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Isolation of Phosphate-Solubilizing Microorganisms Naturally Colonizing the Rice Rhizosphere and Evaluation of Their Phosphate-Solubilizing Performance

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Abstract: Phosphate-solubilizing microorganisms (PSMs) are able to dissolve insoluble phosphorus in the soil. They play an important role in promoting soil nutrient cycling, plant growth, and dissolving insoluble phosphorus in the soil, and are therefore important microbial resources in biological fertilizers. In this study, the PSMs in rice rhizosphere soil were isolated and purified. Twenty-one phosphate-solubilizing strains were screened, including 6 bacteria and 15 fungi. Morphological analysis and molecular identification of the phosphate-solubilizing bacteria were conducted to determine the species. According to 16S rRNA molecular identification, the phosphate-solubilizing bacteria belonged to *Pseudomonas aeruginosa*, *Bacillus albus*, *Bacillus subtilis*, and *Rhizobium* sp. The phosphate-solubilizing ability of the PSMs was determined using a plate containing tricalcium phosphate (TCP). The results showed that the phosphate-solubilizing index of most of the PSMs increased first and then decreased. The phosphate-solubilizing ability of the PSMs toward the inorganic phosphate TCP, iron phosphate, aluminum phosphate, and organophosphorus of lecithin was measured using the liquid method. The results showed that the phosphate-solubilizing ability of the PSMs was 0.39mg/L–494.13mg/L, and the phosphate-solubilizing ability of TCP was the best. Among them, 13 strains had phosphate-solubilizing ability above 100 mg/L. In this study, Twenty-one strains of highly efficient phosphate-solubilizing bacteria were isolated, providing strain resources and a theoretical reference for the development of phosphate-solubilizing biological fertilizer.

Keywords: Phosphate-Solubilizing Microorganisms, Rice Rhizosphere Soil, Phosphate-Solubilizing Ability

1. Introduction

Phosphorus is an important element in organisms, being a component of ATP, phosphoprotein, nucleic acids, and other significant substances in the body. It participates in the phosphate pentose pathway, oxidative phosphorylation, and other important biological metabolic pathways [1]. However, the form of phosphorus in the soil affects the utilization rate of phosphorus in crops. There are two forms of phosphorus in soil: organic phosphorus and inorganic phosphorus. The content of organic phosphorus is mainly in the form of nucleic acids, phytin, and phospholipids. Inorganic phosphorus, which can be directly used by plants, generally exists in the form of hydrogen phosphate and dihydrogen phosphate, and the content is very small [2]. About two-fifths of the world's arable land lacks available phosphorus, and about two-thirds of China's arable land critically lacks phosphorus. Often, phosphatic fertilizer is added to fulfill the phosphate requirement of plants. However, frequent fertilization is unfavorable, as it negatively impacts soil health, and thus sustainable alternatives are being explored by scientists. With the modernization of agriculture, farmers have indiscriminately applied chemical fertilizer to pursue yield, resulting in soil compaction and degradation, micro-ecological damage, and water holding capacity decline. Concurrently, this has also caused problems such as the

decline of grain product quality and increased nitrate content [3]. Some microorganisms can harness their own growth and metabolism to improve soil, thereby promoting crop growth [4]. Therefore, screening new and efficient phosphate-solubilizing strains and expanding the resource library of phosphate-solubilizing microorganisms (PSMs) to promote the growth of plants in adverse environments are effective ways to address phosphorus deficiency in cultivated land.

PSMs can help plants directly absorb and utilize insoluble phosphorus in the soil, thereby playing an important role in reducing phosphorus fertilizer application and promoting plant growth [5, 6]. According to the substrate types, PSMs can be divided into organic PSMs and inorganic PSMs; however, there is no clear boundary between them, and some strains can use both types of phosphorus [7]. Since *Bacillus megaterium,* which has phosphorus-solubilizing ability, was first isolated from soil in 1935 [8], research on PSMs has become increasingly in-depth. At present, 36 genera and 89 types of PSMs have been identified. The PSMs isolated and identified in the rhizosphere soil of farmland crops mainly include *Bacillus.* sp., *Rhizobium.* sp., *Enterobacter.* sp., and *Micrococcus.* sp. [9]. Many experiments have shown that phosphate-solubilizing bacteria (PSBs) can promote plant growth. *Aspergillus* S29 isolated from the rhizosphere soil of mung bean by Sharma et al. [10] could dissolve insoluble phosphorus and significantly promote the growth of mung bean. Ahmad et al. [11] confirmed that PSBs could promote cotton growth in low-fertility saline-alkali soil. Gupta et al. [4] used *Pseudomonas aeruginosa* and *Bacillus subtilis* along with tri-calcium phosphate (TCP) to test the availability of nutrients and maximum nutrient uptake of nitrogen (N) (6.4%), P (15.8%), and potassium (K) (8.9%). Therefore, PSBs are considered efficient and economical soil-phosphorus activators.

