3 Biotech (2018) 8:461
https://doi.org/10.1007/s13205-018-1485-7

ORIGINAL ARTICLE

Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils

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Received: 24 May 2018 / Accepted: 19 October 2018 / Published online: 27 October 2018

Abstract
Halotolerant phosphate-solubilizing microorganisms (PSMs) capable of producing plant-growth-promoting traits were grown on salt medium containing $Ca_3(PO_4)_2$ or egg yolk. The number of colonies on plates with $Ca_3(PO_4)_2$ was higher than that on plates with egg yolk. Further, a total of 42 PSM isolates were purified. The majority were *Bacillus* spp., while one *Providencia rettgeri* strain was confirmed, for the first time, as a PSM. All PSMs had a phosphate-solubilizing index (PSI) between 1.1 and 2.58 and a strong capacity for dissolving calcium phosphate between 2.25 and 442 $mg{\cdot}L^{-1}$. In contrast, these PSMs were less effective when dissolving aluminum phosphate, ferric phosphate and lecithin. Isolates were also tested for growth-promoting substances. The results showed that all isolates were able to secrete indole-3-acetic acid in amounts ranging from 2.7 to 31.8 $mg{\cdot}L^{-1}$ and exopolysaccharide within the range 74.3 and 225.7 $mg{\cdot}L^{-1}$. Only 12 siderophore-producing strains with siderophore units of 1.9–42.1% were detected. Among them, ten isolates with solubilization rates greater than 200 $mg{\cdot}L^{-1}$ and relatively high NaCl tolerance (1.5 M) were classified as candidate PSMs. Eight different organic acids with different contents were detected in the culture filtrates, and propionic and oxalic acids have been proposed as the main mechanisms for solubilization. The ten isolates have the potential for use as bioinoculants to protect plants in saline environments.

Keywords Saline soil · Phosphate-solubilizing microorganisms · Organic acids · Plant growth-promotion traits

Introduction

Soil salinity is an important environmental stress that threatens agriculture worldwide, limiting the productivity of crop plants because of salt stress and nutrient deficiency. Therefore, managing the uptake of nutrients by crops under salinity stress is an important task that must be solved in agricultural production (Naz and Bano 2010). Inoculation with plant-growth-promoting rhizobacteria (PGPR) is a very effective method to protect crops because it is very effective and environmentally safe (Singh et al. 2011). Many studies have isolated PGPR from soil, but relatively few have focused on PGPR isolates from saline soils. Because of the selection pressure imposed by salt, the adaptation of microorganisms to saline conditions makes them candidates for halotolerant PGPR isolates. Many of these isolates could be phosphate-solubilizing microorganisms (Naz and Bano 2010).

Phosphate-solubilizing microorganisms (PSMs) are a group of PGPR known to dissolve both inorganic and organic phosphorus and to maintain soil nutrient levels. These microorganisms include solubilizing types of both bacteria and actinomycetes (Behera et al. 2014), and they can work with arbuscular mycorrhizal fungi to solubilize and transport phosphate to plants (Mahanta et al. 2018). PSMs are classified into organic P-mineralizing bacteria and inorganic phosphate-solubilizing bacteria according to the type of phosphorus utilized (Tao et al. 2008). The main mechanism underlying inorganic phosphate mineralization is the production of low-molecular-weight organic acids (Puente et al. 2004). These acids acidify phosphate conjugate bases,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13205-018-1485-7) contains supplementary material, which is available to authorized users.

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increasing their solubility as a result of the reduction of soil pH, while also chelating metals (Wei et al. 2018).

PSMs have been isolated from many different types of soil in previous studies (Azziz et al. 2012; Sarkar et al. 2012), but research on the abundance and diversity of PSMs in salt-affected soils remains limited. Based on previous research, isolation and utilization of PGPR from corresponding microbial niches maximizes their use as inoculation agents (Ahemad and Kibret 2012). However, few studies have investigated the diversity of saline soils and how this stressful environmental condition affects PSM diversity and performance (Naz and Bano 2010; Pirhadi et al. 2018). Moreover, the roles of specific organic acids are unclear, and substantial variation has been observed in organic acid types produced by various PSMs (Wei et al. 2018). We studied the distribution and phosphate-solubilizing capacity of PSM isolates present in the rhizosphere of saline soils. The capability of microorganisms to produce organic acids and growth regulators was also investigated. The ultimate objective was to identify potential PSMs from saline soil for future agricultural applications, especially under saline soil conditions.

