnesium contents (Table 2). The pH of the soils ranged from 7.72 to 9.59, EC from 2.96 to 51.51 dS m^{-1}, ESP from 12.62 to 58.52%, calcium from 6.6 to 61.2 meq 100 g^{-1} and magnesium from 1.25 to 22.80 meq 100 g^{-1} soil. Based on these properties, the soil samples were grouped as sodic, saline-sodic and non-saline-non-sodic soils, respectively (Brady 1990). The rhizosphere and non-rhizosphere soil samples from various patches of salt-affected areas were used for enumeration of total bacteria, fungi and actinomycetes as well as phosphate solubilizers (Table 3). The microbial population was enumerated using a spread plate technique. The population of total bacteria ranged from 4.0×10^5 to 34.5×10^5 CFU g soil^{-1}, that of total fungi ranged from 1.5×10^3 to 30.5×10^3, and the actinomycete population ranged from 7.5×10^3 to 75.5×10^3 CFU g soil^{-1}. Pakale and Alagawadi (1993) reported similar populations of total bacteria, fungi and actinomycetes from five soils of northern Karnataka including a sodic soil, whereas Bhardwaj (1974) recorded a similar population of total bacteria in saline-alkali soils. The population of PSB ranged from 19×10^2 to 53×10^3, whereas that of PSF ranged from 6.5×10^2 to 50.5×10^2 CFU g soil^{-1}. While all 19 samples showed the presence of PSB, only 15 samples showed the presence of PSF. Johri et al. (1999) also isolated 4,800 bacterial strains from root-free soil, and the rhizosphere and rhizoplane of *Prosophis juliflora* growing in alkaline soil, out of which 857 strains were P-solubilizers, whereas Das (1963) isolated 18 PSF from a paddy field situated in a saline locality of Falta, West Bengal, India, which is in agreement with the results of the present investigation.

Isolation, characterization and identification of PSMs from salt-affected soils

From plates used for enumeration of PSMs, representative bacterial and fungal colonies with solubilized zones around were subcultured, purified, and maintained for further use. A total of 23 bacteria and 35 fungi were isolated from 19 soil samples. All 23 bacterial isolates were identified to genus

Table 2 Properties of salt-affected soils used for isolation of phosphate-solubilizing microorganisms (PSMs). *EC* Electrical conductivity, *ESP* exchangeable sodium percentage

Places of soil sample collection			pH	EC_e (dS m^{-1})	ESP	Ca (meq 100 g^{-1})	Mg (meq 100 g^{-1})	Soil type
District	Village	Sample number						
Koppal (Karnataka) (15°09′ - 16°03′ N, 75°47′ -76°48′ E)	Gangavathi	G_1	8.42	5.82	17.43	52.2	8.4	Saline-sodic
		G_2	8.56	4.52	16.68	53.9	4.8	Saline-sodic
		G_3	8.00	11.70	18.76	11.4	1.8	Saline-sodic
		G_4	7.72	51.51	41.49	61.2	8.4	Saline-sodic
		G_5	7.98	50.64	47.06	54.6	8.4	Saline-sodic
	Herur	H	9.11	4.47	41.08	35.4	1.8	Sodic
	Marali	M	8.80	3.85	41.83	34.2	1.8	Sodic
	Vaddarahatti	O	8.80	3.85	17.97	17.4	4.8	Sodic
Dharwad (Karnataka) (15°19′ - 15°41′ N, 74°43′ -75°15′ E)	Alagawadi	A	8.63	2.96	20.46	50.4	12.0	Sodic
	Benakoppa	BO	7.97	20.62	19.42	45.0	22.8	Saline-sodic
		BW	8.12	32.24	27.32	6.6	1.3	Saline-sodic
Belgaum (Karnataka) (15°23′-16°58′ N, 74°5′-75°28′ E)	Hooli	WI	8.50	11.28	37.02	39.6	9.2	Saline-sodic
		WII	8.49	11.28	14.77	45.6	4.8	Saline-sodic
		MI	8.23	3.38	12.62	45.6	9.1	Non-saline-non-sodic
		MII	9.01	4.73	26.76	43.8	11.4	Sodic
Indore (Madhya Pradesh) (22°44′ N, 75°50′ E)	Indore	I_1	9.54	7.18	53.02	31.2	6.0	Sodic
		I_2	9.59	7.31	58.52	31.2	6.0	Sodic
		I_3	9.55	7.62	54.30	31.2	5.4	Sodic
		I_4	9.18	4.06	35.85	37.2	7.8	Sodic

level based on the BIOLOG bacterial identification system (Biolog, MicroLog™ release 4.2; http://www.biolog.com/). The isolates were found to belong to the genera *Aerococcus*, *Alteromonas*, *Bacillus*, *Enterobacter*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Out of 23 isolates, 15 belonged to the genus *Pseudomonas*, two each to *Xanthomonas* and *Bacillus*, and one each to *Aerococcus*, *Alteromonas*, *Enterobacter* and *Erwinia*. By using microscopic, morphological and biochemical tests alone, we were able to achieve generic identification of more than 80% of the isolates, which include several frequently occurring genera such as *Aspergillus*, *Pseudomonas*, *Penicillium* and *Enterobacter*. Further reliable generic identification was carried out using 16S and 18S rRNA gene amplification and BLAST homology search. No discrepancies were found between the generic identities determined by conventional and molecular approaches. A total of 24 partial 16S rDNA and 18S rDNA sequences have been submitted to the GenBank database under the accession numbers HQ393855–HQ393878 (Table 1).