Rice is one of the most important food crops, but the soil used for rice cultivation in China—especially in the subtropical regions—is generally limited by a deficiency in available phosphorus [12]. Estrada et al. [13] demonstrated that PSBs are a good strategy for promoting P solubilization and/or N use efficiency in rice plants. Most PSBs are highly specific to a host plant, and host-specificity in bacterial colonization is an important factor for successful biofertilization. A few PSBs that can promote rice growth have been reported, mainly including *Bacillus* sp. and *Agrobacterium* sp. [14]. However, the dissolved inorganic phosphorus and organic phosphorus mineralizing bacteria in the rice rhizosphere have not been comprehensively screened. In this study, the rhizosphere soil of rice was used as the soil sample, and PSMs were isolated and purified in optimized medium. Morphological analysis and molecular identification were then conducted to identify the species of PSMs. Subsequently, the plate method and liquid shake flask culture method were used to explore the phosphate-dissolving capacity of the PSMs. This study provides a theoretical basis for screening efficient PSMs, enriching the PSM resource database, and developing PSM fertilizer in China.

2. Materials and Methods

2.1. Soil Sample

The soil samples were obtained from the rhizosphere soil of a paddy field in Baitu Town (23°00′N, 112°58′E), Gaoyao District, Zhaoqing City, which is located in a subtropical, south subtropical monsoon climate with an average annual temperature of 21.2°C and average annual precipitation of 1650 mm, with precipitation mainly concentrated in April to September. Three points (each point with 1 m of spacing) were randomly selected. After determining the range of sampling points, the dead branches and leaves of plants on the surface of the soil were removed. The soil layer at 15 cm depth was collected at 2 cm from the main stem of the rice plants using a sterilized soil drill with a diameter of 3.5 cm. The soil was removed and mixed in a sterilized bag immediately. The total phosphorus, potassium, and nitrogen and the temperature and humidity of the soil were measured using a soil measuring instrument, and the physicochemical properties of the soil are shown below (Table 1).

Table 1. *Physical and chemical properties of the soil.*

Total N content (mg/kg)	Total P content (mg/kg)	Total K content (mg/kg)	Temperature (°C)	Humidity (%)
38	44	117	29.4	88
51	55	147	31.4	98
55	60	157	30.6	99

2.2. Isolation and Preliminary Screening of PSMs

Nine grams of soil and 90 mL of sterile water were added to a 250 mL conical flask containing 3–5 glass beads and shaken for 30 min in a constant temperature shaker at 200 r/min. Soil suspension concentrations of 10^{-3} to 10^{-5} were prepared using the 10-time dilution method. A soil suspension (0.1 mL) was taken and spread on 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 $FeSO_4.7H_2O$, 0.03 mg $MnSO_4 \cdot H_2O$, 5 g $Ca_3(PO_4)_2$, and 20 g agar. As an alternative to TCP, aluminum phosphate (AP) or iron phosphate (IP) was added to the TPM liquid medium at a concentration of 5 g/L. 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 and was cultured at 28°C for 3 days to observe colony growth. Single colonies with different shapes and large transparent circles around them were picked and inoculated on the corresponding blank plate medium and then purified by the streaking method. After repeating this process 4–5

times, they were inoculated into LB slant medium and stored at 4°C.

2.3. Identification of PSMs

The strains were selected and inoculated in the center of the plate culture medium using the plate spot inoculation method. A single colony was isolated, and the shape, size, and color of the colony were observed and recorded. The cell morphology and Gram-staining reaction were observed under an optical microscope. Preliminarily identification of the strains followed Berger's manual.

