3 Biotech (2018) 8:463
https://doi.org/10.1007/s13205-018-1488-4

ORIGINAL ARTICLE

Characterization of mineral phosphate solubilizing and plant growth promoting bacteria from termite soil of arid region

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Received: 19 September 2017 / Accepted: 21 October 2018 / Published online: 28 October 2018

Abstract

Five highly efficient phosphate solubilizing bacteria, viz., *Pantoea* sp. A3, *Pantoea* sp. A34, *Kosakonia* sp. A37, *Kosakonia* sp. B7 and *Bacillus* sp. AH9 were isolated from termitorial soils of Sanjivani island of southern Maharashtra, India. These isolates were characterized and explored for phosphate solubilization and plant growth promotion. Among these, *Bacillus* sp. AH9 showed highest phosphate solubilization index (3.5) and solubilization efficiency (250%) on Pikovskaya agar. Interestingly, *Pantoea* sp. A34 displayed maximum mineral phosphate solubilization (1072.35 mg/L) in liquid medium and during this period the pH dropped to 3.13. All five isolates had highest P solubilization at 48 h after inoculation. During mineral phosphate solubilization, both gluconic acid and 2-keto gluconic acid were produced by *Kosakonia* and *Bacillus* isolates, while only 2-keto gluconic acid was detected in *Pantoea* isolates. Highest organic acid (39.07 ± 0.04 g/L) production was envisaged in *Bacillus* sp. AH9, while *Pantoea* sp. A34 produced the least amount (13.00 ± 0.01 g/L) of organic acid. Seed bacterization with *Pantoea* sp. A3 and *Kosakonia* sp. A37 resulted in ~ 37% and ~ 53% increase in root length of tomato seedlings, respectively, while *Pantoea* sp. A34 and *Kosakonia* sp. B7 had deleterious effects on root length as well as overall growth of the seedlings. To our knowledge, this is the first report of plant growth promoting potential of microorganisms isolated from termitorial soil of Sanjivani island, which is a drought-prone area. Therefore, such efficient growth promoting P solubilizers can offer an effective solution for sustainable agriculture in arid, dryland farming and drought-prone regions.

Keywords Termite · P solubilizing bacteria · Tri-calcium phosphate · Organic acids · Plant root elongation

Introduction

Phosphorous in soil acts as essential macronutrient required for growth and development of the plant due to its involvement in various critical biological functions (Illmer and Schiner 1992). But, the availability of this important nutrient is very often limited in the soil (Vassilev et al. 2006; Menezes-Blackburn et al. 2018) which is the reason why phosphatic fertilizers have become the world's second most consumed agrochemical after nitrogen (Hameeda et al. 2008). Rapid immobilization of the applied phosphatic fertilizers in soil necessitates frequent and random input of these fertilizers which becomes a costly affair and also deteriorates the soil health (Dey 1988; Tewari et al. 2004). Hence, the release of fixed and insoluble P can increase the bioavailability of this important macronutrient in soil.

Soil bacteria are crucial in the phosphorus cycle and some of them can enhance the available phosphorus pool for the plants by solubilizing the insoluble phosphates. Such bacteria known as phosphate solubilizing bacteria (PSB) may be used as inoculants to increase the phosphorus availability to plants. Solubilization of insoluble phosphate is mediated by a variety of phosphate solubilizing bacteria (PSB), belonging to the genera *Bacillus, Pseudomonas, Rhizobium, Burkholderia, Achromobacter, Azotobacter, Agrobacterium, Micrococcus, Flavobacterium,*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1488-4) contains supplementary material, which is available to authorized users.

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Erwinia through production of organic acids, chelation and exchange reactions (Istina et al. 2015; Kumar et al. 2014; Rodriguez and Fraga 1999; Halder et al. 1990; Craven and Hayasaka et al. 1982; SundaraRao and Sinha 1982). Among different PSBs isolated till date, *Bacillus* spp. and *Pseudomonas* spp. are the most predominant. Especially, *Bacillus* spp. due to their ability to sporulate, offer a significant advantage for the development of biofertilizers because of their survival during storage and in soil (Caesart and Burr 1991). Gram negative PSB have been reported to produce organic acids especially gluconic acid in periplasm by direct oxidation of glucose, while gram positive bacteria like *Bacillus* is known to produce a mixture of organic acids.

Termitarium or termite nest consists of partially digested food, termite excreta and soil particles. The termitarium is enriched with minerals like calcium, phosphorus with high organic matter content, making it a congenial habitat for diverse microorganisms. Firmicutes, Proteobacteria and Actinobacteria have been reported to be the predominant bacterial groups present in both termite guts and termitarium (Moreira et al. 2018; Chew et al. 2018; Manjula et al. 2014, 2016; Fall et al. 2007). However, few reports suggested that Gemmatimonadetes and Bacteroidetes are among the important microbial groups present in termite mounds (Makonde et al. 2015; Ohkuma and Kudo 1996). Members of Bacillaceae and Enterobacteriaceae were the predominant families under Firmicutes and Proteobacteria (Manjula et al. 2016). Fall et al. (2007) reported that members of Nocardiodaceae family dominated the actinobacterial population of termite mounds (Fall et al. 2007). Bacteria with known plant growth promoting traits like diazotrophy, organic matter decomposition, sulfur oxidation have been reported from termite guts and mounds (Chew et al. 2018; Manjula et al. 2014). Recently, phosphate solubilizing bacteria have also been recovered from termitarium (Chauhan et al. 2016). Due to presence of such diverse microbiota with multifarious plant beneficial traits, termite mounds have also been exploited as biofertilizers (Duponnois et al. 2005; Miyagawa et al. 2011). The use of PSB as biofertilizers is well-known as it results in naturally occurring phosphates becoming bioavailable and thereby improving the soil health. There is a huge phosphatic rock deposit in India which can be used as a cheap source of phosphate along with PSB biofertilizers (Hameeda et al. 2008; Niranjan Raj et al. 2006; Richardson 2001).

