



# Construction of Phosphate-Solubilizing Microbial Consortium and Its Effect on the Remediation of Saline-Alkali Soil

Ting Zhang<sup>1</sup> · Xue-li Wang<sup>1</sup> · Juan Zhou<sup>1</sup> · Wei Zhou<sup>2</sup> · Shao-Qi Zhou<sup>1</sup>

Received: 6 July 2024 / Accepted: 23 December 2024  
© The Author(s) 2025

## Abstract

In this study, phosphate solubilizing bacteria (PSB) with good phosphate-solubilizing capability were isolated from phosphogypsum (PG) storage yard, and phosphate-solubilizing bacteria without antagonistic effect were selected to construct phosphate solubilizing microbial consortium (PSMC), and the synergistic effect of PSMC and PG on the physical and chemical properties of saline-alkali soil, soil enzyme activity, soil bacterial diversity, and the growth index and biomass of peanut plants were explored. The results showed that the effect of phosphorus containing soil amendment on saline-alkali soil was better than that of single PSMC or PG. In the T6 group (untreated saline-alkali soil (1.5 kg) + PSMC stock solution (15 mL) + PG (6.0 g)), the pH of saline-alkali soil decreased from 8.54 to 7.03, the content of organic matter increased by 6.64%, the content of alkali hydrolyzable nitrogen, available phosphorus and available potassium increased by 81.68%, 60.31%, and 42.03%, respectively, and the activity of alkaline phosphatase increased by 94.95%. In addition, the electrical conductivity value in T4 group (untreated saline-alkali soil (1.5 kg) + PSMC stock solution (15 mL) + PG (3.0g)) decreased significantly by 20.21%. The diversity and richness of bacterial community in T4 group were the highest, and the growth of peanut plants was the best. The fresh weight of roots and stems increased by 73.34% and 116.6%, respectively. In conclusion, the phosphorus containing soil conditioner prepared by PSMC and PG can effectively improve the soil environment of saline-alkali soil and promote the resource utilization of saline alkali soil.

**Keywords** Phosphate-solubilizing microbial consortium · Phosphogypsum · Phosphorus containing soil conditioner · Saline-alkali soil

## Introduction

Phosphogypsum (PG) is one of the main by-products in the phosphoric acid production process of phosphorus chemical enterprises [1], generally, 4.5–5.5 tons of PG will be produced per ton of phosphoric acid production [2]. Therefore,

PG is recognized as bulk industrial solid waste. At present, the resource utilization rate of PG is low, about 85% of PG in the world is either accumulated on land or discarded in the sea, which can have adverse effects on soil, marine water, and the atmospheric environment [3–5]. PG is acidic, and the content of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  is usually between 80 and 90%, varying based on the source and storage time of PG [6]. In addition, the study also found that PG contains nutrients such as phosphorus (P), sulfur (S), and iron (Fe). Phosphorus mainly exists in the form of available phosphorus, insoluble phosphorus, and eutectic phosphorus [7]. When PG is applied to phosphorus-deficient soil, the soluble phosphorus in PG can be directly absorbed and utilized by plants. Meanwhile, the insoluble phosphorus can be transformed into orthophosphate, which can be absorbed and utilized by plants with the action of phosphate-solubilizing bacteria (PSB) [8, 9]. In recent years, many studies have confirmed that PG can not only help to improve the total phosphorus content in soil, reduce the mining of phosphate rock,

Ting Zhang and Xue-li Wang contributed equally to this article.

✉ Shao-Qi Zhou  
15972986074@163.com

Ting Zhang  
13940474787@163.com

Wei Zhou  
16687871786@163.com

<sup>1</sup> College of Resources and Environmental Engineering, College of Liquor and Food Engineering, Guizhou University, Guiyang 550025, Guizhou, China

<sup>2</sup> Guizhou Academy of Sciences, Guiyang 550001, China

and decrease the use of chemical phosphate fertilizer [10], but also improve soil structure and increase crop yield [11]. Therefore, PG is often considered a soil conditioner used in agricultural fields for purposes such as soil remediation and promoting crop growth [12–14], and PSB are an effective method for enhancing the utilization rate of phosphorus in PG.

Saline-alkali soil is the abbreviation of saline soil, alkaline soil, and saline alkali soil. It is the result of the excessive accumulation of soluble salts on the soil surface and is one of the important environmental factors limiting agricultural production [15]. According to statistics, the global area of saline-alkali land is approximately  $9.54 \times 10^8$  hm<sup>2</sup>, and it is increasing at a rate of  $1.0 \times 10^6$ – $1.5 \times 10^6$  hm<sup>2</sup> per year [16]. The total area of saline-alkali land in China is about  $9.91 \times 10^7$  hm<sup>2</sup>, ranking third in the world [17]. Saline-alkali soil has the characteristics of high pH, with high levels of inorganic salts such as Na<sup>+</sup>, CO<sub>3</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup>, and low organic matter content. These characteristics not only affect the activities of enzymes like catalase and alkaline phosphatase in the soil but also impact the community structure and function of soil microorganisms. This leads to a decrease in the levels of alkali-hydrolyzable nitrogen, available phosphorus, and other nutrients in the soil, hindering crop growth in saline-alkali soil environments [18–20]. Therefore, improving the effective utilization rate of saline-alkali soil is the main problem to be solved [21].

In recent years, the reported methods for improving saline-alkali soil include physical, chemical, and biological methods. Physical and chemical methods have a significant improvement effect on saline-alkali soil in a short time, but they have disadvantages such as heavy workload, high economic costs, and possible secondary pollution. Biological methods include phytoremediation and microbial remediation. Phytoremediation offers wide coverage and high economic benefits, but it also has drawbacks, including the need for large engineering quantities and the challenge of plant disposal post-remediation. Microbial remediation technology offers advantages such as energy efficiency, cost-effectiveness, and stable improvement effects. It can also enhance plant growth in saline-alkali soil, making it a primary focus of research on saline-alkali soil improvement [22–24]. Sahin and other researchers have confirmed that the simultaneous use of microbial agents and chemical amendments can notably enhance the remediation effect of saline-alkali soil [25]. The study found that PSB, mycorrhizal fungi, sulfur-oxidizing bacteria, etc., are commonly used microbial flora in improving saline-alkali soil. Additionally, PG, desulfurization gypsum, and other substances are widely used chemical modifiers for enhancing saline-alkali soil [26, 27]. Al enazy et al. [10] showed that when PSB and PG were applied together, the soil pH decreased from 7.44 to 7.2. Additionally, the content of available phosphorus, the

number of microorganisms, and the activity of dehydrogenase enzyme in the soil significantly increased. Sharma et al. [28] found that the synergistic effect of PSB and PG can promote the absorption of phosphorus by crops like rice and wheat, thereby improving the yield of rice and wheat.

Therefore, the main objective of this study is to isolate and screen bacteria with efficient phosphate-solubilizing capability from PG samples. The study aims to identify strains without antagonistic effects through plate antagonistic tests, construct them into a phosphate-solubilizing microbial consortium (PSMC), and subsequently determine the microbial consortium with the most effective phosphate-solubilizing capability through liquid co-culture experiments. A phosphorus-containing soil conditioner was prepared using a PSMC and PG. The effects of the phosphorus-containing soil conditioner on the physical and chemical properties of saline-alkali soil, soil enzyme activity, soil microbial diversity, and peanut plant growth were investigated through a pot experiment. The research results will provide a new basis for the resource utilization of PG and the treatment of saline-alkali soil.

## Materials and Methods

### Sample Collection and Analysis Methods

Phosphogypsum (PG) samples were collected from the Wengfu phosphogypsum storage yard (106°32'E, 32°39'N) in baiji Village, Machangping Town, Fuquan City, Qiannan Prefecture, Guizhou Province. The storage yard has a total storage capacity of  $1.88 \times 10^8$  m<sup>3</sup>, floor area of 58.70 m<sup>3</sup>. The storage yard is a three-level phosphogypsum warehouse. This study utilizes the five-point sampling method [1] to collect samples at three different locations in the yard: the bottom (where stacking time exceeds 1 year), the middle (where stacking time is approximately 6 months, with a height of about 515 m), and the top (where stacking time is less than 1 month, with a height of around 1030 m). The sampling depth is 0–15 cm, and the samples are placed into sterile sampling bags labeled B-P (bottom phosphogypsum), M-P (middle phosphogypsum) and T-P (Top phosphogypsum) sequentially, then stored at 4°C for standby. According to the P-sequential fractionation method improved by Tiessen and Moir of the Canadian Soil Science Society [29], different forms of phosphorus in the sample were determined, including total phosphorus (TP), available phosphorus (AP), organic phosphorus (OP), and inorganic phosphorus (IP). Inorganic phosphorus primarily determines the content of calcium phosphate (Ca-P), iron phosphate (Fe-P), and aluminum phosphate (Al-P).

Saline-alkali soil samples were collected from the experimental site of the Panjin Saline-Alkali Land Utilization

Research Institute in Liaoning Province. The sampling depth was 0–15 cm. The samples were dried at room temperature. The soil samples that passed through a 2-mm sieve were analyzed for physical and chemical characteristics, including pH, electrical conductivity (EC), organic matter (OM), alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), and available potassium (AK). Soil pH and EC were measured using a pH meter (PHS-3C, Shanghai Aohaosi Instrument Co., Ltd.) and a conductivity meter (DDSJ-308F, Shanghai Yidian Scientific Instrument Co., Ltd.) [30]. The soil organic matter content was determined using the potassium dichromate volumetric method [31]. The alkali-hydrolyzable nitrogen content in the soil was determined using the alkali-hydrolyzable nitrogen diffusion method. The available phosphorus content in the soil was determined using the  $\text{NaHCO}_3$  extraction molybdenum antimony anti-colorimetry method, and the available potassium content in the soil was determined using the ammonium acetate extraction flame atomic absorption spectrometry (AA-3300F, Shanghai Yuanxie Instrument Co., Ltd.) [30]. However, soil samples that passed through the 5-mm sieve were used for the following pot experiments.

## Isolation and Screening of Phosphate-Solubilizing Bacteria

### Preliminary Screening of Phosphate-Solubilizing Bacteria

Ten grams of PG sample were weighed and dissolved in 90 mL of sterile physiological saline solution. It was shaken and cultured at a constant temperature of 30 °C and 180 r/min for 30 min to prepare a PG suspension with a dilution of  $10^{-1}$ . The sample suspension with a concentration of  $10^{-4}$ – $10^{-6}$  was prepared using a tenfold gradient dilution method. 0.1 mL of sample suspension was uniformly coated onto beef extract peptone solid medium, and incubated upside down at 30 °C for 3 days, with three parallel gradients set for each gradient. Selected colonies with different morphological characteristics for streaking culture until pure strains were obtained. Inoculated them on beef extract peptone agar slants and stored them at 4 °C for future use.