The occurrence of *Pseudomonas* as the predominant genus in many soils has been reported (Vikram et al. 2007; Mishra et al. 2009); its predominance could be due to the versatility of the genus to use different substrates as nutrient source. While bacteria belonging to the genera *Vibrio*, *Pseudomonas*, *Alteromonas* and *Acinetobacter* have been isolated from saline habitats (Del Moral et al. 1988), spore-forming *Bacillus* have been isolated from hypersaline habitats (Zahran et al. 1995).

Simultaneously, all fungal isolates were also identified to genus level based on colony morphology and microscopic observations. Out of 35 isolates, 31 belonged to the genus *Aspergillus* and 4 to the genus *Penicillium*. Thomas et al. (1985) found that PSF isolated from coconut (*Cocos nucifera*) plantation soils belonged to the genus *Aspergillus* or *Penicillium*. Kucey (1983) also reported that most of the PSF isolates from prairie soils were either *Aspergillus* or *Penicillium*. The report by Das (1963) of the occurrence of phosphate-solubilizing *Aspergillus niger*, *A. terreus*, *Penicillium*-10 and *P. tardum* in a saline locality of Falta, West Bengal, India, gains support from the present findings.

Determination of P-solubilization activity of PSMs

All 23 PSB and 35 PSF isolates from salt-affected soils were tested for their ability to release Pi from insoluble TCP over different incubation periods.

Bacterial isolates

The amount of Pi released from TCP in general was found to increase with the increase in incubation period, and

Table 3 Microbial population of salt-affected soils in this study. *PSB* Phosphate-solubilizing bacteria, *PSF* phosphate-solubilizing fungi

District	Location of soil sample	Sample number	Total bacteria (CFU×10^5 g^{-1} soil)	Fungi (CFU×10^3 g^{-1} soil)	Actinomycetes (CFU×10^3 g^{-1} soil)	PSB[a] (CFU×10^2 g^{-1} soil)	PSF[a] (CFU×10^2 g^{-1} soil)
Koppal (Karnataka) (15°09′ -16°03′ N, 75°47′ -76°48′ E)	Gangavathi	G_1	17.0	19.5	25.5	309.5	45.5
		G_2	6.0	12.5	14.5	90.0	20.0
		G_3	4.0	10.5	40.0	530.0	32.0
		G_4	27.5	20.0	75.5	108.0	9.0
		G_5	16.5	30.5	59.5	204.5	6.5
	Herur	H	29.5	11.0	23.5	29.5	14.0
	Marali	M	31.0	19.0	14.0	28.0	12.0
	Vaddarahatti	O	20.0	10.0	15.5	20.0	9.0
Dharwad (Karnataka) (15°19'-15°41' N, 74°43' -75°15' E)	Alagawadi	A	16.5	14.0	8.5	49.5	23.0
	Benakoppa	BO	23.0	20.5	8.5	280.0	50.5
		BW	31.0	20.5	13.5	295.0	12.5
Belgaum (Karnataka) (15°23′-16°58′ N, 74°5′-75°28′ E)	Hooli	WI	27.5	21.0	10.5	370.0	22.5
		WII	34.5	18.0	9.0	155.0	10.0
		MI	4.5	18.5	7.5	250.0	11.0
		MII	8.5	29.5	8.0	270.0	7.5
Indore (Madhya Pradesh) (22°44′ N, 75°50′ E)	Indore	I_1	19.0	2.0	8.5	24.5	0.0
		I_2	19.0	1.5	10.5	19.0	0.0
		I_3	19.5	2.5	10.5	39.0	0.0
		I_4	20.0	3.5	9.5	24.0	0.0

[a] Colonies showing clear zones on MSM and Pikovskaya's agar medium

maximum solubilization was observed at 15 DAI with most of the bacterial strains (Table 4). The percent of Pi released by the isolates at 15 DAI ranged from 6.06 to 15.17, whereas the reference strain (*P. striata*) showed 12.17% solubilization. Two of the isolates of salt-affected soils viz., *Pseudomonas* sp. $PSBW_2RH$-1 (15.17%) and *Aerococcus* sp. $PSBCRG_1$-1 (13.41%) recorded significantly higher TCP solubilization than the reference strain. Gupta et al. (1994) also reported that *Bacillus* sp. solubilized 0.33–13.20% TCP, which corroborates the results of the present investigation. The change in pH of the broth at 15 DAI ranged from 3.59 to 5.71 in different isolates.

Fungal isolates

The amount of Pi released from TCP in general was found to increase with increase in incubation period up to 7 DAI (Table 5). Maximum solubilization was observed at 7 DAI by most of the fungal isolates, beyond which time there was a decline in Pi release. The percent Pi released by the isolates ranged from 10.42 to 21.36% as compared to the reference strain (*A. awamori*), which showed 23.64% solubilization. Among the PSF isolates, *Aspergillus* sp. PSFNRH-2 recorded the maximum solubilization (21.36%) followed by *Aspergillus* sp. $PSFCRG_5$-1 (21.20%), *Aspergillus* sp. PSFNRO-2 (21.17%) and *Aspergillus* sp. PSFNRM-1. These results clearly indicate the superiority of fungal isolates over bacterial isolates to release Pi from TCP in broth medium. The greater P-solubilizing activity in agar and liquid media of fungi compared to bacteria has been reported earlier (Gaur 1986; Mishra et al. 2009). Bopaiah (1985) reported *Aspergillus* sp. of non-rhizosphere soil to solubilize 18.69% TCP and *Penicillium* sp. in the range of 8.34–6.99%, which is in agreement with the results of present investigation. The change in pH of the broth medium at 9 DAI ranged from 2.77 to 6.94.