The present study reports isolation, molecular identification and characterization of highly efficient mineral phosphate solubilizing bacteria from termitorial soils of Sanjivani island, India, which is microbiologically unexplored and famous for rare medicinal herbs.

Materials and methods

Samples and isolation of phosphate solubilizing bacteria (PSB)

Sanjivani island (also known as Wadwal Bet) is located between 73.25°N and 18.7°E in Latur district, India. The island having a rocky undulating topography, harbors a number of rare medicinal herbs (Satpute et al. 2013). This unusual habitat has not yet been explored for its microbial wealth. Termitarium samples were collected from Sanjivani island. The samples were stored at 4 °C in sterile containers. For each sample, 1 g soil was serially diluted in physiological saline (0.85% NaCl w/v). 100 µL aliquots from 10^{-4}, 10^{-5} and 10^{-6} dilutions were plated in Pikovskaya (PVK) agar (Pikovskaya 1948), International Streptomyces Project (ISP) 4 medium, ISP7, Actinomycetes agar and incubated at 37 °C for 48–72 h (for PVK) and at 28 °C for 72–144 h (for ISP4, ISP7 and Actinomycetes agar). 10^{-4} dilution of the above-mentioned sample was heated at 60 °C for 30 min and 100 µL aliquot were spread on Pikovskaya agar plates with the intention to specifically isolate spore-forming bacteria. Colonies forming clear halo around them were again streaked on Pikovskaya agar plates for further purification. Purified bacterial isolates were preserved as glycerol (50%) stocks at − 80 °C. All isolates were grown in National Botanical Research Institute's Phosphate growth medium with Bromophenol blue (NBRIP-BPB) broth (Nautiyal 1999) and monitored at every 24 h (up to 72 h) for a preliminary screening of potential phosphate solubilizing bacteria. After initial screening, selected PSB were again grown in NBRIP-BPB broth for quantification of mineral phosphate solubilization for finally selecting a few isolates for further characterization.

Growth medium and conditions

Pikovskaya agar medium, ISP4, ISP7 and Actinomycetes agar were procured from HiMedia (India) and NBRIP-BPB broth was prepared using the following composition—glucose 10 g/L, tri-calcium phosphate 5 g/L, magnesium chloride 5 g/L, magnesium sulfate 0.25 g/L, potassium chloride 0.20 g/L, ammonium sulfate 0.10 g/L, and bromophenol blue 0.025 g/L. For nutrient broth, the following composition was used—beef extract 10 g/L, sodium chloride 5 g/L, and peptone 10 g/L. All isolates were inoculated (@ 1% inoculums from glycerol stock) in 10 mL NBRIP-BPB broth and kept under shaking (140 rpm) at 37 °C up to 72 h. Samples were collected aseptically at different points of time for quantification of

mineral phosphate solubilization. After final screening, the selected isolates were inoculated with 1% inoculum (overnight grown culture) in 100 mL NBRIP-BPB broth and incubated at 37 °C under continuous shaking (140 rpm). Samples were drawn aseptically at every 24 h for quantification of mineral phosphate solubilization and determination of pH of the medium. This procedure was continued up to 144 h after incubation. 10 µL of overnight grown cultures of the selected isolates was spot inoculated on Pikovskaya agar plates and incubated for 72 h for determination of solubilization index and solubilization efficiency.

Mineral phosphate solubilization assay

Phosphorus in the culture supernatant was estimated by the method described by Fiske and Subbarow (1925). The solubilized phosphorus was calculated by subtracting the amount of inorganic phosphorus in the control tubes from the amount of inorganic phosphorus in the inoculated tubes.

Determination of solubilization index (SI) and solubilization efficiency (SE)

After 72 h incubation at 37 °C, colony diameter and halo diameter of each selected isolate on Pikovskaya agar plates were measured to determine SI and SE (Edi-Promono et al. 1996; Nguyen et al. 1992) using the following formula:

$$SI = (\text{Colony diameter} + \text{Halo diameter})/\text{Colony diameter},$$

$$SE = (\text{Halo diameter}/\text{Colony diameter}) \times 100.$$

Identification and quantification of organic acids produced during P solubilization

For HPLC analysis, 1 mL NBRIP-BPB broth containing bacterial suspension was collected aseptically and centrifuged for 2 min at 10,000 rpm. 500 µL supernatant was transferred in fresh 1.5 mL micro-centrifuge tubes and to it 500 µL 1N HCl was added to solubilize any traces of insoluble phosphate. 100 µL of this suspension was diluted five times in the mobile phase (8 mM sulphuric acid). Organic acids present in the suspension were detected by high-performance liquid chromatography (HPLC) system (Dionex India Ltd.) equipped with refractive Index (RI) detector. An ion exclusion column (Aminex, HPX-87H, Biorad, Hercules, CA) was used at a temperature of 30 °C with 8 mM sulphuric acid as mobile phase at flow rate of 0.6 mL/min. Injection volume of the sample was 50 µL. Standard organic acids, viz., gluconic acid, 2-keto gluconic acid, lactic acid, glucuronic acid, malic acid, succinic acid, oxalic acid, citric acid procured from Sigma-Aldrich were run in HPLC for quantification. For this purpose, all standards were prepared in mobile phase with a final concentration of 1 mg/mL.