The primary screening medium for PSB is an inorganic phosphorus-selective solid medium, slightly modified from the National Botanical Research Institute's Phosphate Growth Medium (NBRIP) [32, 33]. It contains glucose 10.0 g,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  5.0 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.25 g, KCl 0.2 g,  $(\text{NH}_4)_2\text{SO}_4$  0.1 g,  $\text{Ca}_3(\text{PO}_4)_2$  25.0 g, agar 15 g, distilled water 1000 mL, pH 7.0.  $\text{Ca}_3(\text{PO}_4)_2$  needs to be sterilized separately from other components and then mixed in. The pure single strain was inoculated onto NBRIP solid medium, and the formation of a phosphate-solubilizing halo was observed after 4 days of incubation at 30 °C [34]. Each strain was cultured in three parallel cells. The

phosphate-solubilizing capability of the strain was preliminarily identified based on the size of the phosphate-solubilizing halo and the Phosphate Solubilizing Index (PSI) ( $\text{PSI} = D/d$ ), where D represents the diameter of the phosphate-solubilizing halo and d represents the diameter of the colony [35].

### Re-screening of Phosphate-Solubilizing Bacteria

Johri et al. have pointed out that using the size of the phosphate-solubilizing halo in plate culture as an index to determine the phosphate-solubilizing capacity of PSB is not reliable [36]. Wang et al. also pointed out that there is not a linear relationship between the PSI index and the actual phosphate-solubilizing capacity of the strain [9]. Therefore, to investigate the strain's effective phosphate-solubilization capacity, it is necessary to carry out liquid phosphate-solubilization test. The strains screened in the "Isolation and Screening of Phosphate-Solubilizing Bacteria" section were inoculated in 50 mL of beef extract peptone liquid medium and cultured at 30 °C for 18 h at 180 RPM to obtain a stock solution of  $1 \times 10^7$  CFU/mL. The stock solution mentioned above was inoculated into NBRIP liquid medium at a 1% inoculum volume. The composition of other substances was the same as that of NBRIP solid medium, with the exception that agar was not added. At the same time, a control group with 1% sterile normal saline was cultured at 30 °C and 180 RPM for 7 days. Each strain was set up with three parallel groups. According to the molybdenum antimony resistance colorimetry [37], the available phosphorus content in the culture medium of each strain was determined. This method was used for the construction of PSMC.

## Construction of Phosphate-Solubilizing Microbial Consortium

### Antagonistic Test Between Phosphate-Solubilizing Bacteria

The premise for preparing a microbial consortium is that there is no antagonistic effect between strains [38]. The most commonly used method to explore whether there is an antagonistic effect between strains is to cross-mark on the plate. If the strain does not grow or grows poorly at the cross mark, it has an antagonistic effect. If the bacteria at the intersection grow well, there is no antagonistic effect [39]. The phosphate-solubilizing strains isolated and screened in the "Re-screening of Phosphate-Solubilizing Bacteria" section were inoculated into NBRIP solid medium in pairs using the cross-streak method and cultured at 30 °C for 4 days. The growth of the strains at the crossroads was observed, and the

phosphate-solubilizing strains without antagonistic effects were identified.

### Liquid Co-Culture Test

The phosphate-solubilizing strains without antagonistic effects were selected and mixed them in a 1:1 (v/v) ratio to obtain PSMC. The stock solution with a concentration of  $1 \times 10^7$  CFU/mL was prepared following the method of the “Re-screening of Phosphate-Solubilizing Bacteria” section. The experimental group was inoculated with a 1% inoculum of the stock solution containing microbial consortium into the NBRIP liquid medium, while the control group was inoculated with 1% sterile normal saline. The experimental group and the control group were cultured at 30°C for 7 days with a rotation speed of 180 RPM. The available phosphorus content of each culture solution was determined according to the method described in the “Re-screening of Phosphate-Solubilizing Bacteria” section, and the PSMC with good phosphorus-solubilizing capacity were selected for the subsequent test.

### Identification of Phosphate-Solubilizing Bacteria

#### Morphological Identification

The strains of the PSMC were inoculated into a beef extract peptone solid medium and cultured at 30°C for 2 days. The morphological characteristics of each colony were observed, and their size, shape, surface gloss, edge morphology, and color were recorded. After Gram staining, the single-cell morphology of the strain was observed under a 1000× microscope (Olympus, Tokyo, Japan).

#### Molecular Biological Identification

The strains of PSMC were inoculated in a beef extract peptone liquid medium and cultured at 30 °C for 2 days at 180 RPM. The DNA of each bacterium was extracted following the method in the Bacterial Genomic DNA Extraction Kit (Beijing Solaibao Technology Co., Ltd.). Subsequently, the extracted DNA was amplified via PCR using bacterial

universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTACCTTGTTACGACTT-3'). The PCR amplification reaction system contained 25 μL. Bacterial DNA template 2 μL, 2×Taq PCR Master Mix 12.5 μL with primer each for upstream and downstream at 50 μL, ddH<sub>2</sub>O 8.5 μL. PCR reaction procedure: predenaturation at 94°C for 5 min, denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, 35 cycles, final extension at 72°C for 10 min, and preservation at 4°C. PCR amplification products were sent to Shanghai Sangon Bioengineering Co., Ltd. for sequencing. The nucleotide sequences were compared with the GenBank database using BLAST (<http://www.ncbi.nlm.nih.gov/>). The available standard sequences were compared in Mega 6.0 software, and the phylogenetic tree was constructed using the neighbor-joining method [40].

### Application of Phosphate-Solubilizing Microbial Consortium in the Remediation of Saline-Alkali Soil

Phosphorus-containing soil amendments were prepared by combining PSMC with PG. A 35-day pot experiment was conducted on the restoration of saline alkali soil using peanuts treated with alcohol disinfection. The volume of the flowerpot (24 cm in height and 24 cm in diameter) is approximately 10,852 cm. Three peanut seeds were planted in each experimental pot and 3 replicate groups were set up for each experiment. The following four aspects were completed: (1) determination of soil physicochemical properties, including changes in soil pH, electrical conductivity (EC), organic matter (OM), available nitrogen (AN), available phosphorus (AP), and available potassium (AK), (2) exploration of the changes in soil enzyme activity, (3) analysis of the changes in soil bacterial community; (4) objective analysis of the effects of phosphorus-containing soil amendments on the growth of peanut plants.

#### Preparation of Phosphorus-Containing Soil Conditioner and Design of Pot Experiment

The stock solution of a PSMC was evenly stirred with PG according to the proportions outlined in Table 1 to prepare

**Table 1** Pot experiment design plan

Group	Design scheme
T0	Untreated saline-alkali soil (1.5 kg)
T1	Untreated saline-alkali soil (1.5 kg) + peanut seeds
T2	Untreated saline-alkali soil (1.5 kg) + PSMC stock solution (15 mL) + peanut seeds
T3	Untreated saline-alkali soil (1.5 kg) + PG (3.0 g) + peanut seeds
T4	Untreated saline-alkali soil (1.5 kg) + phosphorus containing soil conditioner 1 + peanut seeds
T5	Untreated saline-alkali soil (1.5 kg) + phosphorus containing soil conditioner 2 + peanut seeds
T6	Untreated saline-alkali soil (1.5 kg) + phosphorus containing soil conditioner 3 + peanut seeds

soil conditioners with different PG contents. Phosphorus-containing soil conditioner 1 (15 mL of PSMC stock solution + 3.0 g of PG); Phosphorus-containing soil conditioner 2 (15 mL of PSMC stock solution + 4.5 g of PG); Phosphorus-containing soil conditioner 3 (15 mL of PSMC stock solution + 6.0 g of PG). The pot experiment details are provided in Table 1.

### Determination of Physical and Chemical Indexes of Saline-Alkali Soil

After 35 days of pot culture, the physical and chemical indexes of potted soil, such as pH, EC value, the content of OM, AN, AP, and AK were determined according to the method described in the “Sample Collection and Analysis Methods” section.

### Determination of Soil Enzyme Activity in Saline-Alkali Soil

The activities of soil urease (S-UE), catalase (S-CAT), and alkaline phosphatase (S-ALP) were determined using specific kits (S-UE determination kit, S-CAT kit, S-ALP determination kit) purchased from Shanghai Jining Industrial Co., Ltd. S-UE and S-CAT levels were determined by microphotometry using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific (China) Co., Ltd.), while S-ALP levels were determined by spectrophotometry using a spectrophotometer (Model 721, Shanghai Jinghua Technology Instrument Co., Ltd.). S-ALP was determined according to the steps of kit (S-UE determination kit, S-CAT kit, S-ALP determination kit, appeal kit was purchased from Shanghai Jining Industrial Co., Ltd.). S-UE and S-CAT were determined by microphotometry using a microplate reader (multiskan skyhigh, Thermo Fisher Scientific (China) Co., Ltd.), and S-ALP was determined by spectrophotometry (spectrophotometer, 721 Shanghai Jinghua Technology Instrument Co., Ltd.).

### Determination and Analysis of Soil Bacterial Diversity

In order to analyze the bacterial diversity of soil samples in different treatment groups, the total bacterial DNA of each sample was extracted from 0.25 g potted soil samples using the Soil DNA Extraction Kit (DP812, Beijing Tianjin Biochemical Technology Co., Ltd., China). Subsequently, the V3-V4 hypervariable region of bacteria was amplified following the general protocol for bacterial amplification [41, 42]. The PCR amplification products were sent to Beijing BmK Technology Co., Ltd. (Beijing, China) after 2% agarose gel electrophoresis using an electrophoresis apparatus (Compact M, Jena, Germany). Sequencing analysis was conducted on the Illumina Novaseq 6000 platform.

### Determination of Plant Growth Index and Biomass

The peanut plant was pulled out completely from the pot and the soil around its roots washed. The appearance of plants under different treatments was observed. The root length and stem length were measured, and the number of leaves on each plant was recorded. The shoot and the root of the peanut plant were separated. The fresh weight of the different parts was weighed, then subject the plant to 120°C for 30 min to kill it, followed by drying at 80°C until a constant weight is achieved. Finally, weigh the dry weight of the different parts.