Materials and methods

Study site and soil sampling

We collected soil samples from sites in the Dongying area located in the Yellow River Delta in China (longitude: 118.3, latitude: 37.5) during the summer of 2015. These areas had saline soil. We collected 10 samples at an interval of 1 m between each sampling point in a desert salt flat. The samples were scraped off from encrusted environmental surface soils; in addition, 15-cm soil cores were collected. After collection, samples were homogenized and stored in sterile containers at 4 °C until use in further experiments.

Isolation of the PSMs

To process the soil for PSM isolates, 5 g of each soil sample was placed into 45 mL of sterile water and shaken (150 rpm) for 30 min. Then, 1 mL of soil suspension was transferred into a tube containing 9 mL of sterile water. This mixture was shaken well and diluted (10^{-1} dilution), with subsequent serial 10^{-1}-fold dilution until a 10^{-6} dilution was reached. Next, 100 μL of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were cultured on tricalcium phosphate medium (TPM) and yolk medium (YM) agar plates. The TPM contained 10.0 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.3 g NaCl, 0.3 g KCl, 0.03 g $FeSO_4{\cdot}7H_2O$, 0.03 mg $MnSO_4{\cdot}H_2O$, 5 g $Ca_3(PO_4)_2$ and 20 g agar. The YM agar plate contained 10.0 g peptone, 5.0 g NaCl, 10.0 g beef extract, one fresh egg yolk, 20 g agar and 1000 mL distilled water (Nautiyal 1999). Bacteria with soluble phosphorus circles on both plate types were counted and purified. Once purified, each isolate was stored in glycerol-containing LB medium for subsequent analysis.

Molecular identification of isolates

The PSMs were identified using the gene-encoding 16S rRNA sequence. DNA extraction of the isolates was conducted following the procedure specified by the manufacturers of the Bacteria kit (Omega Bio-Tek, Inc., US). We used the universal primers F27 (5′-AGAGTTTGATCCTGG CTCAG-3′) and R1387 (5′-GGGCGGWGTGTACAAGGC -3′) to amplify the sequence using a PCR system (Biometra, Germany), which contained 1 μL of GoTaTaq polymerase, 1 × Go Taq buffer, 25 mM $MgCl_2$, 0.2 mM of each dNTP, and 1 μL of each primer. Sterile water was added to reach a final volume of 25 uL. The PCR was performed as follows: 95 °C for 3 min, followed by 35 cycles of 95 °C for 1 min, 56 °C for 1 min and 72 °C for 2 min, with a final extension step at 72 °C for 3 min. The amplified rRNA was compared with sequences in the GenBank database (National Center for Biotechnology Information, NCBI) to identify the closest relatives. Then, the neighbor-joining method in MEGA version 4.0 software was used to construct the evolutionary tree. The sequences were deposited into GenBank, and the accession numbers were obtained.

Assessment of the ability to dissolve soluble phosphorus

To determine the capacity of PSMs to dissolve phosphorus, 20 μL of inoculum was cultured on TPM plates overnight at 28 ± 2 °C. This capacity was determined based on visual confirmation of clearing zones after 3, 7 and 10 days. All experiments were performed in triplicate. The phosphate-solubilizing index (PSI) was determined using the formula described by Sarkar et al. (2012): PSI = A/B, where A is the diameter of the colony plus the cleared area around the colony, and B is the diameter of only the colony.