Phenotypic and biochemical characterization

Colony morphology of the selected isolates was recorded after growing the bacteria on Pikovskaya agar plates. Biochemical tests and the ability to utilize different carbon sources were performed using the Hi25^{TM}Enterobacterial identification kit procured from HiMedia (India). The ability of the bacterial isolates to grow under different temperatures was determined. For this, bacterial isolates were grown on nutrient agar plates at different temperatures 4 °C, 37 °C, 45 °C and 50 °C and the growth was recorded after 24 h of incubation. Tolerance of the isolates to NaCl was determined by growing them on NaCl (0–10% w/v) supplemented nutrient agar at 37 °C. Nutrient agar with pH ranging from 3 to 11 (at a pH 2 interval) was used to determine the optimum pH for growth of the isolates.

Molecular identification of the selected isolates

Genomic DNA extraction from the overnight grown bacterial isolates was carried out using the HiPur DNA Express kit (HiMedia, India).Genomic DNA was quantified spectrophotometrically. The 16S rRNA gene was amplified using the universal primers (16S F27 and 16S R1525). The amplification was obtained in reaction volume of 50 µL containing 1 µL (~ 100 ng) genomic DNA, 2.5 µL forward primer, 2.5 µL reverse primer, 5 µL (2.5 mM) dNTPs, 5 µL 10X PCR Buffer S with 17.5 mM $MgCl_2$ (HiMedia, India), 1 Unit Taq Polymerase (HiMedia, India) and sterile water. The following conditions were used for amplification using a thermal cycler (Applied Biosystems, USA)—initial denaturation at 94 °C for 3 min, 34 cycles of (1) denaturation at 94 °C for 30 s, (2) primer annealing at 55 °C for 30 s, (3) primer extension at 72 °C for 2 min followed by final extension at 72 °C for 7 min. PCR products were purified using the HiPurA™ PCR Product Purification Kit (HiMedia, India). Purified PCR products were sequenced and based on partial sequences of 16S rRNA gene, phylogenetically related bacteria were aligned using a BLAST search against the GenBank database. Multiple sequence alignment of the 16S rRNA gene sequences of the related taxa were implemented using the *CLUSTAL W* option in MEGA 7 (Kumar et al. 2016). Jukes–Cantor coefficient was used to calculate the evolutionary distances for construction of a neighbor-joining phylogenetic tree (Saitou and Nei 1987; Jukes and Cantor 1987). Topology of the phylogenetic tree was evaluated by bootstrapping with 1000 replicates (Felsenstein 1985).

Plant root elongation assay

To assess the ability of the selected isolates to promote the elongation of plant roots, tomato (*Lycopersicon esculentum*) seeds were used. The seeds were procured from local

market. Seeds were soaked overnight in water and the next day they were surface sterilized using 70% ethanol for 30 s followed by rinsing the seeds in sodium hypochlorite (2%) for 10 min and then washed twice with sterile distilled water. Then, the seeds were inoculated in overnight grown bacterial suspension (~ 10^8cfu/mL) and kept at room temperature for 2 h. After that, three seeds were placed aseptically on semi-solid (0.5%) agar plates along with control plates containing seeds soaked in sterile water. The plates were incubated at 37 °C under light for 10 days and measurement of the root length was performed using centimeter scale.

Statistical analysis

All assays including plant root elongation assay were carried out in triplicates. Selection of the bacterial isolates based on ability to solubilize mineral phosphate was carried out through Duncan's multiple range test (DMRT) using SPSS version 16 program. Calculation of standard error of mean was performed in Microsoft Excel 2007. All the experiments (except isolation of bacteria) were performed thrice with two replications.

Results

Isolation and screening of PSB

A total of 200 bacterial isolates were obtained after spreading the soil sample on Pikovskaya agar, ISP4, ISP 7, Actinomycetes agar plates including five isolates after plating the soil sample were heated at 60 °C. When all these bacterial isolates were subjected to qualitative screening for mineral phosphate solubilization based on color change in NBRIP-BPB broth, 23 isolates showed color change within 24 h, 26 isolates showed color change after 48 h and 151 isolates did not show any color change even after 72 h.

The 23 bacterial isolates which showed rapid change in color of NBRIP-BPB broth were selected for further screening, as they were assumed to be good mineral phosphate solubilizer. When these isolates were subjected to quantitative screening for mineral phosphate solubilization over a period of 72 h, it was found that five isolates, viz., A3, A34, A37, B7 and AH9 were superior over the others throughout 72 h period of time as indicated by their mean phosphate solubilization values which were statistically significantly higher over the others (Table 1).

Table 1 Quantitative screening of selected 23 bacterial isolates for P solubilization