### Data Analysis

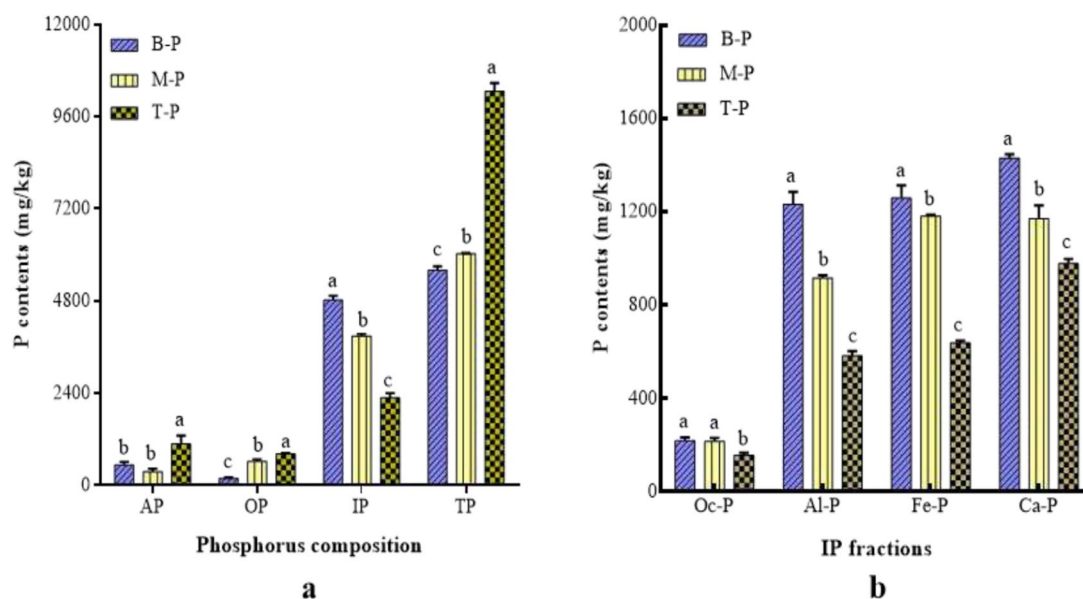
Microsoft Excel 2019 software was used to calculate and summarize the experimental data. All values represent the mean  $\pm$  standard error from the three groups of repeated experimental data. SPSS 26.0 software (IBM Corporation, New York, USA) was used for statistical analysis using one-way ANOVA and Tukey post event test to evaluate the differences among all treatment groups. The statistical significance level was set at  $p < 0.05$ . Use GraphPad Prism 9.3 and Origin Software (Version Origin 2021) to draw the histogram and line graph in the paper.

## Results

### Analysis of Phosphorus Content in PG Samples

The phosphorus containing components in PG samples that were collected from different locations were shown in Fig. 1a, and there were differences in the phosphorus containing components among different samples. The TP content in the three PG samples was the highest, ranging from 5670 to 10,100 mg/kg. The component with the second-highest concentration was IP content, with a content ranging from 2357 to 4799 mg/kg. The AP content ranged from 345 to 1020 mg/kg, ranking third in concentration. The OP content, with concentrations ranging from 191 to 822 mg/kg, had the lowest levels. The AP and OP contents were significantly lower than those of IP and TP.

Based on the differences in the contents of IP components (Fig. 1b), the content of closed storage phosphorus (Oc-P) was significantly lower than that of Al-P, Fe-P, and Ca-P. The so-called closed storage phosphorus (Oc-P) refers to phosphate coated with an iron oxide gel film. Since the outer iron coating is hard to remove, plants find it challenging to absorb and utilize [43]. The content ranged from 163 to 232 mg/kg. The content of Al-P was lower than that of Fe-P and Ca-P. In all PG samples, the IP components in the order of



**Fig. 1** Phosphogypsum sample analysis. (a) Phosphogypsum sample contains phosphorus component. (b) inorganic phosphorus component. (B-P: bottom phosphogypsum. M-P: middle phosphogypsum. T-P: Top phosphogypsum. AP: available phosphorus. Op: organic phosphorus. IP: inorganic phosphorus. Oc-p: Occluded P. Al-P: Al-bound P. Fe-P: Fe-bound P. Ca-P: Ca-bound P)

decreasing contents were as follows: Ca-P, Fe-P, Al-P, and then Oc-P.

Analysis of phosphorus components in PG samples (Fig. 1a) showed that TP contents were the highest, followed by IP content, and the  $\text{Ca}_3(\text{PO}_4)_2$  content was the highest in IP (Fig. 1b). In addition, the culture substrate containing  $\text{Ca}_3(\text{PO}_4)_2$  is the only phosphorus source that can be used by PSB [44]. Therefore,  $\text{Ca}_3(\text{PO}_4)_2$  was selected as the only phosphorus source to screen PSB in PG and the soil near its storage yard. Previous studies have shown that PSB isolated using  $\text{Ca}_3(\text{PO}_4)_2$  as the sole phosphorus source can degrade  $\text{AlPO}_4$  and  $\text{FePO}_4$  [45]. The phosphorus-solubilizing performance of the screened PSB for  $\text{AlPO}_4$  and  $\text{FePO}_4$  needs further investigation.

## Isolation and Screening of Phosphate-Solubilizing Bacteria

### Preliminary Screening of Strains

Forty-six strains with different shapes were enriched from PG samples using culturable technology with beef extract peptone solid medium. Among them, 26 strains formed distinct phosphate-solubilizing halos on NBRIP solid medium. The PSI index of each strain is shown in Table 2. The results showed that the PSI index of 26 strains was greater than 1. The PSI index of strains TA8, tA8, tB4 and LA4 was greater than or equal to 2, while 12 strains had PSI index between 1.5 and 2.0, and 10 strains had PSI index between 1.0 and 1.5.

T-P: Top phosphogypsum. AP: available phosphorus. Op: organic phosphorus. IP: inorganic phosphorus. Oc-p: Occluded P. Al-P: Al-bound P. Fe-P: Fe-bound P. Ca-P: Ca-bound P)

### Re-screening of Phosphate-Solubilizing Bacteria

Twenty-six strains with obvious phosphate-solubilizing halos were inoculated into NBRIP liquid medium, and the soluble phosphorus content in the supernatant of each strain was obtained after 7 days of culture. The results showed differences in the phosphorus-solubilizing capacity among the strains, with the available phosphorus content in the culture medium of 26 strains ranging from 25.61 to 168.88 mg/L. Among them, 7 strains of bacteria exhibited good phosphate-solubilizing capability, with the available phosphorus content in the culture medium ranging between 153.02 and 168.88 mg/L (as depicted in Fig. 2). Consequently, these 7 strains of PSB were selected for further tests.

### Construction of Phosphate-Solubilizing Microbial Consortium

The results of the plate antagonistic test among the seven PSMC are shown in Table 3. The results showed that the three groups of microbial consortia tA7-TA8, tA7-TB12 and TA8-TB12 had no antagonistic effect. The phosphate-solubilizing test of the above-mentioned microbial consortium was conducted using liquid co-culture. The results showed that the available phosphorus content of the microbial consortia tA7-TA8, tA7-TB12 and TA8-TB12 was  $225.69 \pm 0.76$  mg/L,  $221.88 \pm 0.79$  mg/L, and  $234.84 \pm 0.41$  mg/L, respectively. The available

**Table 2** Phosphorus Solubility Index (PSI) of 26 phosphorus-solubilizing bacteria

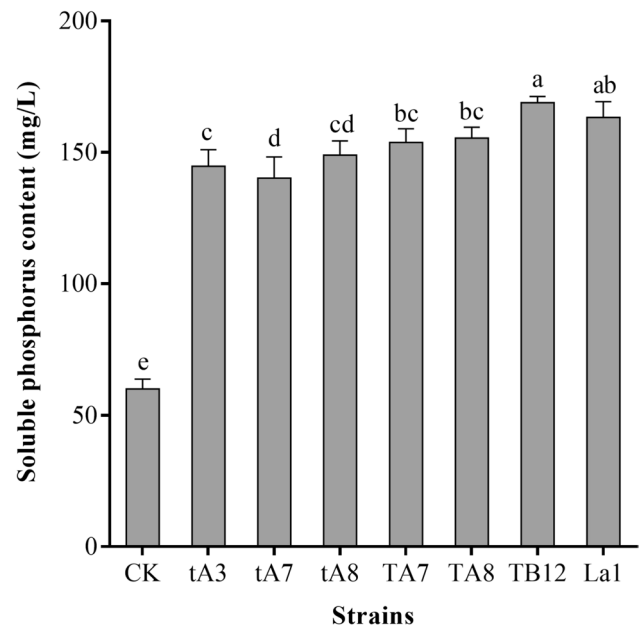
Strains	Diameter of phosphate-solubilizing halo (cm) (D)	Diameter of the colony (cm) (d)	PSI D/d
TA4	2.10±0.01	1.93±0.01	1.09±0.00
TA7	0.97±0.02	0.53±0.00	1.81±0.03
TA8	1.57±0.03	0.80±0.03	2.04±0.19
TA9	4.43±1.20	3.40±0.96	1.32±0.01
tA2	0.62±0.00	0.37±0.00	1.71±0.02
tA3	1.17±0.00	0.80±0.00	1.45±0.00
tA5	0.83±0.00	0.55±0.00	1.51±0.01
tA7	0.66±0.00	0.50±0.00	1.35±0.03
tA8	1.27±0.01	0.62±0.00	2.03±0.00
tA15	0.61±0.00	0.38±0.00	1.62±0.03
TB12	1.10±0.00	0.57±0.00	1.91±0.00
tB1	0.53±0.00	0.38±0.00	1.39±0.01
tB4	0.87±0.00	0.37±0.00	2.39±0.08
tB6	0.53±0.00	0.37±0.00	1.50±0.13
LA2	0.43±0.00	0.33±0.00	1.31±0.00
LA4	1.10±0.02	0.43±0.00	2.55±0.11
La1	0.92±0.01	0.57±0.00	1.63±0.01
La2	0.57±0.00	0.40±0.00	1.42±0.01
La4	0.73±0.00	0.47±0.00	1.61±0.01
LB2	0.67±0.00	0.47±0.00	1.43±0.00
LB3	0.61±0.00	0.51±0.00	1.20±0.00
LB5	0.39±0.00	0.30±0.00	1.28±0.00
LB11	0.57±0.00	0.46±0.00	1.25±0.02
Lb1	1.07±0.00	0.57±0.00	1.51±0.06
Lb2	1.13±0.03	0.69±0.01	1.64±0.02
LC1	0.59±0.00	0.37±0.00	1.60±0.00

phosphorus content of the three groups of PSMC was greater than that of any single strain in the corresponding combination. According to the results of the plate antagonistic test and liquid phosphate-solubilizing co-culture test, TA8 and TB12 were selected as the experimental object and used for the pot experiment of improving saline-alkali soil.