The genomic DNA of the PSBs was extracted according to the requirements of the DNA extraction kit (Suzhou Jinweizhi Biotechnology Co., Ltd.). The extracted DNA was amplified by PCR using the bacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and R1387 (5'-GGGCGGGWGTGTACAAGGC-3'). The amplified products were sent to Suzhou Jinweizhi Biotechnology Company for sequencing. The sequencing results were submitted to NCBI, and the accession number was obtained. The Blast program was used for comparison with the sequences on the GenBank database, and sequences with high homology were selected. A phylogenetic tree was constructed using MEGA11 to determine the species of the strains.

2.4. Assessment of the TCP-Dissolving Ability on Plate Medium

The purified strain was inoculated in the center of TPM plate medium with $Ca_3(PO_4)_2$ as the insoluble phosphorus source, and the PSBs and phosphate-solubilizing fungi (PSFs) were respectively placed in incubators at 37°C and 28°C for cultivation. The dissolved phosphorus circle diameter (D) and colony diameter (d) were measured on days 3, 5, and 7, and the ratio between them was calculated (HC=D/d). This ratio constituted the phosphate-solubilizing index.

Assay of phosphorus released from different phosphorus sources.

To estimate the differences in released phosphorus, all the preliminarily screened PSBs were inoculated into 20 mL LB liquid medium and cultured at 28°C and 180 r/min for 12 h. One milliliter of bacterial liquid was added to 30 mL YM and TPM liquid medium, and as an alternative to TCP, aluminum phosphate (AP) or iron phosphate (IP) was added to the TPM liquid medium at a concentration of 5 g/L and cultivated at 28°C with 180 r/min shaking for 3 days. For the PSF fermentation broth, the preliminarily screened PSFs were inoculated into 200 mL YM and TPM liquid medium and then cultured at 28°C and 180 r/min for 7 days. Two milliliters of fermentation broth were then centrifuged at a speed of 12000 r/min for 5 min, and 0.5 mL supernatant was used to determine the soluble phosphorus content by the molybdenum antimony colorimetric method (three replicates per strain).

2.5. Experimental Data Processing

The soluble phosphorus was determined by the molybdenum antimony anti-colorimetric method and was sorted, analyzed, and plotted in Excel 2016 (Microsoft Corp., Redmond, WA, USA). SPSS 21.0 (IBM Corp., Armonk, NY, USA) was used for single-factor analysis of variance (significance level of 0.05).

3. Results

Isolation and Morphological Identification of PSMs

Through the isolation and purification of PSMs in the collected rice rhizosphere soil samples, a total of 21 strains exhibiting good growth and obvious phosphate-solubilizing circles were preliminarily isolated, 6 of which were identified as PSBs and 15 of which were identified as PSFs after colony morphology identification. Among them, five bacteria were isolated from TCP medium and named CAP-1~CAP-5. One bacterium was isolated from lecithin medium and named LP-1. Ten fungi were isolated from lecithin medium and named LP-2~LP10. Three fungi were isolated from IP medium and named FEP-1–FEP-4. Four fungi were isolated from AP medium and named ALP-1–ALP-4. The morphological characteristics of the PSMs are indicated in Tables 2 and 3.

Table 2. *Morphological characteristics of the phosphate-solubilizing bacteria.*

Strain name	Morphological characteristics			Gram straining
	Color	Texture	Shape	
TCP medium				
CAP-1	Cinnamon	Sticky wet and smelly, easy to pick up	Round with smooth margins	Negative
CAP-2	Milky white	Dry and smelly, difficult to pick up	Round with smooth margins,	Positive
CAP-3	Milky white,	Sticky wet and smelly, easy to pick up	Round with smooth margins	Positive
CAP-4	White	Sticky wet and smelly, easy to pick up	Irregular with smooth margins, small and flat.	Positive
CAP-5	White	Smooth and dry, difficult to pick up	Irregular with toothy margins, small and raised,	Negative
Lecithin medium				
LP-1	Red	Smooth, viscous, and smelly	Round with smooth margins	Positive

Table 3. *Morphological characteristics of the phosphate-solubilizing fungi.*

Strain	Morphological characteristics		
	Color	Texture	Shape
Lecithin medium			
LP-2	Milky white	Dry and flocculent, difficult to pick up	Round, with toothy margins
LP-3	Pink	Sticky, wet and shiny, difficult to pick up	Round with smooth margins