Sl. no.	Isolates	Solubilized phosphorus (mg/L)		
		24 h	48 h	72 h
1	A1	$223.61^{c,d}$	313.19^{e}	406.25^{a}
2	A3	$326.04^{a,b,c,d}$	$450.34^{a,b,c,d,e}$	642.71^{a}
3	A5	$321.80^{a,b,c,d}$	690.97^{a}	609.72^{a}
4	A11	$264.58^{b,c,d}$	$530.90^{a,b,c,d,e}$	574.30^{a}
5	A12	$226.73^{c,d}$	$507.64^{a,b,c,d,e}$	606.60^{a}
6	A13	$306.60^{b,c,d}$	$413.54^{b,c,d,e}$	564.23^{a}
7	A14	$298.61^{b,c,d}$	$628.82^{a,b,c}$	669.10^{a}
8	A19	$322.15^{a,b,c,d}$	$642.36^{a,b}$	664.23^{a}
9	A29	$324.79^{a,b,c,d}$	$576.73^{a,b,c,d}$	636.46^{a}
10	A32	192.36^{d}	$379.86^{c,d,e}$	418.75^{a}
11	A34	$376.73^{a,b,c,d}$	$536.11^{a,b,c,d,e}$	604.17^{a}
12	A37	$478.82^{a,b,c}$	$667.36^{a,b}$	652.43^{a}
13	A38	202.43^{d}	$360.41^{d,e}$	501.73^{a}
14	A40	$296.18^{b,c,d}$	$564.58^{a,b,c,d,e}$	594.10^{a}
15	A41	199.30^{d}	$494.79^{a,b,c,d,e}$	495.14^{a}
16	A53	$286.80^{b,c,d}$	705.55^{a}	710.42^{a}
17	B1	$323.25^{a,b,c,d}$	$658.33^{a,b}$	697.57^{a}
18	B5	$321.94^{a,b,c,d}$	$565.27^{a,b,c,d,e}$	569.44^{a}
19	B6	$281.25^{b,c,d}$	$535.76^{a,b,c,d,e}$	613.19^{a}
20	B7	$501.70^{a,b}$	696.87^{a}	682.99^{a}
21	AC4	$262.65^{b,c,d}$	$402.97^{c,d,e}$	587.76^{a}
22	AC24	$302.12^{b,c,d}$	$424.87^{c,d,e}$	601.23^{a}
23	AH9	586.32^{a}	647.12^{a}	681.21^{a}

Values bearing same letters are not significantly different at 95% confidence limit

Molecular and phenotypic characterization

All the five isolates were identified based on 16 s rRNA gene sequence, colony morphology, biochemical tests, carbon source utilization and some physiological attributes (Table S1 and Table S2). A3, A34, A37 and B7 were found to be gram negative short rods, while AH9 was found to be gram positive long rod. A3 and A34 showed 100% and 99.63% similarity with *Pantoea dispersa* LMG 2603^T and *Pantoea septica* LMG 5345^T, respectively. A37 showed 99.25% similarity with *Kosakonia cowanii* JCM 10956^T (Basonym *Enterobacter cowanii*), while B7 also showed 99.37% similarity with the same type strain of *K. cowanii*. AH9 showed 99.81% similarity with *Bacillus megaterium* NBRC 15308^T (Fig. 1). Partial 16S rRNA gene sequences of these five isolates were submitted to NCBI with accession numbers KC588504.2, KC588506.2, KC588510.2, KC588509.2 and KC588519.2. A comparison of biochemical test and carbon source utilization with the type strains based on the earlier reports is presented in Table S1. *Pantoea* sp. A3 was found to have similar phenotypic attributes with the type strain *P. dispersa* LMG2603^Texcept lysine utilization, rhamnose utilization and lactose utilization while *Pantoea* sp. A34 also differed from *P. septica* LMG5345^T with respect to phenylalanine deaminase activity, Voges–Proskauer test, cellobiose, mellibiose and

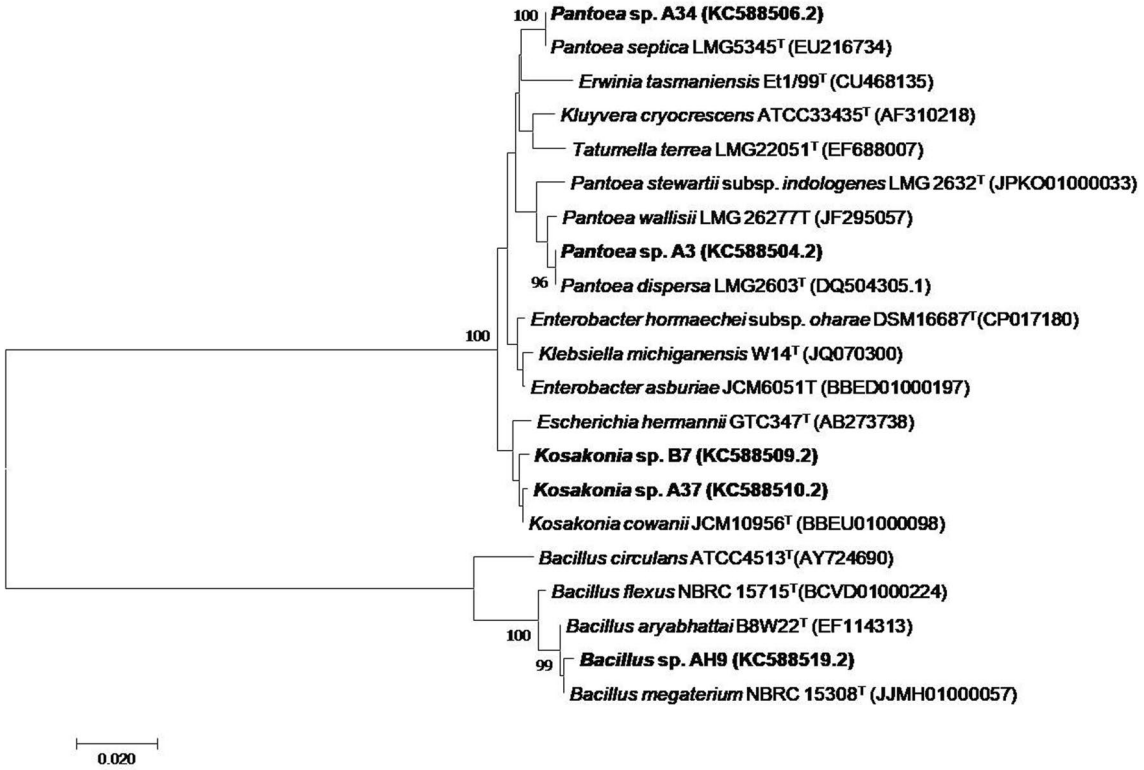

Fig. 1 Neighbor-joining (NJ) phylogenetic tree based on 16S rRNA gene sequences showing the position of the P solubilizing bacterial isolates (shown in bold) with their relatives. The scale bar indicates 0.02 nucleotide exchanges per nucleotide. Bootstrap values > 95% are shown at the inter-nodes