## Identification of Phosphate-Solubilizing Bacteria

### Morphological Identification

Strains TA8 and TB12 were cultured on beef extract peptone solid medium for 2 days, the colony morphology and single-cell morphology of each strain are shown in Fig. 3. The diameters of strains TA8 and TB12 are 0.8 cm and 0.57 cm, respectively. The colonies are regular and round, with an upward uplift, moisture, low transparency, and smooth edges. Strain TA8 was pale yellow, and strain TB12 was milky white. The staining test revealed that all strains were



**Fig. 2** Available phosphorus content of 7 phosphate-solubilizing bacteria. CK: Dissolved amount of corresponding calcium phosphate in NBRIP medium without adding bacterial strains

Gram-positive bacteria. Strain TA8 exhibited a spherical single-cell microstructure, while strain TB12 had a rod-shaped microstructure.

### Molecular Biological Identification

The 16S rDNA gene sequences of the two strains were submitted to the NCBI database for BLAST comparison. The homology of the two phosphate-solubilizing bacteria and their model strains with close genetic relationships was analyzed using Mega 6.0 software, and a phylogenetic tree was constructed. The strains ta8 and TB12 were identified as *Pseudomonas*. TA8 was proposed to be a *Pseudomonas chlororaphis* strain, and TB12 was nearly related to *Pseudomonas koreensis*. The 16S rDNA gene sequences of strains TA8 and TB12 were submitted to GenBank, and their accession numbers were OQ674427 and OQ674424, respectively (Fig. 4).

## Application Effect Analysis of Phosphate-Solubilizing Microbial Consortium Bacteria Saline-Alkali Soil Remediation

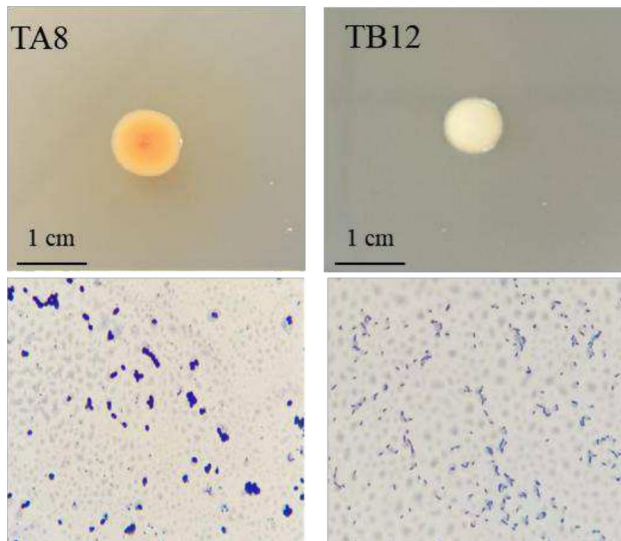
### Impact on Physical and Chemical Properties of Saline-Alkali Soil

The change in soil pH is depicted in Fig. 5a. The pH of the untreated T0 group soil sample is 8.54, which was higher than that of any treatment group. The study found that the

**Table 3** Plate Antagonism Test of 7 phosphorus-solubilizing bacteria

Strain	tA3	tA7	tA8	TA7	TA8	TB12
tA3						
tA7	+					
tA8	+	+				
TA7	+	+	+			
TA8	+	-	+	+		
TB12	+	-	+	+	-	
La1	+	+	+	+	+	+

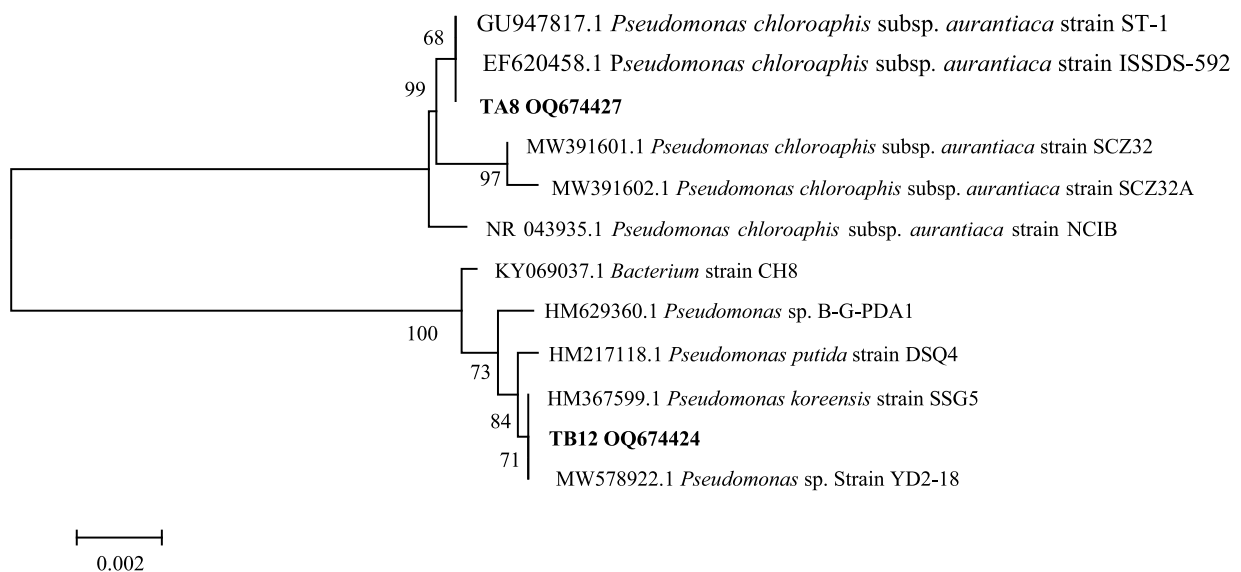
Note: “+” means there is antagonism, “-” means there is no antagonism



**Fig. 3** Single colony morphology and microstructure of TA8 and TB12

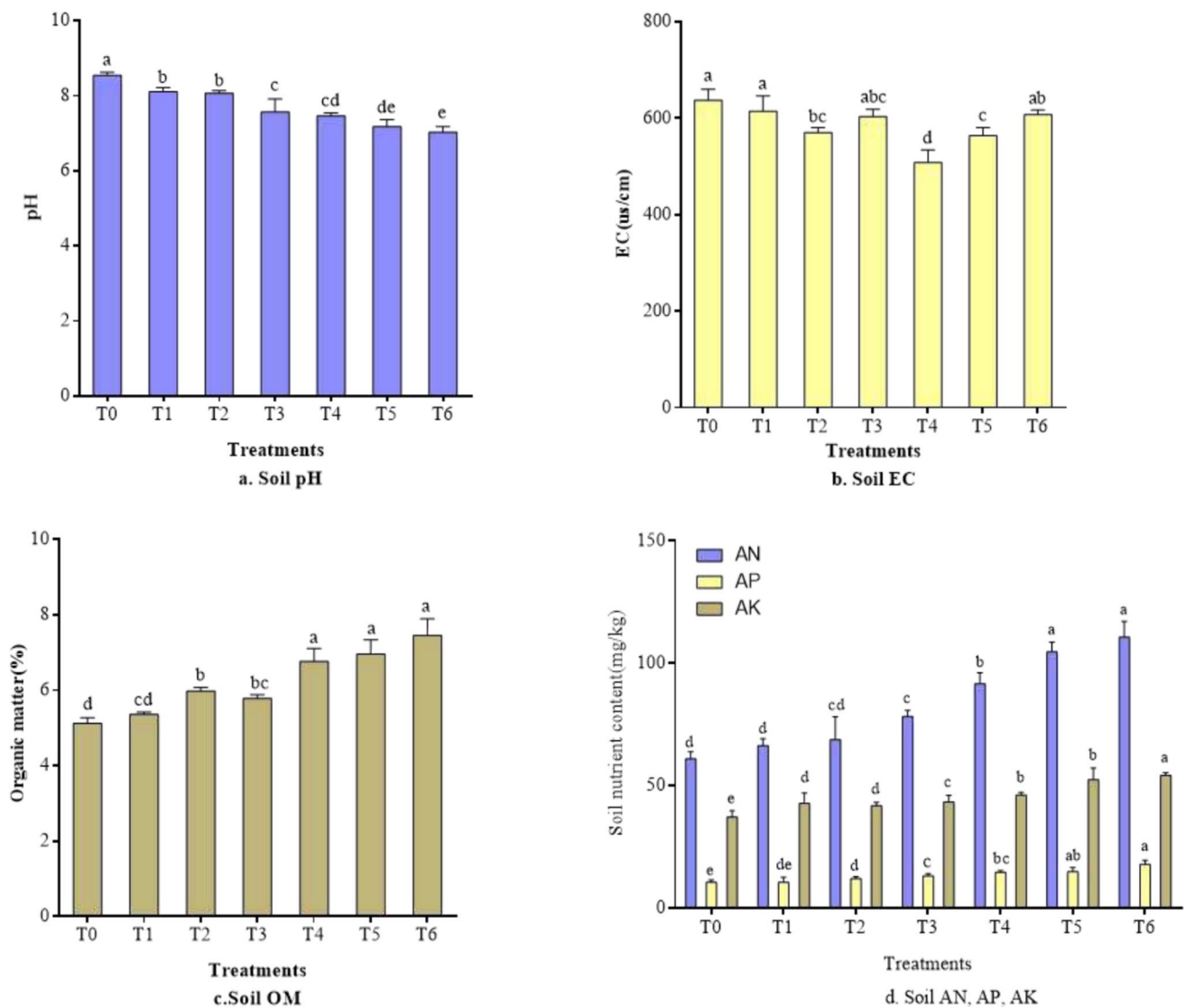
remediation effect of a phosphorus-containing soil conditioner on saline-alkali soil was superior to that of adding a PSMC or PG alone. The pH of the T4, T5, and T6 groups decreased by 12.30%, 16.04%, and 17.68%, respectively, compared to the T0 group, reaching a significant level. The pH of soil samples decreased as the PG content in the soil conditioner increased. The change in soil EC was similar to that of pH (Fig. 5b). The soil EC of the six treatment groups was reduced to varying degrees, with the most significant decrease observed in the T4 group, which decreased by 20.21%. In contrast to the pH change, the EC of the soil increased with the content of PG in the soil conditioner.