Assay of phosphorus released from different phosphorus sources

To estimate the differences in phosphorus released, all strains were inoculated (1% v/v) into YM liquid medium containing $MgCl_2{\cdot}6H_2O$ 5 $g{\cdot}L^{-1}$, $MgSO_4{\cdot}7H_2O$ 0.25 $g{\cdot}L^{-1}$, KCl 0.2 $g{\cdot}L^{-1}$, $(NH_4)_2SO_4$ 0.1 $g{\cdot}L^{-1}$, glucose 10 $g{\cdot}L^{-1}$ and into TPM liquid medium, and the pH of both media was adjusted to 7.0. Isolates with same starting concentration of approximately 1×10^8 $CFU{\cdot}mL^{-1}$ were inoculated into 20 mL of liquid medium at 28 ± 2 °C for 3 days. Unamended media served as the control. All experiments were performed in triplicate. The soluble phosphorus content in the broth was estimated by the phosphomolybdic blue method (Jackson 1973). Briefly, the bacterial cultures were centrifuged at 12,000 rpm for 10 min. Then, the supernatant (500 µL) was mixed with 40 µL 2,4-dinitrophenol, after which 20 µL of dilute sulfuric acid was added, followed by 5 mL of chromogenic reagent, and the volume was diluted to 50 mL using sterilized water. We measured the OD value at 700 nm using an ultraviolet spectrophotometer (Shanghai, China).

Salt tolerance of isolates

Cultures were tested for their tolerance to sodium chloride (NaCl) at 0.5, 1, 1.2 and 1.5 M, using LB agar (10 g tryptone, 5 g yeast extract, 10 g NaCl, 18 g agar, and 1000 mL distilled water). Growth of colonies on the plate was recorded using the naked eye after 7 days of cultivation. Every treatment was repeated three times. Colonies larger than 5 mm in diameter were considered effectively growing colonies.

Assessment of organic acids using high-performance liquid chromatography (HPLC)

Isolates were inoculated into 20 mL TPM liquid culture contained 0.5% (m/v) $Ca_3(PO_4)_2$ and were shaken (180 rpm) for 3 days at 28 ± 2 °C. Subsequently, the samples were centrifuged (12,000 rpm) for 10 min, and the supernatant was passed through a 0.22-µm membrane film. Detection and quantification of organic acids were conducted using a Waters 996 HPLC equipped with a ZORBAX SB-Aq 250 mm × 4.6 mm column (Merck, Germany). The mobile phase was 0.01 M monopotassium phosphate at a flow rate of 0.6 mL min^{-1}. Eluates were detected at $\lambda = 210$ nm and were identified based on their retention time.

Growth-promoting substances

For indole acetic acid production, overnight cultures of PSM were transferred to medium containing L-tryptophan and were examined according to the procedure described by Glickmann and Dessaux (1995). For siderophore production, overnight cultures of PSM were transferred to MKB medium (5 g proteose peptone, 15 mL glycerol, 2.5 g K_2HPO_4, 2.5 g $MgSO_4$ and 1000 mL distilled water) and were cultured at 28 ± 2 °C (150 rpm) for 48 h. Siderophore production was estimated using the equation $SU = [(Ar - As)/Ar] \times 100$, where SU is the activity unit of siderophores, Ar is the absorbance value of the control, and As is the absorbance value of the isolates (Machuca and Milagres 2003). For exopolysaccharide production, all of the isolates were inoculated into LB medium and were cultivated at 28 ± 2 °C for 48 h. Exopolysaccharide production was then estimated using the method described by Patel et al. (2010).

Results

Isolation and identification of the PSMs

We used TPM and YM containing $Ca_3(PO_4)$ and egg yolk plate medium for screening PSM colonies. In total, 26 types of bacteria were isolated using inorganic phosphorus, whereas 16 were isolated from organic phosphorus (i.e., egg yolk). These strains were amplified using 16S-rRNA genes as the target. The BLAST algorithm for the sequences showed that most of the isolates that clustered together were *Bacillus* and each of these were different species (Fig. 1). Moreover, five strains belonged to *Pseudomonas* sp., two strains belonged to *Streptomyces* sp. and single isolates were obtained for *Arthrobacter* sp, *Providencia rettgeri* sp. and *Acinetobacter* sp. This appears to be the first discovery of *Providencia rettgeri* sp. as a PSM. All the sequencing results were deposited into the GenBank sequence data library under the accession numbers KX834824–KX834865.