Production of plant-growth-promoting substances by PSM isolates

The PSB and PSF isolates of salt-affected soils were further examined for the production of IAA and GA by quantitative methods. All PSB and PSF isolates were able to produce both IAA and GA (Fig. 1). These results are in line with the observations of Barea et al. (1976), who tested 50 PSB and found all of them to produce one or more plant-growth-promoting substances (PGPS) like IAA, GA, cytokinins or a combination thereof. Production of IAA and GA by PSB has been reported by various researchers (Gaur and Gaind 1992; de Freitas et al. 1997; Mishra et al. 2009; Bianco and Defez 2010). The amount of IAA produced by the bacterial isolates ranged from 0.74 to 9.53 μg 25 ml^{-1} broth, and that of GA ranged from 2.08 to 12.55 μg 25 ml^{-1} broth,

Table 4 Percent Pi released from tricalcium phosphate (TCP) in broth medium by bacterial isolates of salt-affected soils over different incubation periods. *DAI* Days after inoculation

Strain	Incubation period (days)			pH of broth at 15 DAI[a]
	5	10	15	
Pseudomonas sp. $PSBM_2RH$-1	2.80	6.66	9.48	5.22
Pseudomonas sp. $PSBW_1RH$-1	4.50	9.75	12.00	5.10
Pseudomonas sp. $PSBW_2RH$-1	3.55	4.58	15.17	4.65
Aerococcus sp. $PSBCRG_1$-1	7.16	12.65	13.41	3.59
Alteromonas sp. $PSBCRG_2$-1	6.36	9.94	11.67	4.23
Pseudomonas sp. $PSBCRG_4$-1	6.12	6.56	8.12	5.15
Pseudomonas sp. $PSBNRG_5$-1	5.32	7.26	9.51	5.38
Pseudomonas sp. PSBORB-1	8.15	10.56	12.02	5.25
Bacillus sp. $PSBCRG_3$-1	6.18	10.52	10.01	4.24
Pseudomonas sp. PSBNRM-1	8.77	10.71	11.37	5.50
Pseudomonas sp. $PSBNRG_3$-2	5.28	7.39	8.95	4.57
Bacillus sp. PSBNRH-1	4.13	9.74	6.06	5.09
Enterobacter sp. PSBWRB-1	4.73	5.88	8.64	4.78
Pseudomonas sp. $PSBM_1RH$-1	4.55	7.21	8.99	4.97
Pseudomonas sp. $PSBCRG_3$-3	4.74	6.77	9.44	3.76
Pseudomonas sp. $PSBI_1$-1	6.86	6.97	7.47	4.35
Alteromonas sp. $PSBI_2$-1	4.88	7.11	9.81	5.37
Pseudomonas sp. $PSBI_2$-2	3.66	5.21	6.84	5.27
Pseudomonas sp. $PSBI_3$-1	4.66	7.06	7.33	5.53
Xanthomonas sp. PSBNRA-1	4.15	6.91	7.40	3.80
Xanthomonas sp. PSBNRO-1	5.08	5.83	6.31	5.71
Erwinia sp. $PSBI_4$-1	5.36	7.44	7.80	5.26
Pseudomonas sp. $PSBI_4$-2	5.50	6.98	8.76	5.24
Pseudomonas striata (reference strain)	6.82	8.62	12.17	4.08
SEM±	0.108	0.104	0.096	0.026
CD (P=0.01)	0.427	0.411	0.380	0.105

[a] Initial pH of the medium was 7.00

which is comparable to earlier reports (Leinhos and Vacek 1994; de Freitas et al. 1997; Bianco and Defez 2010) in different PSB strains. Maximum IAA production was recorded by the isolate *Bacillus* sp. $PSBCRG_3$-1 (9.53 μg), which was, however, significantly lower than the reference strain (*P. striata*) (23.50 μg), although significantly higher than all other isolates. The isolate *Enterobacter* sp. PSBWRB-1showed the highest production of GA (12.55 μg), which was significantly higher than all other isolates as well as the reference strain, *P. striata* (8.40 μg 25 ml^{-1}).

The amount of IAA produced by PSF isolates ranged from 2.33 to 8.69 μg 25 ml^{-1} broth, while GA production ranged from 3.44 to 14.80 μg 25 ml^{-1} broth (Fig. 2). Production of IAA by *Verticillium* and GA by *Aspergillus*, *Penicillium*, *Gibberella*, *Rhizopus*, *Alternaria* and *Sphaceloma* has been reported by Subba Rao (1977). Maximum IAA production was recorded by the strain *Penicillium* sp. $PSFNRG_5$-3 (8.69 μg), which was statistically equivalent to the reference strain, *A. awamori* (8.32 μg). The fungal isolate *Aspergillus* sp. $PSFCRG_3$-3 produced the maximum amount of GA (14.80 μg) followed by *Aspergillus* sp. $PSFM_1RH$-1 (13.18 μg), both of which were significantly superior to all other isolates as well as to the reference strain, *A. awamori* (7.81 μg). The results of the present investigation support the view that the production of PGPS is common among P-solubilizers isolated from rhizosphere and non-rhizosphere soils (Barea et al. 1976; Leinhos and Vacek 1994). Furthermore, the large variation in the amount of IAA and GA produced by different isolates indicates the metabolic variability among the isolates, as suggested by Leinhos and Vacek (1994). Thus, the strains isolated in this study could provide additional plant growth promotional activity apart from releasing Pi into the rhizosphere.