The change of OM content is shown in Fig. 5c. The OM content increased in all six treatment groups, with the most significant increases observed in the T4, T5, and T6 groups. the OM content increased by 1.65%, 1.84%, and 6.64%, respectively, compared to the T0 group. The changes in AN, AP, and AK contents are shown in



**Fig. 4** Phylogenetic tree of two phosphate-solubilizing bacteria



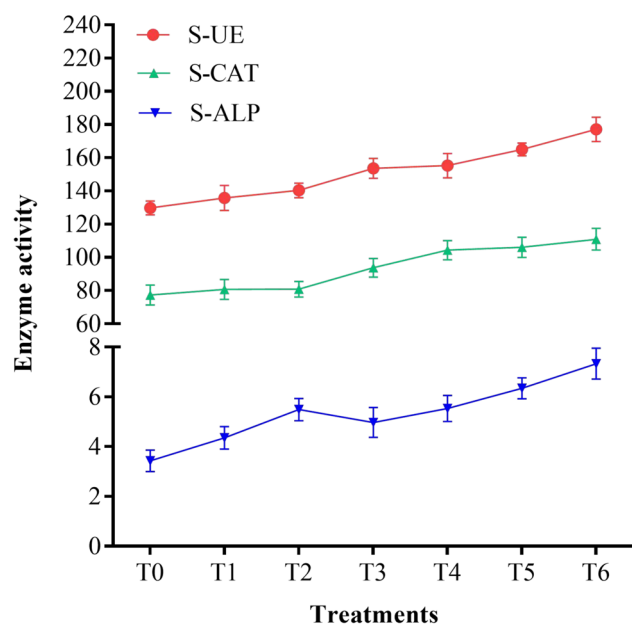


**Fig. 5** Physical and chemical properties of soil in different treatment groups. (a) Soil pH. (b) Soil Electrical Conductivity (EC). (c) Soil Organic Matter (OM). (d) Soil Available Nitrogen (AN), Available Phosphorus (AP), and Available Potassium (AK)

Fig. 5d. The study found that the content of AN and AP in the T1 group did not change significantly ( $p > 0.05$ ), but the AK content changed significantly ( $p < 0.05$ ). The total nutrient content of the soil increased significantly in all treatment groups except for the T1 group. In addition, the study also found that the total nutrient increase of T2 and T3 groups under a single treatment was less than that of T4, T5, and T6 groups phosphorus-containing soil conditioner. The AN content in the three groups of soil samples increased by 50.49%, 71.83%, and 81.68%, respectively. Additionally, the AP content increased by 41.70%, 49.40%, and 60.31%, respectively. The AK content increased by 25.56%, 31.73%, and 42.03%, respectively.

#### Effect on Soil Enzyme Activity of Saline-Alkali Soil

Soil enzyme activity is an important indicator of soil fertility, closely related to soil properties, types, and environmental conditions [46]. The changes in soil urease (S-UE), catalase (S-CAT), and alkaline phosphatase (S-ALP) activities in different treatment groups are shown in Fig. 6. It can be seen from the figure that compared with the original saline-alkali soil sample T0, the S-UE enzyme activity, S-CAT enzyme activity, and S-ALP enzyme activity of the six treatment groups increased to varying degrees. Among them, the soil enzyme activities of the T1, T2, and T3 treatment groups were lower than those of the T4, T5, and T6 treatment groups. Compared with the T0 group, S-UE activity



**Fig. 6** Change of the content of soil urease (S-UE) ( $\mu\text{g/d/g}$ ), catalase (S-CAT) (U/g) and alkaline phosphatase (S-ALP) ( $\mu\text{mol/d/g}$ )

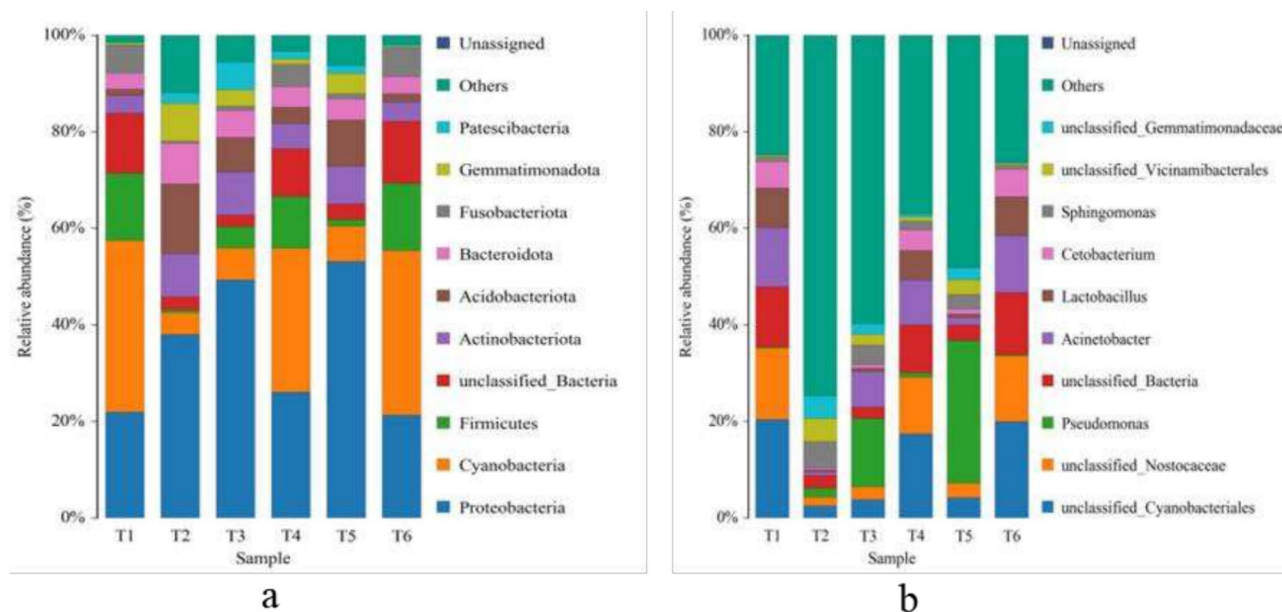
increased by 23.75%, 28.94%, and 33.96%. S-CAT activity increased by 31.13%, 37.90%, and S-ALP activity increased by 56.12%, 77.66%, and 94.95% in the T4, T5, and T6 groups, respectively.

### Impact on Bacterial Diversity in Saline Alkali Soil

Microorganisms are an essential component of the soil ecosystem, with a beneficial influence on the reduction of soil salt and alkali ions, the improvement of soil structure, and the soil fertility. The alteration of soil microbial quantity and community structure serves as a crucial indicator of soil improvement in saline-alkali soil [47]. Alpha diversity can reflect the abundance of microbial communities in soil samples [48]. The Chao1 index and ACE were used to evaluate the species richness in the samples, while the Shannon index and Simpson index were used to evaluate the microbial functional diversity in the samples [49]. It can be seen from Table 4 that the Shannon index and Simpson index of the bacterial community in each treatment group follow the order T2 > T3 > T5 > T4 > T6 > T1. When comparing the T1

**Table 4** Alpha-diversity index in soil samples of each treatment group

Microorganism	Treatments	OTU	ACE	Chao1	Simpson	Shannon
Bacteria	<b>T1</b>	574	574.24	574.00	0.92	5.37
	<b>T2</b>	1067	1067.60	1067.01	1.00	9.19
	<b>T3</b>	953	953.56	953.01	0.99	8.32
	<b>T4</b>	958	958.25	958.00	0.95	6.52
	<b>T5</b>	774	774.57	774.01	0.99	7.86
	<b>T6</b>	703	703.27	703.00	0.93	5.57



**Fig. 7** Relative abundance of bacterial communities at phylum (a) and genus (b) levels in different treatment groups

group, which exclusively planted peanuts, to the remaining five groups of saline-alkali soil samples, it is evident that the diversity of soil bacterial communities is higher in the latter groups than in the T1 group. The order of Chao1 index, OTU number, and ACE of each treatment group was T2 > T4 > T3 > T5 > T6 > T1. The study also found that the abundance of the bacterial community in the soil was greater than that in the T1 group. This difference could be attributed to either a single phosphate-solubilizing microbial consortium, PG, or the influence of phosphorus-containing soil conditioner.

According to the analysis of the distribution of soil bacterial species at the phylum level (see Fig. 7a), the bacterial community composition of the top 10 relative abundance in the soil of each treatment group includes *Proteobacteria*, *Cyanobacteria*, *Firmicutes*, *Actinobacteria*, *Acidobacteria*, *Bacteroidota*, *Fusobacteriota*, *Gemmatimonadota*, and *Patescibacteria*. The relative abundance of *Proteobacteria* was over 10%, remarked as the dominant bacteria in each treatment group. The relative abundance of *Cyanobacteria* was more than 10% in the T1, T4, and T6 treatment groups, surpassing *Proteobacteria* as the predominant bacteria. The composition of dominant bacteria in each treatment group was consistent, while the relative abundance varied. Compared with the T1 control group, the relative abundance of *Proteobacteria*, *Acidobacteria*, *Bacteroidota*, and *Actinobacteria* increased significantly in the T2, T3, and T5 groups, while the abundance of *Cyanobacteria* decreased significantly.

According to the analysis of the distribution of soil bacterial species at the genus level (see Fig. 7b), the top 10 bacteria in each treatment group are *Pseudomonas*, *Acinetobacter*, *Lactobacillus*, *Cetobacterium*, and *Sphingomonas*. The dominant bacteria composition in each treatment group was consistent, while the relative abundance varied. It can be seen from Fig. 7b that the relative abundance of *Pseudomonas* and *Sphingomonas* increased significantly in the T3 and T5 groups compared to T1 in the control group. The abundance of the T4 and T6 treatment groups compared to T1 in the control group remained relatively stable.

### Effect on Growth Index and Biomass of Peanut Plants

The effects of different treatment groups on the growth indexes of peanut plants are shown in Fig. 8. The study found that the root length and stem length of peanut plants in all treatment groups were greater than those in the control group T1 (Fig. 8a). The root length and stem length of the T4 group, which used a phosphorus containing soil conditioner, were the largest, with a root length of 16.53 cm and a stem length of 11.67 cm. In terms of the number of leaves and branches of peanut plants (Fig. 8b), the T4 treatment group had the highest number of leaves and branches. The

number of leaves in the T4 group was significantly greater than that in the T1 group, showing an increase of 75.12%. However, the number of branches was not significantly different from that of other T2, T5 and T6 treatment groups containing phosphate-solubilizing microbial consortium.

It can be seen from Fig. 8c that the most significant change in the fresh weight of the roots and stems of peanut plants occurred in the T4 group, with an increase of 4.41 g and 10.63 g, respectively. This represents 73.34% and 116.6% increase compared to the T1 group. The dry weights of the roots and stems of peanut plants were significantly increased in each treatment group (Fig. 8d). Compared with the T1 group, the root dry weights of the T2, T3, T4, T5, and T6 treatment groups increased by 19.30%, 52.63%, 142.11%, 52.63%, and 26.32%, respectively. The stem dry weight increased by 86.02%, 79.03%, 88.71%, 75.27%, and 94.62%, respectively.