Strain	Morphological characteristics		
	Color	Texture	Shape
LP-4	Milky white	Dry and flocculent, easy to pick up	Irregular with toothy margins and raised
LP-5	White	Dry and difficult to pick up	Irregular with smooth margins and raised
LP-6	The edge is white and the center is black	Dry and difficult to pick up	Irregular with toothy margins
LP-7	The edge is white and the center is dark green	Fuzzy and difficult to pick up	Round with scattered margins and raised
LP-8	The edge is white and the center is gray	Dry and difficult to pick up	Irregular with toothy margins
LP-10	The edge is white and the center is black	Fuzzy and difficult to pick up	Round with smooth margins and raised
IP medium			
FEP-1	Yellow	Fuzzy and easy to pick up	Round with smooth margins and raised
FEP-3	The edge is white and the center is green	Fuzzy and easy to pick up	Round
FEP-4	Orange	Dry and difficult to pick up	Round with smooth margins and raised
AP medium			
ALP-1	White	Dry and flocculent, difficult to pick up	Round with smooth margins and raised
ALP-2	Yellowish white	Dry and flocculent, difficult to pick up	Round with smooth margins
ALP-3	Cinnamon	Dry and easy to pick up	Irregular with smooth margins
ALP-4	Breen	Fuzzy and easy to pick up	Round with smooth margins and raised

Table 4. *Identification results of phosphate-solubilizing bacteria.*

strains	Accession number	Number of bases (bp)	Similar strain		
			Nearest type strain	Accession number	Similarity (%)
CAP-1	ON376807	1473	Pseudomonas aeruginosa	JQ659549.1	99
CAP-2	ON080902	1348	Bacillus albus	MK993460.1	99
CAP-3	ON376809	1452	Bacillus subtilis	MN696247.1	99
CAP-4	ON080903	1430	Rhizobium sp.	OM363673.1	99