Phosphate-solubilizing efficiency of the PSM isolates

An efficiency study was conducted by performing clearing zone formation experiments on TPM plates after incubation for 3, 7 and 10 days at 28 °C. Phosphate-solubilization abilities were detected by calculating the PSI. Figure 2a shows a comparison of the phosphate-solubilizing ability of 26 strains isolated from the TPM plates, while Fig. 2b shows the comparative phosphate solubility of 16 strains isolated on YM plates. Some clearing zones gradually increased over time. Among the PSM isolates, the *Bacillus* spp. (TPM9, YM11 and YM14) and *Providencia rettgeri* sp. (TPM23) had the highest PSI values, i.e., 2.58, 2.37, 2.00 and 2.30, respectively, after 10 days.

Phosphorus released from different sources by PSM isolates and their salt tolerance

The ability of PSMs to solubilize phosphorus was studied in greater detail based on liquid culture (Table S1). In these cases, $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_3$ were added to the TPM medium (5 $g \cdot L^{-1}$). The amount of phosphate solubilized ranged between 2.25 and 442 $mg \cdot L^{-1}$ in the presence of $Ca_3(PO_4)_2$. Ten isolates of *Bacillus* sp. (YM-13, YM-12, YM-11, YM-10, YM-16, YM-4, YM-9, TPM-26), *Providencia* (TPM-23) and *Enterobacter* (YM-14) showing solubilization values > 200 $mg \cdot L^{-1}$ were classified as PSMs (Fig. 3). *Bacillus* isolate YM-13 demonstrated the highest solubilization (442 $mg \cdot L^{-1}$), while two phosphate-solubilizing *Streptomyces* showed much lower

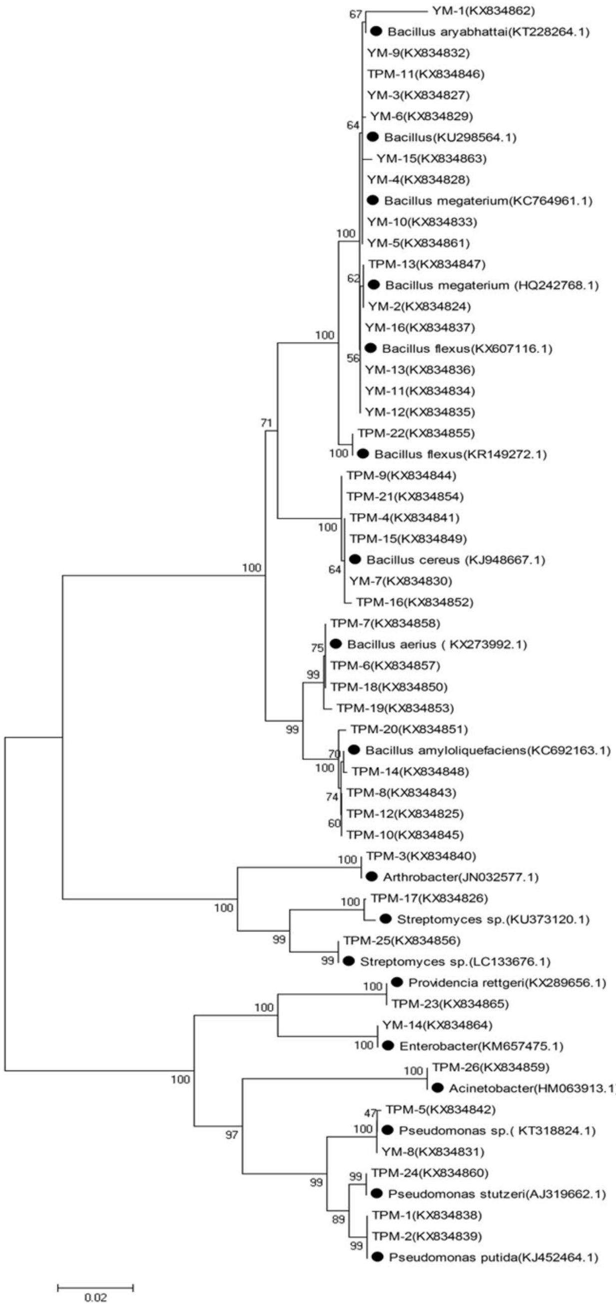

Fig. 1 The phylogenetic tree was established by the neighbor-joining method based on 16S rRNA sequences and closely related sequences. The bar denotes 2 nucleotide exchanges per 100 nucleotides. Bootstrap values > 95% are indicated at the branch points. The accession numbers are presented in parentheses