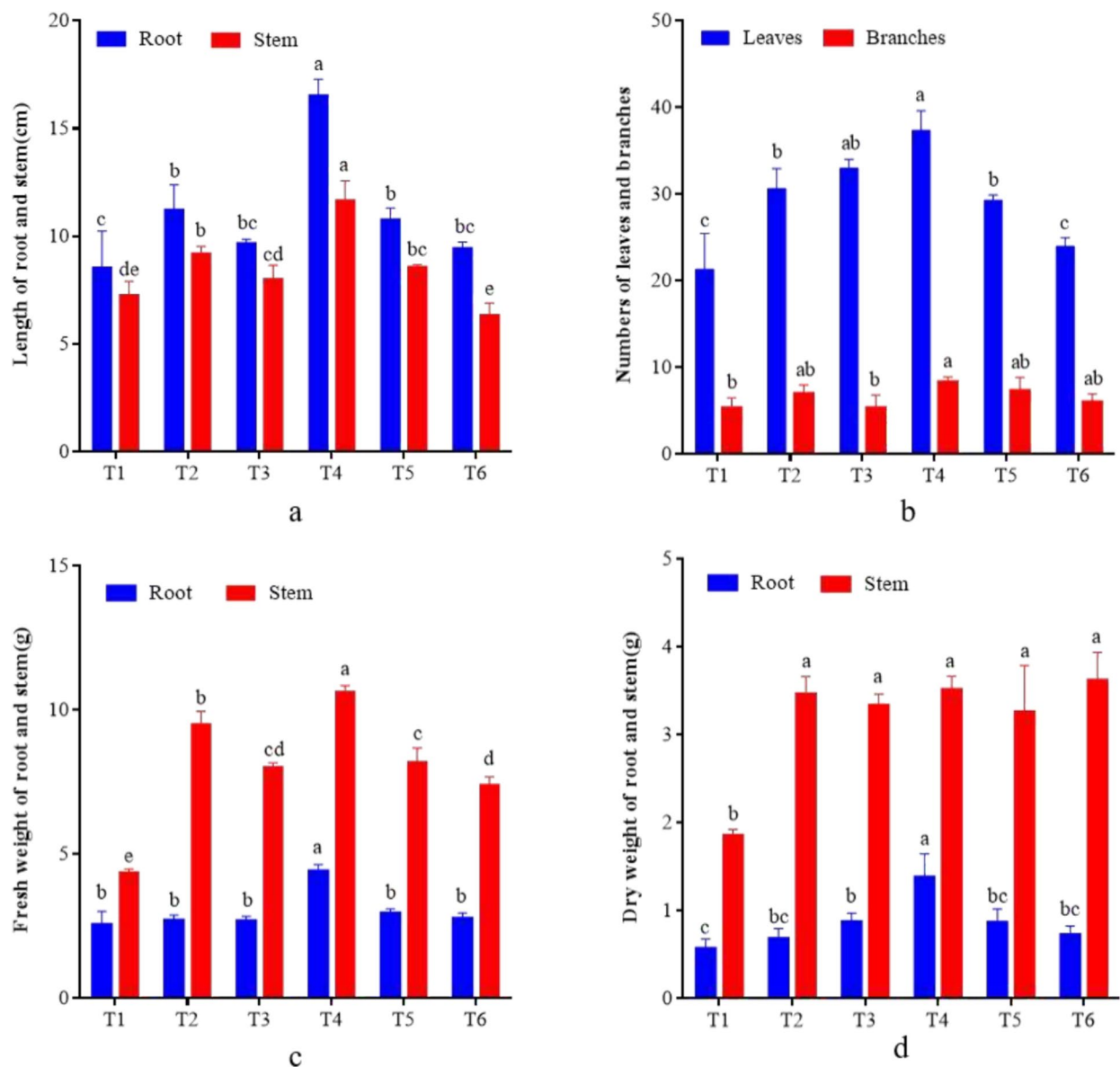
## Analysis and Discussion

### Analysis of Phosphorus Content in PG Samples

PG contains available phosphorus, inorganic phosphorus, and organic phosphorus, and the content of calcium phosphate in insoluble inorganic phosphorus is the largest, which aligns with the conclusions drawn by Zhang [50] and Tang [51]. The study found that the insoluble phosphorus in PG accounted for 23.33% of the total phosphorus content, while calcium phosphate accounted for 40.98% of its content. This percentage was higher than the findings of Xu et al. [52], confirming the value and significance of recycling PG.

### Isolation and Identification of Phosphate-Solubilizing Bacteria

In this study, it was found that the PSI value of the phosphate-solubilizing halo of each strain was not entirely consistent with the phosphate-solubilizing capacity under liquid culture conditions. For example, the PSI index of strain LA4 was 2.55, while the AP content in the corresponding culture medium was 36.91 mg/L, and the PSI index of strain tb4 was 1.09. On the contrary, the AP content in the culture medium was 134.72 mg/L, and the PSI index of strain La1 was 1.63. The corresponding AP content was 160.38 mg/L. David et al. proposed that the production of phosphate-solubilizing halos is the result of calcium phosphate being solubilized by organic acids produced by phosphate-solubilizing microorganisms through osmotic diffusion [53]. Liu et al. [44] found that the size of the phosphate-solubilizing halo produced by the strain on the plate medium did not correlate well with the amount of phosphate solubilized by the strain in liquid culture. This is consistent with the research report by Nacoon



**Fig. 8** Changes in peanut growth indicators of different treatment groups. **(a)** Length of root and stem. **(b)** Total numbers of leaves and branches. **(c)** Fresh weight of root and stem. **(d)** dry weight of root and stem

et al. [54], who isolated and screened 11 phosphorus-solubilizing strains. The PSI index of strain LC373001 was 1.10, and the AP content was 489  $\mu\text{g}/\text{mL}$ . The PSI index of strain LC373004 was 2.10, while the AP content was only 470  $\mu\text{g}/\text{mL}$ . The test results of Johri and Chung also concluded that the AP content of strains with a high PSI index was not necessarily high in liquid culture [36, 55]. Therefore, it is not reliable to screen phosphate-solubilizing bacteria solely using the plate phosphate-solubilizing halo method.

Through the plate antagonistic test and the liquid co-culture test, the strains TA8 and TB12 were selected to construct the PSMC. After morphological and molecular biological identification, it was found that both strains TA8 and

TB12 belonged to *Pseudomonas* spp. In the existing studies, many reports have mentioned that *Pseudomonas* sp. has high phosphate-solubilizing capability [55–59]. The strain TA8 screened in this study is closely related to *Pseudomonas chlororaphis*, with an AP content of 158.11  $\text{mg}/\text{L}$ , which is superior to the strains 3T4-12 (2.89  $\text{mg}/\text{mL}$ ) and 3T4-20 (2.61  $\text{mg}/\text{mL}$ ) isolated by López-Hernández et al. [60]. The study indicates that possibly *Pseudomonas chlororaphis* can effectively enhance the availability of phosphorus in the soil surrounding the root system and stimulate the growth of *Arabidopsis* roots. Strain TB12 screened in this study is related to *Pseudomonas koreansis*. The AP content is 168.88  $\text{mg}/\text{L}$ , higher than that of *Pseudomonas* strain No.5 (81.73

mg/L) [61] as reported by Cao et al. Jabborova [62] discovered that inoculating *Pseudomonas koreansis* IGPEB17 into the ginger rhizosphere can significantly enhance the available phosphorus content of the crop rhizosphere soil and stimulate ginger growth.

### Determination of Physical and Chemical Properties of Saline-Alkali Soil

The study found that compared to the blank control group T0, a single application of phosphorus-solubilizing microbial consortium (T2 group) or PG (T3 group) can reduce the pH and EC values of saline alkali land. When the PSMC and PG are applied together to saline-alkali soil, the pH and EC values of the soil fluctuate more [63]. The results showed that the soil pH value of the T1 treatment group, which only planted peanut plants, decreased compared to the T0 group. The reason may be that with the germination and growth of peanut plants, the growth of their roots increased the soil's permeability and permeability, leading to a decrease in soil pH. The decrease in soil pH in T2 group may be the result of mechanisms such as organic acids secreted by phosphate-solubilizing bacterial solution added, or organic acids produced by plant roots were stimulated [64]. The remediation effect of T4, T5, and T6 groups containing PSMC and PG on soil pH in this study is essentially consistent with the research findings of Taktek et al. [65]. The pH of the PG used in this study is 2.08, and direct application can effectively reduce the pH of saline-alkali soil. In addition, PG can also provide sufficient substrates for phosphorus-solubilizing microorganisms. These microorganisms secrete more organic acids through an acidolysis mechanism, thereby reducing the soil pH [65, 66]. The research results show that PG cannot significantly reduce the EC value of saline-alkali soil. In the T4, T5, and T6 treatment groups, the soil EC value also increases with the addition of PG, which aligns with the findings of Liu et al. [67]. This is because the EC of PG itself is high, which will also cause an increase in soil EC value after being applied to the soil [27].

The findings show that the increase in OM, AN, AP, and AK in T4, T5, and T6 groups with phosphorus-containing soil conditioner was higher than that in T1, T2, and T3 groups with a single treatment. This finding is consistent with Vassilev and other research conclusions [68]. The reason is that PG itself contains calcium, phosphorus, sulfur, and other nutrients, as well as a certain amount of  $P_2O_5$ . Therefore, the PSMC can reproduce in large quantities using PG as a substrate. These bacteria produce organic acids during their metabolism, which helps solubilize insoluble phosphorus sources in the soil and enhance the AP content in the soil. PG promotes the formation of soil aggregates through neutralization reactions,

generating calcium colloids in alkaline soil. This process improves soil structure and contributes to the accumulation of soil organic matter [68, 69]. PG and PSMC not only reduced soil pH and EC but also improved soil physical and chemical properties, which was conducive to increasing AN and AK contents. According to the influence of pH, EC, OM, AP, and other nutrients in saline-alkali soil, the comprehensive improvement effect is better than single improvement methods. This indicates that it is feasible to enhance saline-alkali soil by combining PSMC and plant growth-promoting rhizobacteria (PGPR).