Table 5 Pi released (%) from TCP in broth medium by fungal isolates of salt-affected soils over different incubation periods

Strain	Incubation period (days)				pH of broth at 9 DAI[a]
	3	5	7	9	
Aspergillus sp. PSFCRG$_1$-1	6.81	15.25	19.32	15.65	3.12
Aspergillus sp. PSFCRG$_2$-1	6.52	14.60	15.36	15.16	3.43
Penicillium sp. PSFCRG$_3$-1	6.74	15.54	17.07	15.20	2.98
Aspergillus sp. PSFCRG$_1$-2	7.12	16.28	17.37	15.55	3.18
Aspergillus sp. PSFCRG$_2$-2	6.04	13.71	16.91	14.96	3.16
Aspergillus sp. PSFCRG$_3$-2	5.77	12.22	15.06	12.85	3.51
Aspergillus sp. PSFCRG$_4$-1	5.86	15.24	17.88	15.01	3.28
Aspergillus sp. PSFCRG$_3$-3	6.13	12.27	15.35	17.77	4.43
Aspergillus sp. PSFCRG$_3$-4	5.67	16.26	19.00	14.48	2.99
Aspergillus sp. PSFCRG$_5$-1	6.30	15.97	21.20	15.20	2.91
Aspergillus sp. PSFCRG$_5$-2	5.49	15.38	18.90	14.30	3.08
Penicillium sp. PSFNRG$_5$-3	6.89	15.69	20.72	14.86	2.77
Aspergillus sp. PSFW$_1$RH-1	6.33	15.82	20.26	15.72	3.02
Aspergillus sp. PSFW$_1$RH-2	6.58	16.64	20.56	15.70	3.05
Aspergillus sp. PSFW$_1$RH-3	6.63	16.03	18.30	15.60	3.11
Aspergillus sp. PSFW$_1$RH-4	5.55	15.35	17.12	14.45	3.25
Aspergillus sp. PSFW$_2$RH-1	8.34	18.62	19.43	16.13	2.81
Aspergillus sp. PSFW$_2$RH-2	7.44	14.61	18.25	15.13	4.06
Aspergillus sp. PSFM$_2$RH-1	5.77	14.92	19.37	13.75	3.04
Aspergillus sp. PSFM$_1$RH-1	7.11	15.71	17.64	15.43	4.26
Aspergillus sp. PSFM$_1$RH-2	2.83	12.96	10.42	8.90	6.48
Aspergillus sp. PSFORB-1	7.00	14.28	18.94	15.87	5.24
Aspergillus sp. PSFORB-2	7.11	14.79	17.36	15.87	6.94
Penicillium sp. PSFORB-3	4.35	13.87	18.75	14.22	3.03
Aspergillus sp. PSFORB-4	6.91	14.45	16.50	15.91	3.06
Aspergillus sp. PSFWRB-1	6.79	13.68	16.04	15.48	3.58
Penicillium sp. PSFWRB-2	6.05	17.17	20.52	16.77	5.50
Aspergillus sp. PSFWRB-3	6.53	14.12	16.94	16.47	3.64
Aspergillus sp. PSFWRB-4	7.47	16.10	18.09	17.67	3.07
Aspergillus sp. PSFNRO-1	6.62	14.13	17.58	16.10	2.82
Aspergillus sp. PSFNRH-1	7.31	12.12	16.27	15.88	3.01
Aspergillus sp. PSFNRM-1	7.58	17.49	21.02	16.51	3.02
Aspergillus sp. PSFNRO-2	7.54	17.28	21.17	15.68	2.86
Aspergillus sp. PSFNRA-1	7.50	16.21	19.95	15.75	2.91
Aspergillus sp. PSFNRH-2	8.96	18.98	21.36	14.23	2.83
Aspergillus awamori (reference strain)	7.42	16.16	23.64	18.61	2.80
SEM±	0.126	0.347	0.254	0.238	0.023
CD (P=0.01)	0.459	1.264	0.925	0.867	0.084

[a] Initial pH of the medium was adjusted to 7.00

Effect of inoculation of efficient P-solubilizing isolates on growth and P-uptake of sorghum plants in salt-affected soil under pot culture conditions

Based on the efficiency of P-solubilization and PGPS production as well as tolerance to salt, six isolates each of bacteria and fungi were further examined for their performance to enhance growth and P-uptake of sorghum plants in a salt-affected soil under pot culture conditions. Plant growth parameters, viz., shoot and root length, stem girth, shoot and root dry matter content, were recorded at 45 days of plant growth.

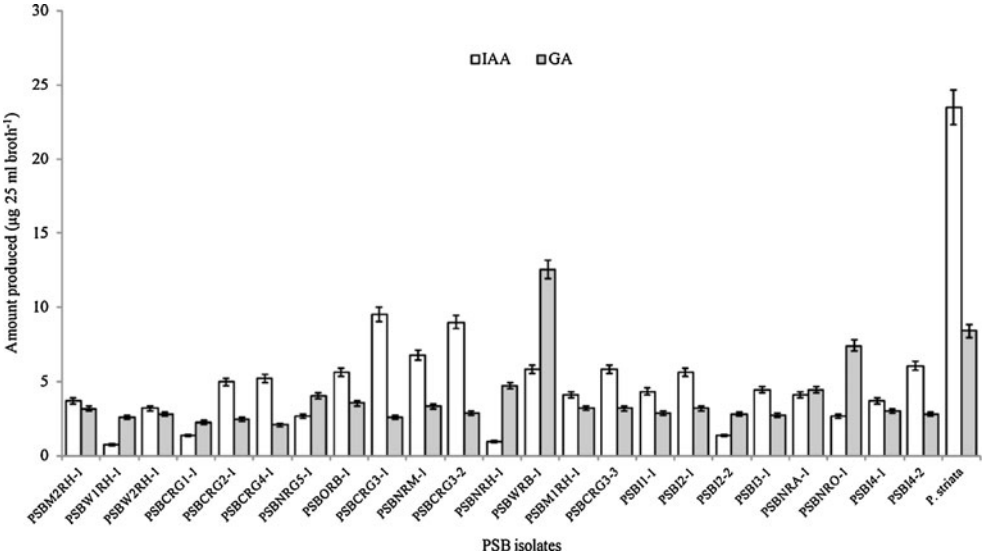

Fig. 1 Production of indole acetic acid (IAA) and gibberellic acid (GA) by phosphate-solubilizing bacteria (PSB) isolates from salt-affected soils