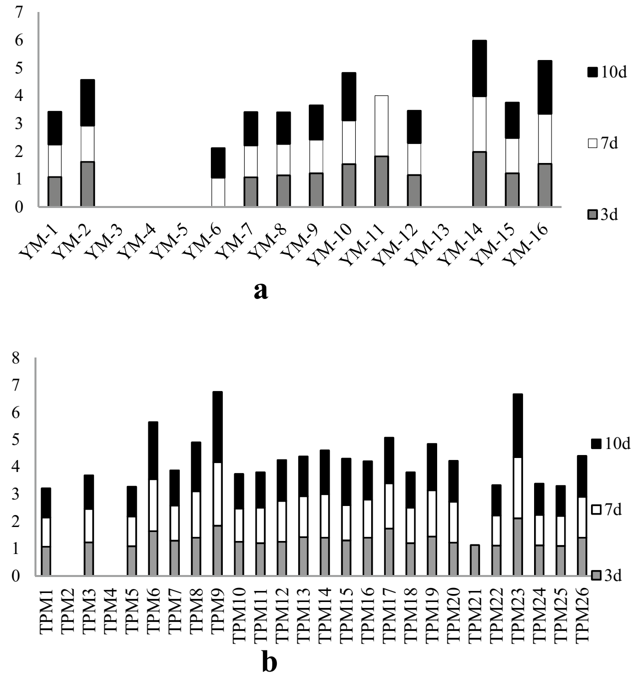

Fig. 2 Phosphate-solubilization index produced by phosphate-solubilizing bacteria after growth for 3 days (gray bars), 7 days (white bars) and 10 days (black bars) on the plate containing tricalcium phosphate medium

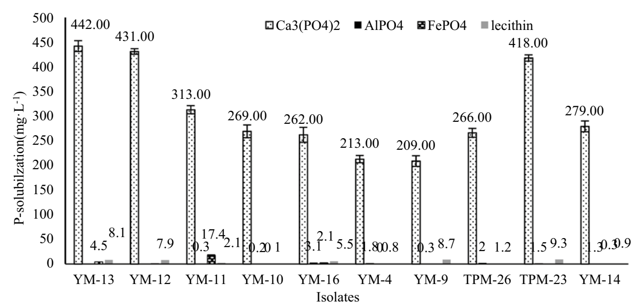

Fig. 3 Phosphate-solubilizing ability of phosphate-solubilizing bacteria in TPM with different phosphorus sources. Bacterial suspensions with the same initial concentration (1×10^8 CFU·mL^{-1}) were transferred into 20 mL of TPM and were shaken (180 rpm) at 28 ± 2 °C for 3 day. The soluble phosphorus content in bacterial suspensions was determined by the phosphomolybdic blue method. Error bars indicate the standard deviations of mean data values

phosphate-solubilization values of 11.8 mg L^{-1} (TPM-17) and 19.10 mg L^{-1} (TPM-25). In contrast, when $FePO_4$ and $AlPO_3$ were added, the soluble phosphorus content in the medium was much lower. Specifically, the amount of solubilized $AlPO_3$ ranged between 0.2 and 14.6 mg·L^{-1}, with isolate TPM-12 showing the highest value of 14.6 mg L^{-1}. The solubilization of $FePO_4$ ranged between 0.3 and 19.72 mg·L^{-1}. The highest amount of solubilized $FePO_4$ was detected in *Bacillus* YM-6 at 19.72 mg·L^{-1}. When lecithin was added to the medium, all the isolates from the solid YM had the ability to solubilize the lecithin at levels between 0.8 and 8.7 mg·L^{-1}. However, many of the TPM isolates were unable to dissolve lecithin. Tolerance for NaCl concentration ranged between 1 and 1.5 M in the medium. The *Providencia* sp. TPM-23 and *Enterobacter*

Table 1 Plant growth-promoting products, siderophores, extracellular polysaccharides (EPS), and indole-3-acetic acid (IAA) from phosphate-solubilizing microorganisms (PSMs) and their salt tolerance