### Determination of Soil Enzyme Activity in Saline-Alkali Soil

This study found that the S-ALP enzyme activity of the T3 treatment group using PG alone changed slightly, increasing by 14.97%, while the S-ALP enzyme activity of the T2 treatment group with PSMC increased significantly, rising by 46.01%. It can be speculated that PSMC can improve the solubility of phosphorus in soil by enhancing the S-ALP activity of the soil. In addition, Balota found that the life activities of phosphate-solubilizing bacteria in saline alkali soil can produce various enzymes that enhance soil enzyme activity when studying the effect of phosphate-solubilizing bacteria on soil S-ALP activity in saline-alkali soil [46]. When PSMC was introduced with PG, the activities of S-CAT, S-UE, and S-ALP enzymes increased significantly. Soil enzyme activity also increased with the rise in PG content in phosphate-containing soil amendments. S-UE enzyme activity, S-CAT enzyme activity, and S-ALP enzyme activity in the T6 treatment group increased by 33.96%, 42.81%, and 94.95%, respectively, because PG increased the content of organic matter in the soil. The PSMC and the original microorganisms in the soil utilized organic matter for reproduction, thereby increasing the microorganism population and soil enzyme activity [69].

### Determination and Analysis of Soil Bacterial Diversity

Soil microorganisms play a crucial role in the saline-alkali soil ecosystem. They can promote the transformation of substances in the soil, improve the soil structure and fertility of saline-alkali soil, reduce soil pH and EC, and have a significant effect on enhancing saline-alkali soil [70]. It was found that *Proteobacteria* was the most dominant phylum in saline-alkali soil planted with peanuts, followed by *Actinobacteria* and *Bacteroidota*. Other scholars [71, 72] have also concluded that *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the dominant phyla when studying the saline-alkali land in Hetao Plain, Ningxia, and the Yellow River Delta. Zhang et al. [73] reported that in the rhizosphere of

peanuts in saline-alkali soil, the levels of *Proteobacteria* and *Actinobacteria* were high, with average abundances of about 35% and 25%, respectively. Wang et al. [74] found that the relative abundance of *Proteobacteria* increases with the improvement of soil nutrient levels. In this study, compared with the T0 group, the soil nutrient contents such as OM and AN in each treatment group were increased to varying degrees, and the relative abundance of *Proteobacteria* also increased accordingly. Bahram et al. [75] found that a weak alkaline environment is suitable for the survival of actinobacteria. In this study, the soil pH of each treatment group decreased to varying degrees, leading to a corresponding increase in the relative abundance of actinobacteria to varying degrees.

Although the species composition at the phylum level is basically the same, there are significant differences in the dominant genera of different improved treatments. This study showed that the relative abundance of *Pseudomonas* increased significantly after treatment, which was closely related to the fact that the best phosphate solubilizing bacteria also belonged to *Pseudomonas*. In addition, *Sphingomonas* is also one of the dominant bacteria. Dihe et al. [15] found that *Sphingomonas* has strong adaptability and is widely distributed in different saline-alkali soil. Wang et al. [76] found that *Sphingomonas* is a group of bacteria with diverse metabolism. The increase of its relative abundance will enhance the activities of catalase and oxidase in microbial cells in soil and enhance the role of oxidase in soil environment. In general, the changes of microorganisms at phylum and genus levels in the treatment group added with soil amendments were greater than those in the single treatment group, indicating that the effect of compound improvement was better than that of single improvement.

Based on the experimental results, it was found that the application of a phosphate-solubilizing microbial consortium and PG had varying degrees of impact on the physical and chemical properties, soil structure, and soil nutrients of the soil. Therefore, the changes in bacterial diversity varied among different treatment groups.

### Effects of Saline-Alkali Soil Improvement on Growth and Biomass of Peanut Plants

Peanut is an important cash crop and oil crop, but due to the high concentration of saline-alkali in the soil, the large-scale planting of peanut is limited. Through the study on the growth index and biomass of peanut plants in different treatment groups, we found that the growth of peanut plants in T4 group was the best, and the fresh weight and dry weight of roots and stems changed significantly compared with the T1 group. T4 group was the treatment group with low content of PSMC and PG. With the increase of PG content, the growth index and biomass of peanut plants decreased, which was

consistent with the conclusion reached by Gomes-Araújo and others when exploring the impact of PG on sugarcane yield [77]. The treatment effect of T4 group is also better than that of T2 group and T3 group. The application of PG directly supplies nutrients such as P, Ca and S to the plants, which promotes the growth and development of plants [78], and the PSMC agent further improves the utilization rate of phosphorus in soil on the basis of PG, which is also conducive to the growth of plants. Phosphate-Solubilizing Bacteria also produce growth-promoting hormones like auxins, cytokinins, and gibberellins, which promote cell division, cell differentiation, shoot growth, root development, flowering, germination, and xylem differentiation [79]. Seema Dharni et al. (2014) inoculated *Pseudomonas monteilii* (PsF84) and *Pseudomonas plecogrossicida* (PsF610) onto rose-scented geranium (*Pelargonium graveolens* cv. bourbon) grown on sludge modified soil. The isolate PsF84 increased the dry biomass of shoot by 44%, root by 48%, essential oil yield 43% and chlorophyll by 31% respectively over uninoculated control. The corresponding increase with the isolate PsF610 were 38%, 40%, 39% and 28%, respectively [80]. The results showed that microbial inoculants combined with PG could improve the aboveground biomass of plants, the availability of nutrients in soil and the absorption of nutrients by plants, and the application of soil amendments made by microbial inoculants and PG in saline soil could promote plant growth and soil productivity.

### Conclusion

Two strains of bacteria without antagonistic effects were selected from a bacterial isolation from the Wengfu PG storage yard through plate phosphorus dissolution halo method, liquid phosphorus dissolution method, and antagonistic experiments. After morphological and molecular biology identification, they were identified probably as *Pseudomonas chlorophoraphis*-TA8 and *Pseudomonas koreensis*-TB12, both belonging to the *Pseudomonas* genus, which is commonly used for biological inoculation to improve the utilization rate of phosphorus in soil. In this study, *Pseudomonas chlorophoraphis*-TA8 and *Pseudomonas koreensis*-TB12 and the phosphate containing soil conditioner made by PG had significant effects on the soil pH, EC, OM and, AP and AK nutrient contents of saline alkali soil. Because the ultimate purpose of saline-alkali soil improvement is to improve soil productivity and promote the growth of crops planted in saline-alkali soil, the study found that T4 group (phosphate solubilizing bacteria (15 mL) and PG (3.0 g)) had the best plant growth, the largest number of leaves and branches, and the fresh weight of rhizome increased by 73.34% and 116.60%, and the dry weight of rhizome increased by

52.63% and 75.27%, respectively. The results are helpful to better understand the improvement effect of PSMC and PG on saline alkali soil and provide useful information for the resource utilization of PG and the comprehensive improvement of saline alkali soil. In addition, due to the high cultivability of phosphate-solubilizing bacterial strains, PG has a significant global production and low cost. Therefore, integrating phosphate-solubilizing bacterial strains with PG to enhance saline-alkali soil offers notable advantages in both technology and economics. This approach is expected to become a primary method for improving saline-alkali soil in the future.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s00248-024-02485-x>.

**Acknowledgements** Thank my teachers, Mr. Wang and Mr. Zhou, for their help in the experiment.

**Author Contribution** Conceptualization, Ting-Zhang.; Software, Ting-Zhang.; Formal analysis, Ting-Zhang.; Investigation, Ting-Zhang.; Data curation, Ting-Zhang.; Writing—original draft, Ting-Zhang.; Writing—review & editing, Xue-li Wang.; Juan-Zhou; Supervision, Xue-li Wang, Juan-Zhou, Wei-Zhou.; Funding acquisition, ShaoQi Zhou, All authors reviewed the manuscript.

**Funding** This research was funded by Science and Technology Support Project of Guizhou Province: (([2020] 1Y116), ([2021] General 273), ([2021] General 121), and ([2022] General 148, ([2022]226)).

**Data Availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethics Approval** This study does not involve any human or animal experimentation; hence, ethical approval is not required for this research.

**Consent to Participate** Informed consent was obtained from all individual participants included in the study.

**Consent for Publication** The authors have stated that their study does not involve experimentation on humans or animals, and as a result, there is no need for consent to publish the data for this study.

**Competing Interests** The authors declare no competing interests.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

## References

- Gu K, Chen B (2020) Loess stabilization using cement, waste phosphogypsum, fly ash and quicklime for self-compacting rammed earth construction. *Constr Build Mater* 231:117195
- Wei Z, Zhang Q, Li X (2021) Crystallization kinetics of  $\alpha$ -hemihydrate gypsum prepared by hydrothermal method in atmospheric salt solution medium. *Crystals* 11(8):843
- Rashad AM (2017) Phosphogypsum as a construction material. *J Clean Prod* 166:732–743
- Lieberman RN et al (2020) The geochemical evolution of brines from phosphogypsum deposits in Huelva (SW Spain) and its environmental implications. *Sci Total Environ* 700:134444
- Torres-Sánchez R et al (2020) Hydrogen fluoride concentrations in ambient air of an urban area based on the emissions of a major phosphogypsum deposit (SW, Europe). *Sci Total Environ* 714:136891
- Qi J et al (2023) Application of phosphogypsum in soilization: a review. *Int J Environ Sci Technol* 20(9):10449–10464
- Liu S, Ouyang J, Ren J (2020) Mechanism of calcination modification of phosphogypsum and its effect on the hydration properties of phosphogypsum-based supersulfated cement. *Constr Build Mater* 243:118226
- Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ - Science* 26(1):1–20
- Wang X-L et al (2023) Phosphate-solubilizing capacity of *Paeecilomyces lilacinus* PSF7 and optimization using response surface methodology. *Microorganisms* 11(2):454
- Al-Enazy AAR et al (2017) Role of microbial inoculation and industrial by-product phosphogypsum in growth and nutrient uptake of maize (*Zea mays* L.) grown in calcareous soil. *J Sci Food Agric* 97(11):3665–3674
- Cánovas CR et al (2018) Valorization of wastes from the fertilizer industry: current status and future trends. *J Clean Prod* 174:678–690
- Chuan LM et al (2018) Phosphogypsum production and utilization in China. IOP Publishing Ltd., 022099 (5pp). <https://doi.org/10.1088/1757-899X/382/2/022099>
- Wu F et al (2022) Utilization path of bulk industrial solid waste: a review on the multi-directional resource utilization path of phosphogypsum. *J Environ Manage* 313:114957
- Rosales J et al (2020) Treated phosphogypsum as an alternative set regulator and mineral addition in cement production. *J Clean Prod* 244:118752
- Yang D et al (2022) Saline-alkali stress reduces soil bacterial community diversity and soil enzyme activities. *Ecotoxicology* 31(9):1356–1368
- Wang Z et al (2018) Types, harms and improvement of saline soil in Songnen Plain. IOP Conference Series: Mater Sci Eng 322(5):052059
- Fang S, Hou X, Liang X (2021) Response mechanisms of plants under saline-alkali stress. *Front Plant Sci* 12:667458
- Singh K (2016) Microbial and enzyme activities of saline and sodic soils. *Land Degrad Dev* 27:70
- Cheng YL et al (2023) Effects of the salt-tolerant gramineous forage *Echinochloa frumentacea* on biological improvement and crop productivity in saline-alkali land on the Hetao Ningxia Plain in China. *Sustainability* 15(6):5319