Shoot and root length

All inoculation treatments, with the exception of two fungal isolates (*Aspergillus* sp. PSFCRG$_4$-1 and *Aspergillus* sp. PSFCRG$_2$-1) and the reference strain of bacteria (*P. striata*), showed a significant increase in shoot length compared to the RP control. The two fungal isolates and *P. striata* were on par with the RP control. The bacterial isolates were in general more efficient than the fungal isolates in enhancing shoot length (Table 6). All bacterial isolates, except *Pseudomonas* sp. PSBI$_3$-1, recorded significantly higher shoot length (35.21–77.4 cm) than the reference strain, *P. striata* (31.00 cm), but *Pseudomonas* sp. PSBI$_3$-1 (32.50 cm) was on par with *P. striata*. The maximum shoot length among the bacterial isolates was recorded by *Aerococcus* sp. PSBCRG$_1$-1 (77.4 cm), which was significantly higher than all other inoculation treatments but was statistically the same as the SSP control (76.30 cm). Among the fungal isolates used, *Aspergillus* sp. PSFNRH-2 exhibited the maximum shoot length (72.20 cm), which was significantly longer than that of the reference strain, *A. awamori* (68.53 cm). However, *Aspergillus* sp. PSFNRH-2 led to shoot lengths statistically shorter than those of *Aerococcus* sp. PSBCRG$_1$-1 and the SSP control.

The root length of sorghum plants was found to increase significantly compared to the RP control with all inoculation treatments. In general, the bacterial isolates performed better than the fungal isolates in increasing root length (Table 6). All the bacterial isolates recorded significantly higher root length (26.10–52.20 cm) than the reference strain, *P. striata* (21.60 cm). The maximum root length among the bacterial isolates was recorded by the strain *Alteromonas* sp. PSBCRG$_2$-1 (52.20 cm), which was significantly higher than all other bacterial isolates and that

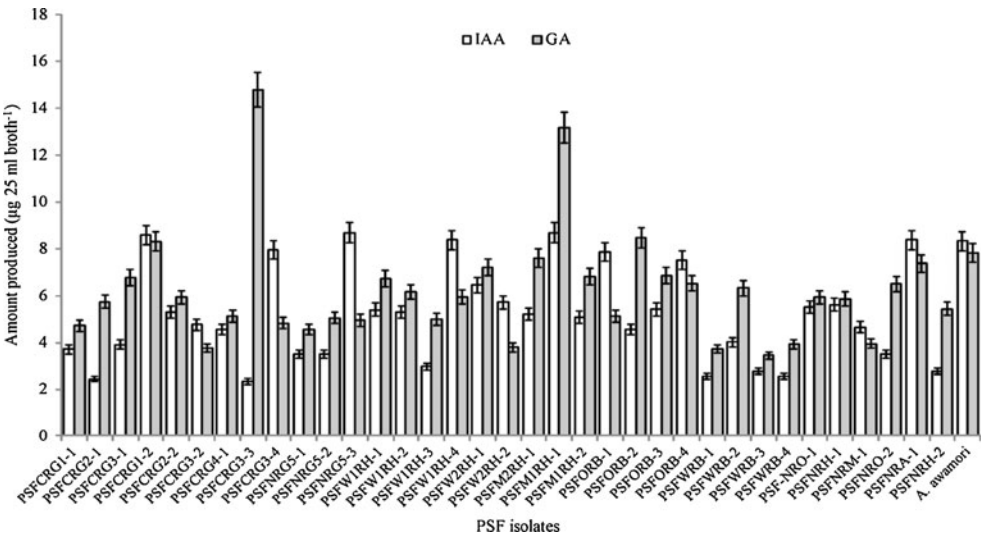

Fig. 2 Production of IAA and GA by phosphate-solubilizing fungi (PSF) isolates from salt-affected soils

of the SSP control (41.90 cm). The fungal isolate *Aspergillus* sp. PSFNRH-2 showed the highest root length (68.10 cm), and was significantly better than all other inoculation treatments including the reference fungal strain, *A. awamori* (40.50 cm) and the SSP control. However, all other fungal isolates showed significantly shorter root lengths (19.80 to 28.93 cm) than the reference strain (*A. awamori*) and the SSP control.

The results of this pot trial also indicated that, in general, the inoculation treatments showed increased plant growth parameters and P-uptake of sorghum plants compared to the RP control at 45 days after sowing. While all the bacterial isolates increased shoot length and root length significantly over the reference bacterial strain (*P. striata*), only one fungal isolate, *Aspergillus* sp. PSFNRH-2 showed a significant increase in shoot and root lengths over the reference fungal strain, *A. awamori*. *Aerococcus* sp. $PSBCRG_1$-1 among bacterial isolates, and *Aspergillus* sp. PSFNRH-2 among fungal isolates recorded maximum shoot and root lengths. Similar increases in shoot and root lengths due to inoculation of PSM have been reported in different crop plants (Dubey 1996; de Freitas et al. 1997; Vikram et al. 2007; Mishra et al. 2009). The increased shoot and root lengths of sorghum plants in inoculated treatments may be attributed to the increased cell elongation and multiplication due to enhanced nutrient uptake by plants (Black 1968) or due to production of PGPS (Brown 1975) in the vicinity of the roots by PSMs. While all the strains of PSM used in the present study enhanced the P-uptake in sorghum plants, they were also able to produce both IAA and GA and thus might have contributed to the enhanced shoot and root growth of sorghum plants. It is well known that IAA and GA play a role in root and shoot elongation as well as in enhancing plant growth (Brown 1975; Mishra et al. 2009).