Isolates*	PGPs (mg·L^{-1})			Salt tolerance (M)
	Siderophore unit%	EPS	Value of IAA	
Bacillus sp				
YM-13	–	107.8 ± 3.8	4.2 ± 0.2	1
YM-12	–	126.3 ± 3.5	5.3 ± 0.3	1
YM-11	40.8	156.7 ± 6.7	8.9 ± 0.1	1
YM-10	4	145.3 ± 5.8	14.9 ± 1.1	1
YM-16	—	115.5 ± 3.8	18.4 ± 1.1	1.5
YM-4	—	108.5 ± 3.1	13.0 ± 1.0	1
YM-9	–	126.7 ± 4.3	6.4 ± 0.2	1.5
TPM-26	–	108.2 ± 1.5	11.6 ± 1.9	1.5
Providencia TPM-23	–	79.7 ± 0.9	3.1 ± 0.7	1.5
Enterobacter YM-14	1.9	115.2 ± 1.3	18.8 ± 1.6	1.5

The prefix for the isolate YM and TPM indicate yolk medium and tricalcium phosphate medium, respectively

EPS extracellular polysaccharide, *IAA* indole-3-acetic acid, *PGPs* plant growth-promoting products

sp. YM-14 were both shown to grow at the 1.5 M NaCl concentration (Table 1).

Organic acid production by PSM isolates

The secretion of organic acids was tested during $Ca_3(PO_4)_2$ solubilization by HPLC to analyze mechanisms involved in the phosphate-solubilizing abilities. Eight different low-molecular-weight organic acids (gluconic acid, formic acid, malic acid, lactic acid, succinic acid, citric acid and propionic acid) were produced by 42 isolates, and many of the isolates produced multiple organic acids. All of the strains produced oxalic acid and citric acid, except for TPM-2, but there was no obvious correlation between the number of acids produced and the ability to solubilize phosphorus. However, the 10 best solubilizers secreted propionic acid, oxalic acid and citric acid (Fig. 4). Thus, the higher solubilization of $Ca_3(PO_4)_2$ could possibly be related to the propionic and oxalic acid production.

Evaluation of plant-growth-promoting (PGP) traits among PSM isolates

Isolates were also checked with respect to their multiple PGP traits (Table 1). All the PSMs produced indole acetic acid (IAA) in quantities ranging from 2.7 to 31.8 mg·L^{-1}. Strains YM2 and YM3 had the highest IAA-production ability, yielding 31.8 and 25.1 mg·L^{-1}, respectively. Both of these isolates are in the genus *Bacillus*. There were 12 (28.6% of the total) siderophore-producing strains. Of these, 3 strains belonged to *Pseudomonas* spp., representing 60% of all *Pseudomonas* spp. isolated in this study. All the strains except YM3 had a weaker capacity for producing siderophores. Strain YM3 had the highest siderophore-producing capacity with a siderophore unit of 63.7%. All the PSMs produced exopolysaccharide in amounts ranging from 74.3 to 225.7 mg·L^{-1}. Among these, 5 strains possessed exopolysaccharide production capacity with final concentrations < 100 mg·L^{-1}. The remaining 37 strains (88.1% of the total) produced exopolysaccharide at > 100 mg·L^{-1}. *Bacillus* strain YM6 had the highest production, i.e., 225.7 mg·L^{-1}.

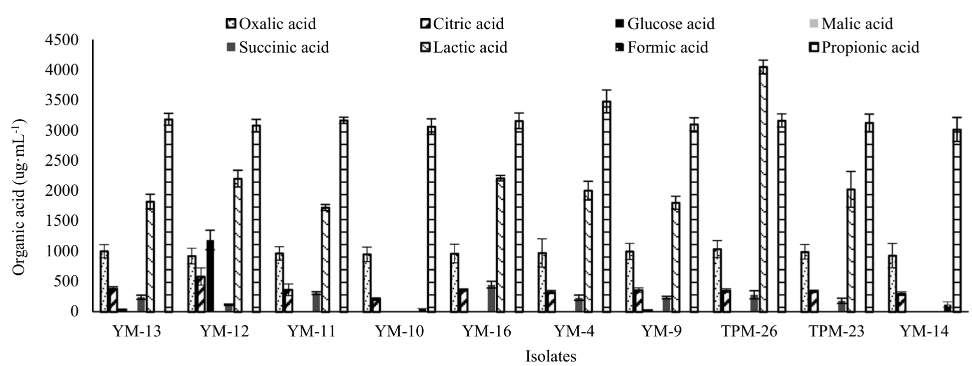

Fig. 4 Organic acids production by PSM isolates in TPM broth with tricalcium phosphate based on high-performance liquid chromatography