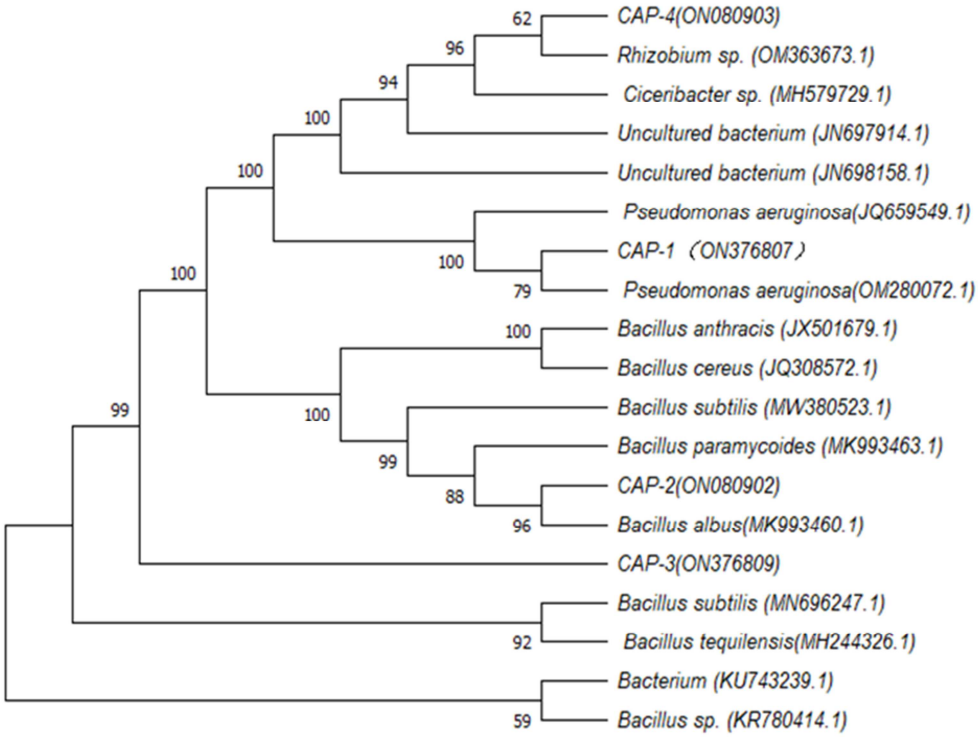

Figure 1. *Phylogenetic tree of the phosphate-solubilizing bacteria.*

3.1. Molecular Identification of PSBs

Polymerase chain reaction (PCR) was performed on the genomic DNA of the PSBs after DNA extraction. The obtained sequences of the PSBs were imported into NCBI Blast and compared with the published 16S rRNA gene sequences of similar strains, following which the sequences with highly homologous expression were downloaded (Table 4). The result of the Blast alignment showed that there was no sequence that was 100% identical to the CAP-1, CAP-2, CAP-3, and CAP-4 strains in the GenBank database. The phylogenetic tree indicated that CAP-1, CAP-2, CAP-3, and CAP-4 were located along the same branch as *Pseudomonas aeruginosa, Bacillus albus, Bacillus subtilis,* and *Rhizobium* sp., respectively, with 99% homology (Figure 1).

3.2. Phosphate-Solubilizing Ability Determined by the Plate Method

All the isolated PSMs were inoculated in the center of inorganic phosphorus medium with tricalcium phosphate as the sole phosphorus source. The D and d values of the strains were measured on the third, fifth, and seventh day. The HC value was calculated according to HC = D/d. The obtained HC values are shown in Figure 2. It can be seen that after 5 days of cultivation, the HC values of the isolated strains were in the order of CAP-1>FEP-3>ALP-2>ALP-3>CAP-4>FEP-4>ALP-1>FEP-1>ALP-4>CAP-2>CAP-5>CAP-3. Among them, the HC value of CAP-1 was more than 2.5, which confirmed that CAP-1 grew well on the medium with tricalcium phosphate as the phosphorus source. Transparent circles of CAP-3, CAP-4, and CAP-5 were not observed until the fifth day. The HC value of these increased from the fifth day to the seventh day. ALP-4, whose mycelia grew vigorously, was a mould. The mycelia of ALP-4 dispersed easily and fell onto the medium during each observation, and so it was difficult to observe a single colony at the later stage. Therefore, the HC value on the seventh day could not be determined. In addition, except for CAP-2, CAP-4, CAP-5, FEP-1, and FEP-4, the variation trend in the HC values of the other phosphate-solubilizing strains was as follows: in the early stage, the HC value showed an upward trend, while at the later stage the HC value decreased, and the maximum value appeared on the fifth day.

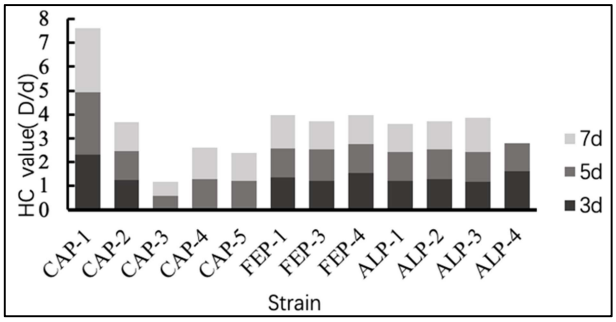

Figure 2. *Hydrolysis circle of the isolated strains.*

3.3. Phosphate-Solubilizing Ability of PSMs Using the Liquid Method

Table 5. *Phosphate solubilization of PSMs in liquid medium contained tricalcium phosphate (TCP), aluminum phosphate (AP), iron phosphate (IP) and lecithin.*