Stem girth

Stem girth of sorghum plants was also enhanced by all inoculation treatments (Table 6). The maximum stem

Table 6 Growth and dry matter content of sorghum plants in salt-affected soil at 45 days after sowing as influenced by efficient PSM isolates of salt affected soils

Treatment	Shoot length (cm)	Root length (cm)	Stem girth (cm)	Shoot dry matter (g $plant^{-1}$)	Root dry matter (g $plant^{-1}$)	Total dry matter (g $plant^{-1}$)
RP control	29.6	15.1	0.67	0.107	0.067	0.173
P. striata	31.0	21.6	0.83	0.120	0.083	0.203
A. awamori	68.5	40.5	2.30	1.623	0.810	2.433
Aerococcus sp. $PSBCRG_1$-1	77.4	34.9	2.60	2.120	0.790	2.910
Alteromonas sp. $PSBCRG_2$-1	35.2	52.2	1.10	0.360	0.430	0.790
Pseudomonas sp. PSBNRM-1	60.1	34.0	1.70	0.970	0.360	1.330
Enterobacter sp. PSBWRB-1	64.5	26.1	2.13	1.330	0.370	1.700
Xanthomonas sp. PSBNRA-1	63.4	46.2	1.57	0.800	0.451	1.250
Pseudomonas sp. $PSBI_3$-1	32.5	27.2	0.90	0.120	0.170	0.290
Aspergillus sp. PSFNRH-2	72.2	68.1	2.63	1.747	1.860	3.607
Penicillium sp. PSFWRB-2	61.2	28.9	1.60	0.950	1.543	1.493
Aspergillus sp. PSFNRM-1	41.4	26.9	1.40	0.480	0.400	0.880
Aspergillus sp. $PSFCRG_4$-1	29.7	19.8	0.83	0.120	0.177	0.297
Aspergillus sp. $PSFW_1RH$-2	58.7	28.0	2.20	1.253	0.760	2.013
Aspergillus sp. $PSFCRG_2$-1	29.6	25.5	0.80	0.130	0.500	0.630
SSP-control	76.3	41.9	2.70	1.940	0.853	2.793
SEM±	0.685	0.734	0.060	0.024	0.017	0.028
CD (P=0.01)	2.85	3.06	0.250	0.100	0.071	0.119

girth among the bacterial isolates was recorded by *Aerococcus* sp. PSBCRG$_1$-1 (2.60 cm), which was significantly superior to that recorded by all other bacterial isolates (0.92–2.13 cm) including the reference strain, *P. striata* (0.83 cm), but was on par with the SSP control (2.70 cm). Among the fungal isolates, *Aspergillus* sp. PSFNRH-2 recorded the highest stem girth (2.63 cm), which was significantly higher than that recorded by all other fungal isolates (0.80–2.20 cm) including the reference strain, *A. awamori* (2.30 cm) but was on par with the SSP control (2.70 cm). The fungal isolate *Aspergillus* sp. PSFNRH-2 also showed significantly increased stem girth over many of the bacterial isolates but was on par with *Aerococcus* sp. PSBCRG$_1$-1. It was also noted that all inoculation treatments enhanced the stem girth of sorghum plants over the RP control. Among the bacterial isolates, the stem girth was increased to the maximum extent by the isolate *Aerococcus* sp. PSBCRG$_1$-1 (2.60 cm) followed by *Enterobacter* sp. PSBWRB-1 (2.13 cm), *Pseudomonas* sp. PSBNRM-1 (1.10 cm) all of which were significantly superior to the reference strain, *P. striata* (0.83 cm). However, among fungal isolates, *Aspergillus* sp. PSFNRH-2 (2.63 cm) recorded significantly greater stem girth than the reference strain, *A. awamori*.

Shoot, root and total dry matter

Five bacterial isolates, four fungal isolates and the reference strain, *A. awamori* showed significant increases in shoot dry matter accumulation in sorghum plants over the RP control (Table 6). However, the bacterial reference strain, *P. striata* (0.120 g), the bacterial isolate *Pseudomonas* sp. PSBI$_3$-1 (0.120 g) and the two fungal isolates (*Aspergillus* sp. PSFCRG$_4$-1 and *Aspergillus* sp. PSFCRG$_2$-1; 0.120 and 0.130 g, respectively) were on par with the RP control (0.107 g). The highest shoot dry matter among all the treatments was recorded by the one receiving inoculation of *Aerococcus* sp. PSBCRG$_1$-1 (2.120 g), which was significantly superior to all treatments including the SSP control (1.940 g). Among the fungal isolates, *Aspergillus* sp. PSFNRH-2 recorded the highest shoot dry matter (1.747 g), which was significantly superior to all other fungal isolates as well as to the reference strain, *A. awamori* (1.623 g), but was statistically inferior to the SSP control (1.940 g).