Discussion

The PSMs were classified into organic phosphate-mineralizing bacteria and inorganic phosphate-solubilizing bacteria depending on their phosphorus source utilization. The preliminary screening method for phosphate-solubilizing bacteria was mainly based their ability to dissolve $Ca_3(PO_4)_2$ on indicator plates (Barroso and Nahas 2005). However, organic and inorganic phosphorus co-screening has rarely been used, and it reveals interesting new information. Although PSMs are present in almost all types of soil, their densities and abundance vary according to the physical and chemical properties of the soil. Little research on compositions and diversity of PSM in saline soils is available (Joshi and Bhatt 2011). Consequently, we used TPM and YM for the initial screening of PSM on solid media to isolate PSM from saline soil. The solubilization zones surrounding bacteria were screened on both plates. However, the number of colonies on TPM plates was greater than that on YM plates. This is consistent with a previous report (Tao et al. 2008), which states that phosphorus in soil mainly exists in bound forms, such as tricalcium phosphate ($Ca_3(PO_4)_2$), which are unavailable to plants, especially in high salinity soils.

Phylogenetic analysis of the phosphate solubilizers showed that the most frequent genus was *Bacillus*, followed by *Pseudomonas*. This finding also supports previous research (Azziz et al. 2012). Isolation studies for PSM identification from saline soils identified endophytic halotolerant *Bacillus* and *Pseudomonas* (Naz and Bano 2010; Pirhadi et al. 2018). Rodríguez and Fraga (1999) demonstrated that *Bacillus* and *Pseudomonas* species were able to dissolve insoluble phosphorus, thereby increasing the available phosphorus content in soil. In addition, *Providencia rettgeri* sp. was reported for the first time as a phosphate solubilizer.

The low availability of phosphorus in saline soil is attributed to its fixation with calcium, aluminum and iron that is then unavailable to plants (Sashidhar and Podile 2010). With this in mind, we also used $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$ as phosphorus sources in liquid medium to test the phosphate-solubilizing efficiency. All strains could dissolve tricalcium phosphate and the small value of PSI on plates eventually indicated significant phosphate-solubilization potential in liquid. There was no correlation between the phosphate-solubilizing capacity of the plate and liquid culture (Chung et al. 2005; Baig et al. 2010). The phosphate-solubilization activities ranged from 2.25 to 442 mg L^{-1} when $Ca_3(PO_4)_2$ was used as the phosphorus source. However, the activities were much lower with respect to $AlPO_4$ and $FePO_4$. This finding was also demonstrated by Delvasto et al. (2006) and Pérez et al. (2007). The two *Streptomyces* strains had very low phosphate-solubilization capacity and no ability to dissolve $AlPO_4$, $FePO_4$ and organic phosphorus. This illustrates that the phosphorus-solubilization ability of *Streptomyces* is low and indicates that bacterial agents are more suitable for use as PSMs. In addition, the 10 best solubilizers were classified as *Bacillus* sp, *Providencia* and *Enterobacter*, indicating that these candidates have greater potential for use as biological inoculants for alleviating the negative effects of salt on plants in saline soil.

PSMs can convert insoluble phosphates into soluble phosphates by producing organic acids (Rodríguez and Fraga 1999). However, there was no correlation between the total molar organic acid production and soluble phosphorus content in liquid culture. Our study also showed that there was no obvious trend between the soluble phosphorus content and the number of acids produced. This finding is consistent with the results of Vyas and Gulati (2009), which suggest the types and amounts of organic acids differed among the PSM strains and were potentially responsible for the mechanism of solubilization. All of the 10 best PSMs solubilizers with a phosphorus content greater than 200 $mg \cdot L^{-1}$ produced oxalic acid, propionic acid and citric acid. In addition, these 10 PSMs produced large amounts of lactic acid except *Enterobacter* (YM14). Overall, the data suggest that the production of key acids, such as propionic acid and oxalic acid, is especially important for the main mechanism used by PSM for dissolving phosphorus in saline environments.