Strains	the ability of solubilizing phosphrus (mg/L)			
	TCP	AP	IP	Lecithin
CAP-1	370.26±0.19d	42.43±0.19ef	7.46±0.11g	0.39±0.00d
CAP-2	78.49±0.00p	96.56±6.31c	4.31±0.11i	—
CAP-3	155.95±0.59i	—	10.61±0.19e	—
CAP-4	5.46±0.11v	1.93±1.93n	10.03±0.19f	—
CAP-5	127.34±0.11 l	—	3.28±0.03j	—
LP-1	200.43±0.11g	6.24±0.11m	6.49±0.11h	0.39±0.00d
LP-2	71.22±0.11q	48.60±0.19d	309.45±0.11a	4.44±0.00b
LP-3	48.34±0.11s	17.42±0.11k	—	—
LP-4	158.33±0.19h	1.35±0.33n	—	—
LP-5	140.84±0.11j	12.28±0.11 l	—	1.73±0.01c
LP-6	87.68±0.22n	40.70±0.33fg	—	—
LP-7	300.90±0.11e	42.74±0.22ef	25.46±0.33d	—
LP-8	287.66±0.22f	26.42±0.19j	—	—
LP-10	110.76±0.11m	44.74±0.00e	—	17.48±0.11a
FEP-1	494.13±0.11a	38.83±0.11gh	—	—
FEP-3	7.84±0.11t	30.08±0.19i	—	—
FEP-4	406.65±0.22c	37.93±0.11gh	—	—
ALP-1	6.94±0.19u	256.87±0.88a	—	—
ALP-2	80.35±0.11o	44.29±0.22e	26.93±0.11c	—
ALP-3	138.21±0.11k	167.78±0.00b	114.61±0.29b	—
ALP-4	429.66±0.00b	37.54±0.29h	—	—

After the same column of data, the lowercase letters showed that the amount of dissolved phosphorus of each strain reached 5 % significant difference ($p < 0.05$).

To determine the phosphate-solubilizing ability of different strains for different types of phosphorus, the pure cultures were further screened in a liquid medium containing TCP, AP, IP, and lecithin as insoluble P sources at a concentration of 5 g/L. The results are shown in Table 5. The phosphate-solubilizing ability of the strains varied from 0.39 mg/L to 494.13 mg/L. All of the isolated strains had the ability to dissolve TCP, and the ability was 6.94 mg /L–494.13 mg/L. Most of the strains could dissolve AP, and the ability was 1.35 mg/L–256.87 mg/L. Ten strains could dissolve IP, and the capacity was 3.28 mg/L–309.45 mg/L. There were five strains that exhibited the ability to dissolve organic phosphorus lecithin, with amounts of 0.39 mg/L–17.48 mg/L. Overall, the strains isolated in this study exhibited the best dissolving capacity for tricalcium phosphate, and 13 strains had a dissolving capacity higher than 100 mg/L. There were three strains that could dissolve both inorganic phosphorus and organic phosphorus, namely,

CAP-1, LP-1, and LP-2, all of which had high dissolving ability toward tricalcium phosphate or ferric phosphate.

4. Discussion

PSMs can dissolve insoluble or insoluble phosphorus in the soil, such that this unused phosphorus can be directly absorbed and utilized by plants, thereby improving the utilization rate of phosphorus in the soil. Studying such microorganisms can therefore promote the development of microbial fertilizers with agricultural applications [15, 16]. The isolation and identification of PSBs in plant rhizosphere soil has become an increasingly relevant research topic. Chen et al. [17] isolated 36 strains of PSBs using TCP medium. Chawngthu et al. isolated four PSBs from current jhum fields and different-aged fallow soil based on their ability to solubilize TCP in Pikovskaya agar medium 18]. The preliminary screening method for PSBs was mainly based their ability to dissolve $Ca_3(PO_4)_2$ on indicator plates. However, inorganic phosphorus in the soil binds with calcium, iron, and aluminum. Additionally, up to 30–50% of the phosphorus in the soil is in the form of organophosphorus [19]. The co-screening of different organic and inorganic phosphorus has rarely been done. To efficiently isolate the PSMs, four phosphorus sources (TCP, AP, IP, and lecithin) were used as substrates. A total of 21 PSMs were isolated from rice rhizosphere soil, 6 of which were bacteria and 15 of which were fungi. According to 16S rRNA molecular identification, the four PSBs were *P. aeruginosa, B. albus, B. subtilis,* and *Rhizobium* sp. There are presently few studies on the role of *P. aeruginosa* in promoting phosphorus absorption, and the available studies on this species have mainly focused on improving crop disease resistance and stability [20–22]. Phosphorus-solubilizing *Bacillus* is an important class of PSB. *Bacillus* has strong vitality, and the spores formed during stress can be transformed into nutritional cells under environmental conditions, which plays an important role in soil improvement. *Bacillus megaterium* screened from calcareous rhizosphere soils by Liu et al. [23] and *Bacillus thuringiensis* screened by Sauka et al. [24] both exhibited a strong ability to promote the transformation of soluble phosphorus sources. Phosphorus-solubilizing rhizobia are nitrogen-fixing bacteria that can promote the absorption of phosphorus sources. Currently, about 60 types of PSFs have been identified, and they mainly belong to *Alternaria, Aspergillus, Fusarium, Penicillium, Talaromyces,* and *Trichoderma* [25, 26]. In the present study, only a general morphological observation of the fungi was made, and the molecular identification of the fungi and the potential use of the isolates for further study are discussed.