All inoculation treatments except reference strain *P. striata* recorded significantly higher root dry matter than the RP control. *P. striata* (0.083 g) was on par with the RP control (0.067 g) (Table 6). Among the bacterial isolates, *Aerococcus* sp. PSBCRG$_1$-1 recorded the highest root dry matter (0.790 g) and was significantly superior to all other bacterial isolates and to the reference strain, *P. striata* (0.083 g) but was on par with the SSP control (0.853 g). The highest root dry matter production among the fungal isolates was recorded by *Aspergillus* sp. PSFNRH-2 (1.862 g) followed by *Penicillium* sp. PSFWRB-2 (1.543 g), both of which were significantly superior to all other treatments, including the SSP control (0.853 g). However, *Aspergillus* sp. PSFNRH-2 was significantly superior to *Penicillium* sp. PSFWRB-2.

All inoculation treatments except *P. striata* and the bacterial isolate *Pseudomonas* sp. PSBI$_3$-1, recorded a significant increase in the total dry matter content of sorghum plants (Table 6). Among the bacterial isolates, *Aerococcus* sp. PSBCRG$_1$-1 recorded the maximum total dry matter (2.910 g), which was significantly higher than that recorded with all other bacterial strains, including the reference strain, *P. striata* (0.203 g), but was on par with the SSP control (2.793 g). Among the fungal isolates, *Aspergillus* sp. PSFNRH-2 recorded the maximum total dry matter (3.607 g), and was significantly superior to all other fungi (0.297–2.013 g), bacteria (0.290–2.910 g) and the SSP control (2.793 g).

The isolate *Aerococcus* sp. PSBCRG$_1$-1 recorded 18 and 20 times more shoot dry matter, and 10 and 12 times more root dry matter than that of the reference strain (*P. striata*) and RP control, respectively. Increase in shoot length, root length and dry matter with the inoculation of phosphobacteria of up to 25–50% have been observed in various crop plants (Ocampo et al. 1975; Siddikee et al. 2010). The isolate *Aerococcus* sp. PSBCRG$_1$-1 (2.910 g) among bacteria and *Aspergillus* sp. PSFNRH-2 (3.607 g) among fungi recorded significantly higher total dry matter of sorghum plants compared to all other inoculation treatments including the two reference strains. While *Aerococcus* sp. PSBCRG$_1$-1 was on par with the SSP control, *Aspergillus* sp. PSFNRH-2 was significantly superior to the SSP control. The isolate *Aerococcus* sp. PSBCRG$_1$-1 led to a 14-fold increase in total dry matter of sorghum plants over the reference strain (*P. striata*), whereas *Aspergillus* sp. PSFNRH-2 showed an increase in total dry matter of 1.5 times over the reference strain, *A. awamori*. Similar increases in dry matter yields of crop plants due to inoculation with PSMs have been reported by several workers (Salih et al. 1989; Alagawadi and Gaur 1992; Tomar et al. 1994, 1996; Mudalagiriyappa et al. 1997; Siddikee et al. 2010).

Plant microbe–microbe interactions are complex and dependent on multiple traits (Barea et al. 2004). The strong promotion of growth of sorghum, percent Pi release by PSM, and P uptake by plants due to combined interactions of plants and microbes provides new findings with which to improve crop productivity, particularly for soils poor in available phosphorus and affected by salinity.

References

Alagawadi AR, Gaur AC (1992) Inoculation of *Azospirillum brasilense* and phosphate solubilizing bacteria on yield of sorghum (*Sorghum bicolor* L. Moench) in dry land. Trop Agric 69:347–350

Barea JM, Navarro E, Montoya E (1976) Production of plant growth regulators by rhizosphere phosphate solubilizing bacteria. J Appl Bacteriol 40:129–134

Barea JM, Azcon R, Azcon-Aguilar C (2004) Mycorrhizal fungi and PGPR. In: Kamp RM, Calvete JJ, Choli-Papadopoulou T (eds) Principles and practice: methods in proteome and protein analysis. Springer, Berlin, pp 409–417

Baruah TC, Barthakur HP (1997) A textbook of soil analysis. Vikas, New Delhi

Bhardwaj KKR (1974) Numbers of bacteria in saline-alkaline soils determined by a plate method. Soil Biol Biochem 6:69–70

Bianco C, Defez R (2010) Improvement of phosphate solubilization and Medicago plant yield by an indole-3-acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76(14):4626–32

Black CA (1968) Soil plant relationships, 2nd edn. Wiley, New York

Bopaiah BM (1985) Occurrence of phosphate solubilizing micro-organisms in root region of arecanut palm. J Plant Crops 134:60–62

Brady NC (1990) Characteristics of saline and sodic soils. In: Brady NC, Buckman HO (eds) The nature and properties of soils, 10th edn. Macmillan, New York, pp 243–246

Brown ME (1975) Rhizosphere microorganisms—opportunists bandits or benefactors. In: Walker N (ed) Soil Microbiology. Halsted, New York, pp 21–38

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Das AC (1963) Utilization of insoluble phosphates by soil fungi. J Indian Soc Soil Sci 11:203–207

de Freitas JR, Banerjee MR, Germida JJ (1997) Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biol Fertil Soils 24:358–364

Edwards U, Rogall T, Blocker H, Emde M, Bottger EC (1989) Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. Nucleic Acids Res 17:7843–7853

Del Moral A, Prado B, Quesada E, Garcia T, Ferrer R, Ramos-Cormenzana A (1988) Numerical taxonomy of moderately halophilic gram-negative rods from an in-land saltern. J Gen Microbiol 134:733–741

Dubey SK (1996) Response of soybean to rock phosphate applied with *Pseudomonas striata* in a typic chromustert. J Indian Soc Soil Sci 44:252–255

El-Din SMSB, Saber MSM (1983) Effect of phosphate dissolving bacteria on P uptake by barley plants growth in salt-affected calcareous soil. Z Pflanzenernaehr Bodenkd 146:545–550