rafiinose utilization. *Kosakonia* sp. A37 and *Kosakonia* sp. B7 also differed from type strain *K. cowanii* JCM 10956^T with respect to lysine utilization, ornithine utilization, raffinose utilization and lactose utilization. *Bacillus* sp. AH9 differed from the type strain *B. megaterium*15308^T with respect to orinithine and cellobiose utilization. All the isolated strains were able to grow in a pH range of 7–9, at a temperature of 37 °C except *Bacillus* sp. AH9 which was also able to grow even at 50 °C. *Pantoea* strains (A3 and A34) were able to grow at salt concentrations ranging from 0 to 10%, while the rest of the strains was able to grow at a range of 0–8% NaCl concentration.

P solubilization and root elongation potential of the selected isolates

When SI and SE for the five isolates were determined on the basis of halo zone formation in Pikovskaya agar plates, it was found that *Bacillus* sp. AH9 had maximum SI (3.5) and SE (250%), while *Pantoea* sp. A34 had minimum SI (1.8) and SE (75%). SI and SE for all the isolates are presented in Table 2. These five isolates were inoculated in NBRIP-BPB broth to validate their performance in the experiment performed in the culture tubes. Phosphate solubilization of all the isolates was maximum after 48 h after inoculation for all the isolates (Table 2). Although, SI and SE of *Pantoea* sp. A34 were lowest among all, it showed maximum (1072.35 mg/L) solubilization of tri-calcium phosphate (Table 2). Increase in pH values of the culture medium was in compliance with the decrease in P solubilzation in the medium. During the course of phosphate solubilization, the organic acids involved in P solubilization were identified using HPLC and for this purpose, samples after 48 h of incubation were used as P solubilization which was maximum during this period. 2-keto gluconic acid was found to be the only organic acid produced during the phosphate solubilization in *Pantoea* sp. A3, *Pantoea* sp. A34 while in *Kosakonia* sp. A37, *Kosakonia* sp. B7 and *Bacillus* sp. AH9 both gluconic acid and 2-keto gluconic acid were produced, but gluconic acid was found to be produced in a higher amount in these three strains (Table 3; Fig. 2). Among all the six strains, *Bacillus* sp. AH9 produced the highest amount of organic acid (39.07 g/L), while the lowest amount of acid was produced by *Pantoea* sp. A34 (13.0 g/L).

After treating the seeds with individual bacterial isolates, it was observed that *Pantoea* sp. A3 and *Kosakonia* sp. A37 (root length—10 cm and 11.2 cm, respectively, for A3 andA37) could enhance the tomato root elongation

Table 2 Phosphate solubilization by the selected isolates at different time intervals

Bacterial isolates	Time period (h)	P solubilized (mg/L)	Final pH	SI (determined after 72 h)	SE (%)
Pantoea sp. A3	24	$^{c}856.35 \pm 2.19$	3.53	2.0	100.0
	48	$^{a}874.54 \pm 2.67$	3.31		
	72	$^{b}864.12 \pm 1.00$	3.32		
	96	$^{d}796.06 \pm 2.10$	3.40		
	120	$^{e}791.46 \pm 6.46$	3.36		
	144	$^{d}795.46 \pm 2.10$	3.36		
Pantoea sp. A34	24	$^{b}1067.33 \pm 6.62$	3.35	1.8	75.0
	48	$^{a}1072.35 \pm 2.85$	3.33		
	72	$^{c}1037.08 \pm 2.50$	3.37		
	96	$^{d}1033.54 \pm 3.13$	3.39		
	120	$^{e}1026.67 \pm 1.67$	3.40		
	144	$^{e}1024.98 \pm 1.89$	3.40		
Kosakonia sp. A37	24	$^{d}828.64 \pm 2.60$	3.48	2.6	162.5
	48	$^{a}853.48 \pm 3.69$	3.32		
	72	$^{b}844.68 \pm 3.23$	3.79		
	96	$^{c}837.50 \pm 4.16$	4.03		
	120	$^{d}827.40 \pm 2.19$	4.26		
	144	$^{e}813.32 \pm 2.12$	4.50		
Kosakonia sp. B7	24	$^{b}903.21 \pm 1.67$	3.73	2.7	166.7
	48	$^{a}920.54 \pm 2.54$	3.56		
	72	$^{c}889.68 \pm 2.62$	3.93		
	96	$^{d}872.87 \pm 1.62$	4.25		
	120	$^{e}845.73 \pm 3.23$	4.62		
	144	$^{f}831.21 \pm 3.21$	5.13		
Bacillus sp. AH9	24	$^{b}863.54 \pm 3.12$	3.48	3.5	250
	48	$^{a}907.29 \pm 2.98$	3.55		
	72	$^{c}776.46 \pm 3.01$	3.63		
	96	$^{d}744.79 \pm 1.74$	3.70		
	120	$^{e}722.92 \pm 1.11$	3.77		
	144	$^{f}686.46 \pm 2.12$	3.73		

Values bearing same letters are not significantly different at 95% confidence limit

Table 3 Identification and quantification of organic acids produced during P solubilization