Halotolerant PGPR have several beneficial traits, and certain bacteria have evolved one or more mechanisms to mitigate plant salt stress. Bahadur et al. (2016) also reported that PSM isolated from soils can produce IAA and can increase the extension of roots in the soil, thus causing phosphorus to become more available to the roots and PSM, promoting plant growth under saline conditions. In our study, we also noticed that all PSM produced IAA. Nishma et al. (2014) demonstrated that all fluorescent pseudomonads produced IAA, and the maximum production was observed in JUPF58, i.e., 85.33 ± 1.53 $mg \cdot L^{-1}$. Wahyudi et al. (2011) reported 15–20 $mg \cdot L^{-1}$ IAA production by *Bacillus* species isolated from a soybean rhizosphere. However, in our study, the maximum production of pseudomonads was 20.8 $mg \cdot L^{-1}$ and that of *Bacillus* spp. was 25.1 $mg \cdot L^{-1}$. Therefore, the isolates from saline soil showed many more beneficial traits for promoting plant growth in the saline soil. Another important property is extracellular polysaccharide (EPS) secretion, which can bind Na^+, thus decreasing the uptake of Na^+ by plants, helping to alleviate salt stress (Qurashi and Sabri 2012). In addition, EPS can promote the formation of micro-aggregates or large soil particles, thus forming a barrier against salt toxicity in plants (Han and Lee 2005). In our experiments, we found all PSM isolates produced EPS, and the quantity between strains was similar. Therefore, PGPR from local saline soil has good potential for eliminating salt stress and for application. Siderophore production is another important trait of PGP bacteria that increases the uptake of iron under saline stress. Sadgir et al. (2016) observed that a high number of phosphate-solubilizing isolates can produce siderophores. In our study, we also found that 25% of isolates produced siderophores. This phenomenon can be explained by the fact that bacteria can solubilize phosphorus by secreting organic acids and can exert siderophore-like functions. In our study, 3 out of 5 *Pseudomonas* spp. strains produced siderophores. This confirmed the previous findings of O´Sullivan and O´Gara (1992), who demonstrated that most *Pseudomonas* spp. can secrete siderophores.

To survive and proliferate in the rhizosphere, bacteria possess mechanisms to resist stress in salt-affected soil. Isolation of PSMs from saline soil can lead to taxa with maximum potential application. Also, substantial research demonstrates the efficacy of PGPR as biofertilizers in promoting plant growth in both normal soil and soil under stress. With respect to soil salinity, bacteria with traits for phosphate solubilization, production of IAA, exopolysaccharides and siderophores, as well as high salt tolerance, have been shown to be effective PGPRs (Qurashi and Sabri 2012; Liu et al. 2014; Shao et al. 2015). Therefore, investigation of the PSM distribution and abundance in indigenous saline soil and preliminary study of their functional characteristics could improve the future possibility for application of PSMs as biofertilizers. In-depth studies on the PGPs and survival ability in saline soil conditions are needed before PGPs can be used commercially as biofertilizers.

Acknowledgements This study was supported with grants from the State Key Laboratory of Urban Water Resource and Environment (2017DX15), the National Ten Thousand Youth Talents Plan of 2014, China Agriculture Research System (CARS14), the National Natural Science Foundation of China (31000728 and 31200211), and the Natural Science Fund of Shandong Province (ZR2014YL011 and ZR2014YL012).

Author contributions All authors have seen and approved the manuscript and its contents, and declare they are aware of the responsibilities connected to authorship. Pei-shi Qi and Xiao-yuan Chi designed the study and performed critical revision of the manuscript; Huan-huan Jiang and Ming-na Chen performed the experiment and data analysis; Huan-huan Jiang composed the manuscript; Tong Wang and Na Chen collected the samples; and Mian Wang and Li-juan Pan guided the research.

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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