20. Hongna C et al (2021) Exogenous salicylic acid signal reveals an osmotic regulatory role in priming the seed germination of *Leymus chinensis* under salt-alkali stress. *Environ Exp Bot* 188:104498
21. Zhang K et al (2023) Advances and future research in ecological stoichiometry under saline-alkali stress. *Environ Sci Pollut Res* 30(3):5475–5486
22. Abou-Shady A (2016) Reclaiming salt-affected soils using electro-remediation technology: PCPSS evaluation. *Electrochimica Acta* 190:511–520
23. Zhao D et al (2021) Impact of saline stress on the uptake of various macro and micronutrients and their associations with plant biomass and root traits in wheat. *Plant Soil Environ* 2:67
24. Sun Z et al (2022) Enhanced improvement of soda saline-alkali soil by in-situ formation of super-stable mineralization structure based on CaFe layered double hydroxide and its large-scale application. *Chemosphere* 300:134543
25. Sahin U, Eroğlu S, Sahin F (2011) Microbial application with gypsum increases the saturated hydraulic conductivity of saline-sodic soils. *Appl Soil Ecol* 48(2):247–250
26. Wang SJ et al (2017) Research on saline-alkali soil amelioration with FGD gypsum. *Resour Conserv Recycl* 121:82–92
27. Zhao Y et al (2018) Extensive reclamation of saline-sodic soils with flue gas desulfurization gypsum on the Songnen Plain. *Northeast China Geoderma* 321:52–60
28. Sharma S, Prasad R (2003) Yield and P uptake by rice and wheat grown in a sequence as influenced by phosphate fertilization with diammonium phosphate and Mussoorie rock phosphate with or without crop residues and phosphate solubilizing bacteria. *J Agric Sci* 141(3–4):359–369
29. Bueis T et al (2019) Phosphorus availability in relation to soil properties and forest productivity in *Pinus sylvestris* L. plantations. *Ann Forest Sci* 76(4):1–13
30. Bao SD (2000) Soil and agricultural chemistry analysis [J]
31. Sparks DL et al (2020) Methods of soil analysis, part 3: chemical methods, vol 14. John Wiley & Sons
32. Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol Lett* 170(1):265–270
33. Wang W et al (2020) Screening, identification and growth promotion ability of phosphate solubilizing bacteria from soybean rhizosphere under maize-soybean intercropping systems [J]. Cold Spring Harbor Laboratory. <https://doi.org/10.1101/2020.12.15.422997>
34. Chen J-X et al (2018) Isolation, identification of phosphate-solubilizing bacteria derived from phosphate tailing soil and their capacities. *Biotechnol Bull* 34(6):183
35. Ponnuragan P, Gopi C (2006) Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *J Agron* 5(4):600–604
36. Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH, and temperature-tolerant, phosphate-solubilizing bacteria in alkaline soils. *Curr Microbiol* 39(2):89–93
37. Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
38. Agyeman FO, Tao W (2014) Anaerobic co-digestion of food waste and dairy manure: effects of food waste particle size and organic loading rate. *J Environ Manage* 133:268–274
39. Lawson CE et al (2019) Common principles and best practices for engineering microbiomes. *Nat Rev Microbiol* 17(12):725–741
40. Chen J et al (2021) Isolation and screening of multifunctional phosphate solubilizing bacteria and its growth-promoting effect on Chinese fir seedlings. *Sci Rep* 11(1):9081
41. Johnson JS et al (2019) Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nat Commun* 10(1):5029
42. Sun T et al (2020) Bacterial compatibility and immobilization with biochar improved tebuconazole degradation, soil microbiome composition and functioning. *J Hazard Mater* 398:122941
43. Xie F, Ying-Fei LI (2011) Discussion on phosphorus forms and transformation in soil. *J Yangling Vocat Tech College* 10:4
44. Liu Z et al (2015) Characterization of phosphate-solubilizing bacteria isolated from calcareous soils. *Appl Soil Ecol* 96:217–224
45. Park K-H et al (2010) Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. *Appl Microbiol Biotechnol* 86(3):947–955
46. Balota et al (2010) Enzymatic activity and mineralization of carbon and nitrogen in soil cultivated with coffee and green manures. *Revista Brasileira de Ciência do Solo* 86:947
47. Hemkemeyer M et al (2021) Functions of elements in soil microorganisms. *Microbiol Res* 252:126832
48. Li B et al (2013) Characterization of tetracycline resistant bacterial community in saline activated sludge using batch stress incubation with high-throughput sequencing analysis. *Water Res* 47(13):4207–4216
49. Grice EA et al (2009) Topographical and temporal diversity of the human skin microbiome. *Science* 324(5931):1190–1192
50. Zhang W et al (2022) Research on the process mineralogy of phosphogypsum using mineral liberation analysis system. *Multipurp Util Miner Resour* 1:205–210
51. Ming-zhu T et al (2022) Characterization of the impurity phases in phosphogypsum by the EBSD-XPS method
52. Li X, Lv X, Xiang L (2023) Review of the State of Impurity Occurrences and Impurity Removal Technology in Phosphogypsum. *Materials* 16(16):5630
53. Jones DL, Darrah PR (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil* 166(2):247–257
54. Nacoon S et al (2020) Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. *Sci Rep* 10(1):4916
55. Chung H et al (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. *Soil Biol Biochem* 37(10):1970–1974
56. Biswas JK et al (2018) Potential application of selected metal resistant phosphate solubilizing bacteria isolated from the gut of earthworm (*Metaphire posthuma*) in plant growth promotion. *Geoderma* 330:117–124
57. Teng Z et al (2019) Characterization of phosphate solubilizing bacteria isolated from heavy metal contaminated soils and their potential for lead immobilization. *J Environ Manage* 231:189–197
58. Rfaki A et al (2020) Isolation, selection and characterization of root-associated rock phosphate solubilizing bacteria in Moroccan wheat (*Triticum aestivum* L.). *Geomicrobiol J* 37(3):230–241
59. Chen Y et al (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl Soil Ecol* 34(1):33–41
60. López-Hernández J et al (2023) Screening of phosphate solubilization identifies six *Pseudomonas* species with contrasting phyto-stimulation properties in *Arabidopsis* seedlings. *Microb Ecol* 86(1):431–445
61. Ying C et al (2018) Phosphorus solubilizing and releasing bacteria screening from the rhizosphere in a natural wetland. *Water* 10(2):195



62. Jabborova D (2022) The effects of *Pseudomonas koreensis* IGPEB 17 and arbuscular mycorrhizal fungi on growth and physiological properties of ginger. *Turk J Agric For* 46(4):488–495
63. Shah C et al (2022) Combined inoculation of phosphate solubilizing bacteria with mycorrhizae to alleviate the phosphate deficiency in banana. *Biologia* 77(9):2657–2666
64. Sun X et al (2021) Plant growth-promoting effect of the chitosanolytic phosphate-solubilizing bacterium *Burkholderia gladioli* MEL01 after fermentation with chitosan and fertilization with rock phosphate. *J Plant Growth Regul* 40(4):1674–1686
65. Taktek S et al (2017) Igneous phosphate rock solubilization by biofilm-forming mycorrhizobacteria and hyphobacteria associated with *Rhizoglossum irregulare* DAOM 197198. *Mycorrhiza* 27(1):13–22
66. Zhang Y et al (2020) Short-term effects of biochar and gypsum on soil hydraulic properties and sodicity in a saline-alkali soil. *Pedosphere* 30(5):694–702
67. Liu Y et al (2018) Optimum combination of soil amendments under drip irrigation with different water sources in coastal areas of East China. *Span J Agric Res* 16(3):e1102–e1102
68. Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Appl Microbiol Biotechnol* 71(2):137–144
69. Huan Huan J (2019) Saline-alkali soil remediation by the combined application of Halotolerant phosphate solubilizing microorganism and rock phosphate. Harbin Institute Technol
70. Liu Y et al (2023) Effects of Phosphogypsum and Suaeda salsa on the soil moisture, salt, and bacterial community structure of salinized soil. *Huan Jing Ke Xue= Huanjing Kexue* 44(4):2325–2337
71. Li M, Ma F, Zhang JH (2021) Effects of rice planting years on physicochemical properties and bacterial community structure in saline-alkali soil. *Agric Res Arid Areas* 39(4):194–202
72. Zhao J, Xie HJ, Zhang J (2020) Microbial diversity and physicochemical properties of rhizosphere microenvironment in *saline-alkali soils of the Yellow River Delta*. *Environ Sci* 41(3):1449–1455
73. Zhang Y et al (2017) The microbially mediated soil organic carbon loss under degenerative succession in an alpine meadow. *Mol Ecol* 26(14):3676–3686
74. Wang D et al (2020) Effects of microbial fertilizers on soil improvement and bacterial communities in saline-alkali *Soils of Lycium barbarum*. *J Agric Biotechnol* 28(8):1499–1510
75. Bahram M et al (2018) Structure and function of the global topsoil microbiome. *Nature* 560(7717):233–237
76. Wang M et al (2019) The responses of a soil bacterial community under saline stress are associated with Cd availability in long-term wastewater-irrigated field soil. *Chemosphere* 236:124372
77. Araújo LG et al (2019) How does gypsum increase the organic carbon stock of an Oxisol profile under sugarcane? *Geoderma* 343:196–204
78. Al-Enazy A-A et al (2018) Effect of phosphogypsum application and bacteria co-inoculation on biochemical properties and nutrient availability to maize plants in a saline soil. *Archives of Agronomy and Soil Science* 64(10):1394–1406
79. Rawat P et al (2021) Phosphate-solubilizing microorganisms: mechanism and their role in phosphate solubilization and uptake. *J Soil Sci Plant Nutr* 21(1):49–68
80. Dharni S et al (2014) Impact of plant growth promoting *Pseudomonas monteilii* PsF84 and *Pseudomonas plecoglossicida* PsF610 on metal uptake and production of secondary metabolite (monoterpenes) by rose-scented geranium (*Pelargonium graveolens* cv. bourbon) grown on tannery sludge amended soil. *Chemosphere* 117:433–439

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.