Bacterial isolates	Organic acids (g/L) detected by HPLC after 48 h		
	GA	2-KGA	Total
Pantoea sp. A3	ND	14.37 ± 0.03	14.37 ± 0.03
Pantoea sp. A34	ND	13.00 ± 0.01	13.00 ± 0.01
Kosakonia sp. A37	23.47 ± 0.02	4.30 ± 0.01	27.77 ± 0.02
Kosakonia sp. B7	17.76 ± 0.01	1.19 ± 0.01	18.95 ± 0.01
Bacillus sp. AH9	34.06 ± 0.05	5.01 ± 0.02	39.07 ± 0.04

Although standards were run for citric acid, oxalic acid, succinic acid, malic acid, tartaric acid, glucuronic acid, these were not detected

The values are represented as mean ± SE

GA gluconic acid, *2-KGA* 2-keto gluconic acid, *LA* lactic acid, *ND* not detected

as compared to control (root length—7.30 cm) where seeds were soaked in distilled water (Table 4). But, *Kosakonia* sp. B7 and *Pantoea* sp. A34 were found to have a deleterious effect on root elongation, where seedlings were found to be crippled with very short roots (Fig. 3). The bacterial isolate *Bacillus* sp. AH9 did not have any beneficial or deleterious effect on root length as it was on par with the control (Table 4; Fig. 3). Three bacterial cultures, viz., *Pantoea* sp. A3, *Kosakonia* sp. A37 and *Bacillus* sp. AH9 were deposited to National Collection of Industrial Microorganisms (NCIM) at CSIR-National Chemical Laboratory (NCL), Pune, India with the accession numbers NCIM-5679, NCIM-5680 and NCIM-5681, respectively.

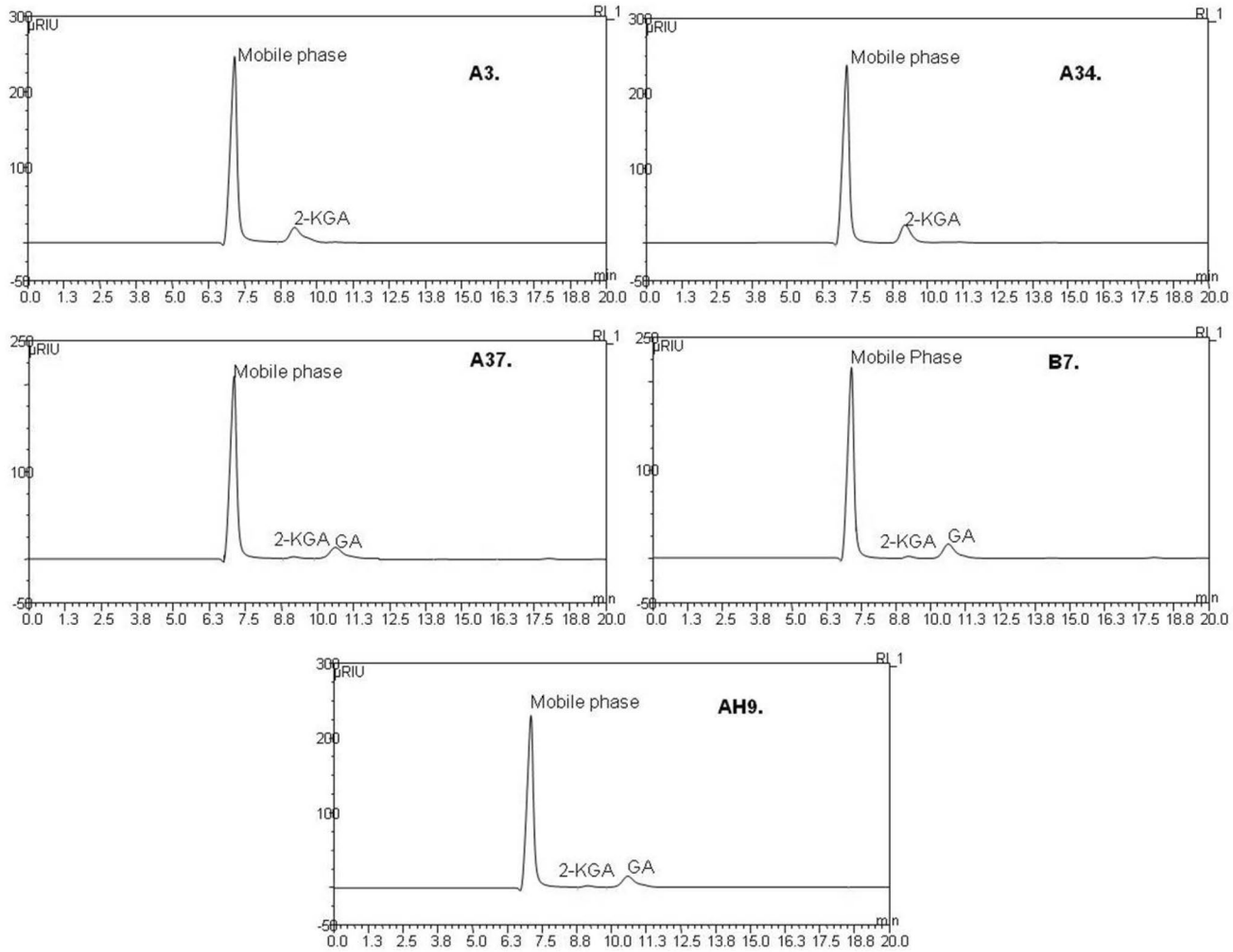

Fig. 2 Chromatograms showing the presence or organic acids produced during solubilization of tri-calcium phosphate. GA: gluconic acid; 2-KGA: 2-keto gluconic acid