The plate method was used to determine the phosphorus-solubilizing ability of the PSMs isolated in this experiment. The results showed that the HC of different PSMs varied, and the peaks of the HC values differed, with the HC values of CAP-2, CAP-4, and CAP-5 peaking around day 7. The variation in HC is related to the mechanism used by the PSBs. At present, there are many explanations for the phosphate-solubilizing mechanism of PSMs, such as the production of organic acids, inorganic acids, or phosphate-solubilizing enzymes, all of which have the common function of altering the pH value of the living environment of the PSBs [27]. In the present study, during the growth process of the PSBs, the HC value increased first and then decreased. This may be related to the metabolic mechanism of the strain. The initial extreme phosphorus deficiency environment promotes the secretion of organic acids by the strain, which improve its phosphorus solubility. At the later stage, when the soluble phosphorus in the environment reaches a certain content, a negative feedback inhibition is formed, and the phosphorus-solubilizing ability of PSMs is weakened.

The formation of phosphorus-soluble halos by colonies on flat plates containing insoluble phosphorus sources can be used as the main method for screening PSMs, but some experimental studies have proved that some colonies that cannot produce visible phosphorus-soluble rings on the plates still have high phosphorus-soluble capacity in liquid medium. In addition, in acidic soil, the fixation of phosphate by aluminium and iron is very pronounced, which limits the availability of soluble phosphate for plant uptake [28]. The phosphate-solubilizing ability of 21 PSMs was determined by liquid media containing $FePO_4 \cdot 2H_2O$, $AlPO_3$, $Ca_3(PO_4)_2$, and lecithin. The phosphate-solubilizing ability of inorganic phosphorus was 494.13 mg/L–1.93 mg/L. Overall, the phosphate-solubilizing ability of TCP was the strongest, which was consistent with the results of Liu et al. [29] and Jiang et al. [30]. The PSMs occurring naturally in soil include phosphate-solubilizing bacteria and fungi. However, PSFs have a stronger capacity to dissolve phosphate rock powder than PSBs. Yang et al. conducted a comparative study on the phosphate-solubilizing ability of some bacteria and fungi and found that the capacity for bacteria to dissolve phosphate rock powder ranged from 26.92 to 43.34 mg/L, whereas the capacity of most fungi was 59.64–145.36 mg/L [27]. Also, in this study, the phosphorus-dissolving ability of the fungi was better than that of the bacteria, and so PSFs have greater potential for development and utilization. This provides an experimental foundation for the subsequent development of phosphorus-deposing microbial fertilizers.

5. Conclusion

PSMs can dissolve insoluble phosphorus in the soil such that this insoluble phosphorus can be directly absorbed and utilized by plants, thereby improving the utilization rate of phosphorus in the soil. Therefore, the study of such microorganisms can promote the development of microbial fertilizers in agricultural applications. In this study, a total of 21 PSMs were screened in the rhizosphere soil of rice, including 6 bacteria and 15 fungi. The PSMs belonged to *P. aeruginosa, B. albus, B. subtilis,* and *Rhizobium* sp. The strains dissolved different insoluble phosphorus (TCP, AP, IP) as substrates, and their phosphorus-dissolving capacity differed. Among the three types of insoluble inorganic phosphorus, the strains had the strongest solubility towards

TCP. Furthermore, based on the analysis of the PSI and phosphorus-dissolving amount in liquid medium, the phosphorus-dissolving ability of the phosphorus-dissolving fungi was better than that of the phosphorus-dissolving bacteria. Phosphate-solubilizing fungi have greater potential for development and utilization, providing an experimental foundation for the development of phosphate-solubilizing microbial fertilizers. This research has significance for enriching the resource database of PSBs and promoting the application of microbial fertilizers in agriculture.

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