El-Gibali MH, El-Reweiny FM, Abdul-Nasser M, El-Dahtory TA (1977) Studies on phosphate solubilizing bacteria in soil and rhizosphere of different plants I. Occurrence of bacteria-acid producers and phosphate dissolvers. Zentralbl Bacteriol Parasitenkd Infektionskr Hyg 132:233–239

Gaur AC (1986) Particle size of rock phosphate and microbial solubilization. Zentralbl Microbiol 141:103–105

Gaur AC, Gaind S (1992) Role of phosphorus solubilizing micro-organisms in crop productivity and enriched organic manure. In: Rai MM, Verma IN (eds) National Seminar on Organic Farming held during 28–29 September, 1992 at College of Agriculture, Indore, p 134

Gilman JC (1957) A manual of soil fungi, 2nd edn. Oxford/IBH, New Delhi

Goldstein AH (1986) Bacterial solubilization of mineral phosphates: Historical perspective and future prospects. Am J Altern Agric 1:51–57

Gordon SA, Paleg LG (1957) Quantitative measurement of indole acetic acid. Plant Physiol 10:37–48

Gupta RD, Singal R, Shankar A, Kuhad RC, Saxena RK (1994) A modified assay for screening phosphate solubilizing microorganisms. J Gen Appl Microbiol 40:255–260

Holt JG, Kreig WR, Sneath PHA, Staley JT, William ST (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore

Jackson ML (1973) Soil chemical analysis. Prentice Hall, New Delhi

Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH and temperature tolerant, phosphate solubilizing bacteria in alkaline soils. Curr Microbiol 39:89–93

Katznelson H, Bose B (1959) Metabolic activity and phosphate dissolving ability of bacterial isolates from wheat root rhizosphere and non rhizosphere. Can J Microbiol 5:79–85

Kucey RMN (1983) Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678

Kucey RMN, Janzen HH, Leggett ME (1989) Microbially mediated increases in plant-availability of phosphorus. Adv Agron 42:199–228

Kundu BS, Nehra K, Yadav R, Tomar M (2009) Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. Indian J Microbiol 49:120–127

Leinhos V, Vacek O (1994) Biosynthesis of auxins by phosphate solubilizing rhizobacteria from wheat and rye. Microbiol Res 149:31–35

Mishra PK, Mishra S, Bisht SC, Selvakumar G, Kundu S, Bisht JK, Gupta HS (2009) Isolation, molecular characterization and growth-promotion activities of a cold tolerant bacterium *Pseudomonas* sp. NARs9 (MTCC9002) from the Indian Himalayas. Biol Res 42:305–313

Mudalagiriyappa S, Agasimani CA, Veeranna HK, Nanjappa HV (1997) Growth analysis and pattern of dry matter accumulation in groundnut (*Arachis hypogea*) as influenced by phosphate solubilizers. Crop Res 13:541–546

Ocampo JA, Barea JM, Montoya E (1975) Interactions between *Azotobacter* and "phosphobacteria" and their establishment in the rhizosphere as affected by soil fertility. Can J Microbiol 21:1160–1165

Pakale NV, Alagawadi AR (1993) Nitrification potential of soils as influenced by soil microbial population. Karnataka J Agric Sci 6:70–72

Paleg LG (1965) Physiological effects of gibberellins. Annu Rev Plant Physiol 16:291–322

Pikovskaya RI (1948) Mobilization of phosphates in soil in connection with the vital activities of some microbial species. Mikrobiologiya 17:362–370

Promod KC, Dhevendaran K (1987) Studies on phosphobacteria in Cochin backwater. J Mar Biol Assoc India 29:297–305

Raj J (1980) Role of microorganisms in the release of soil phosphates to plants. PhD thesis, University of Agricultural Sciences, Bangalore

Rajankar PN, Tambekar DH, Wate SR (2007) Study of phosphate solubilization efficiencies of fungi and bacteria isolated from saline belt of Purna river basin. Res J Agric Biol Sci 3:701–703

Salih HM, Yahya AI, Abovl-Raheni AM, Munam BH (1989) Availability of phosphorus in a calcareous soil treated with rock phosphate or super phosphate as affected by phosphate dissolving fungi. Plant Soil 120:181–185

Siddikee MA, Chauhan PS, Anandham R, Han GH, Sa T (2010) Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20 (11):1577–1584

Subba Rao NS (1977) Microbial products influencing plant growth. In: Soil Microbiology, 4th edn. Oxford/IBH, New Delhi, pp 277–278

Surange S, Wollum AG, Kumar N, Nautiyal CS (1997) Characterization of *Rhizobium* from root nodules of leguminous trees growing in alkaline soils. Can J Microbiol 43:891–894

Thomas GV, Shantaram MV, Saraswathy N (1985) Occurrence and activity of phosphate-solubilizing fungi from coconut plantation soils. Plant Soil 87:357–364

Tomar SS, Abbas M, Khandkar UR (1994) Availability of phosphorus to urdbean as influenced by phosphate solubilizing bacteria and phosphorus levels. Indian J Pulses Res 7:28–32

Tomar RKS, Namdeo KN, Raghu JS (1996) Efficacy of phosphate solubilizing bacteria for biofertilizer with phosphorus on growth and yield of gram (*Cicer arietinum*). Indian J Agron 41:413–415

Vikram A, Hamzehzarghani H, Alagawadi AR, Krishnaraj PU, Chandrashekar BS (2007) Production of plant growth promoting substances by phosphate solubilizing bacteria isolates from vertisols. J Plant Sci 2(3):326–333

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, New York, pp 315–322

Zahran HH, Ahmad MS, Afkar EA (1995) Isolation and characterization of nitrogen fixing moderate halophilic bacteria from saline soils of Egypt. J Basic Microbiol 35:269–275