Table 4 Effect of seed treatment with bacterial isolates on tomato root

Treatments	Root length (cm)
Pantoea sp. A3	$^{b}10.00 \pm 0.25$
Pantoea sp. A34	$^{e}2.70 \pm 0.32$
Kosakonia sp. A37	$^{a}11.20 \pm 0.15$
Kosakonia sp. B7	$^{d}3.30 \pm 0.03$
Bacillus sp. AH9	$^{c}7.70 \pm 0.03$
Control	$^{c}7.30 \pm 0.19$

Values bearing same letters are not significantly different at 95% confidence limit

Discussion

Termites are known to influence the soil and carbon cycling significantly by degrading lignocellulosic material in the soil with help of their active intestinal microflora (Breznak and Brune 1994). Apart from lignocellulose degradation, the gut microflora is also involved in methanogenesis, acetogenesis and nitrogen fixation. Members of the family Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, etc., have been attributed as major bacterial groups present in termite guts. Manjula et al. (2016) reported unique phyla like Acidobacteria and Verrucomicrobia from termite gut. The presence of phylum Acidobacteria in termite gut was attributed to its ability to survive under acidic conditions, while Verrucomicrobia is well-documented for degradation of cellulose, fixation of nitrogen, and acetogenesis (Manjula et al. 2016). Earlier, the genera *Bacillus* and *Paenibacillus* involved in breakdown of polysaccharides and aromatic

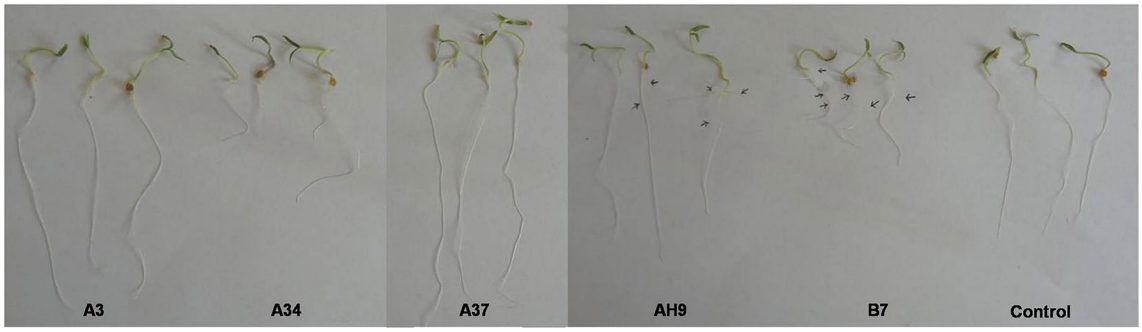

Fig. 3 Effect of bacterial inoculation on root elongation of tomato seedlings

compounds have been reported (Kuhnigk 1994; Kuhnigk and Konig 1997). Long back, nitrogen fixing *Enterobacter agglomerans* was also reported from termite gut (Potrikus and Breznak 1977). As the termite nest/mound is built from the termite feces and soil, it is likely that the termite nest will be dominated by a similar kind of microflora (Manjula et al. 2016). Microbial groups endowed with nitrogen fixation, polysachharide degradation have also been reported from termite mounds (Chew et al. 2018; Miyagawa et al. 2011; Potrikus and Breznak 1977).

In the present study, PSBs were isolated from the termitorial soils from Sanjivani island in southern Maharashtra which is famous for presence of rare herbs and this place is unique due to its topography with small hillocks with almost complete rocky surfaces. In this study, PSBs were isolated based on halo zones formed on Pikovskaya agar plates, but the final screening was done based on color change and solubilization of tri-calcium phosphate in NBRIP-BPB broth, as the halo-based technique is questioned because of several studies reporting bacterial isolates which could solubilize insoluble phosphates without showing halo zones (Gupta et al. 1994; Louw and Webley 1959). The five bacterial isolates belonging to the genera *Pantoea, Kosakonia* and *Bacillus* hold considerable promise for mineral phosphate solubilization. Members of the genera, viz., *Enterobacter, Pantoea* are known for their phosphate solubilizing ability (Rodriguez and Fraga 1999; Dastager et al. 2009; Oliveira et al. 2009). Perez et al. (2007) reported solubilization of tri-calcium phosphate to the tune of 338 mg/L and 363 mg/L, by *P. agglomerans* (strain MMB051) and *Pantoea ananatis* (strain MMB047), respectively, after 3 days of incubation. While Oliveira et al. (2009) reported mineral phosphate solubilization of only 10.8 mg/L and14.6 mg/L in *P. ananatis* (strain B52) and *P. agglomerans* (strain B53), respectively, isolated from cultivated Brazilian soil. Dastager et al. (2009) reported 28 mg/L/day of tri-calcium phosphate solubilization by *Pantoea* NII-186 isolated from soils of Western Ghat forest in India. In the present, the isolated *Pantoea* strains, viz., A3 and A34 were recorded with tri-calcium phosphate solubilization to the tune of 856.35 mg/L and 1067.33 mg/L after 24 h which increased to their maximum values after 48 h. Hameeda et al. (2008) reported phosphate solubilizing ability of *Enterobacter cloaceae* strain EB27, while Gyaneshwar et al. (2002) reported mineral phosphate solubilizing activity in *E. asburiae*. Gupta et al. (2012) reported 150 mg/L P solubilization by a *Enterobacter hormaechei* strain. The Enterobacterial strains isolated in this study showing close match to *K. cowanii* JCM 10956^T (Basonym *E. cowanii*), showed high tri-calcium phosphate solubilization activity. However, no Enterobacteriaceae members isolated from termitarium have been reported to solubilize mineral phosphate. To the best of our knowledge, this is the first report of P-solubilizing Enterobacterial members from soils derived from termite activity. Members of Enterobacteriaceae are known to produce different organic acids for solubilization of Phosphate. Sulbaran et al. (2008) reported production gluconic acid during mineral phosphate solubilization by *P. agglomerans* MMB051, while Perez et al. (2007) also reported the gluconic acid production during mineral phosphate solubilization by *P. ananatis* and *P. agglomerans* isolates. 2-Ketogluconic and gluconic acid were the major organic acids produced by these bacteria. Among gram positive bacteria, the genus *Bacillus* is well-studied for their ability to solubilize mineral phosphates. *B. megaterium* var. *phosphoricum* is well-recognized as an efficient PSB and has been successfully used in India and former Soviet Union (Rodriguez and Fraga 1999). *Bacillus* sp. AH9 isolated in this study also showed very high phosphate-solubilization activity with tri-calcium phosphate solubilization to the tune of 1079.73 mg/L and 907.29 mg/L, respectively, after 24 h. *B. megaterium* has also been reported to be present in the guts of both higher and lower termites, however, only cellulolytic activity could be detected in those (Krasil'nikov and Satdykov 1969; Wenzel et al. 2002). Recently, Chauhan et al. (2017) reported P-solubilizing *B. endophyticus* and *B. cereus* isolated from termitarium. However, the range of P solubilization reported by Chauhan et al. seems too high (16.2 g/L). *B. megaterium* strain 573 isolated from coral

reefs of Gulf of Mannar showed very high (0.906 mmol/L P) mineral phosphate solubilization (Kannapiran and Ravindran 2012). Chen et al. (2006) reported P solubilization by *B. megaterium* (CC-BC10) to the tune of 270.2 mg/L. *Bacillus* sp. AH9 showed the presence of both gluconic acid and 2-keto gluconic acid, although gluconic acid was predominant. Chen et al. (2006) reported the presence of citric acid, lactic acid, propionic acid and one unidentified organic acid from different strains of *B. megaterium*, while Kannapiran and Ravindran (2012) reported production of only 2-keto gluconic acid by *B. megaterium* strain 573. Strains of *B. lichenformis s*and *B. amyloliquefaciens* could produce diverse organic acids like lactic, isovaleric, isobutyric and acetic acids (Rodriguez and Fraga 1999). It will be worth to mention that the termite mound/termitatrium contains a significantly higher amount of P as compared to adjacent soils and inorganic P content which is significantly higher compared to organic P (López-Hernández 2001). Results of our study indicate that the presence of highly efficient PSB may be one of the reasons for enrichment of inorganic P in mound materials. Earlier, Duponnois et al. (2005) showed that the application of termite mound powder enhanced the dissolution of rock phosphate which in turn indicates the presence of specific group of microorganisms involved in inorganic P solubilization.

In India, P-deficient soils are abundant and especially the low P status in Indian semi-arid tropics (SAT) has been substantiated by a number of agronomic experiments (Tandon 1985). Arid and semi-arid soils have a large amount of insoluble phosphates like tri-calcium phosphate of which only ~ 2–4% is plant available and ~ 80% remains in unavailable form (Rao and Tarafdar 2002). Moreover, in the arid zone, temperature goes up to 50 °C with low rainfall (< 200 mm) making the survival of the living beings difficult (Bhatnagar and Bhatnagar 2005).Under such edapho-climatic conditions, ecologically adapted specific plant growth promoting bacteria with the ability to solubilize insoluble calcium phosphate can be very useful for supplementing plant nutritional requirements. In the present study, all the five isolates could solubilize more than 800 mg/L of tri-calcium phosphate within 24 h and hence, hold considerable promise as PSB specifically for arid/semi-arid regions.

In general, a number of plant growth promoting bacteria belonging to *Kosakonia, Pantoea, Bacillus* mostly isolated from rhizosphere have been characterized and shown to improve plant growth (Hameeda et al. 2008; Dastager et al. 2009; Gupta et al. 2012; Selvakumar et al. 2008; Son et al. 2006). In the present study, *Pantoea* sp. A3 and *Kosakonia* sp. A37 were able to significantly enhance the tomato root elongation after treating the seeds with bacterial suspension. Although, AH9 was on par with the control, bacterial isolates *Kosakonia* sp. A37 and *Pantoea* sp. A34 showed negative effects manifested by decreased root length and crippled seedling. The effect of these two bacterial isolates indicates their possible pathogenic nature which needs further studies for confirmation. The three bacterial isolates showing enhancement in plant root elongation holds considerable promise for exploitation as biofertilizers especially for arid and semi-arid regions as they were isolated from an arid region. Earlier, Barua et al. (2012) reported *Bacillus* sp. isolated from saline soil was effective for growth promotion of lady's finger under arid saline soils, while Minaxi et al. (2012) reported multifarious plant growth promoting *Bacillus* isolated from rhizosphere of green gram grown in semi-arid regions of Rajasthan as bioinoculant for semi-arid desert crops. Phosphatic biofertilizers can help to meet up the phosphorus requirement of the plants effectively by solubilization of insoluble mineral phosphates. In this context, the present study showed potential for highly efficient PSB isolates for their possible exploitation in enhancement of plant growth under semi-arid/arid agro-ecosystems.

Acknowledgements Authors are grateful to Directors of CSIR-National Chemical Laboratory (NCL), Pune, India and ICAR-National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, India.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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