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# 锥栗外生菌根际细菌对难溶性无机磷风化作用及其耦联碳酸盐形成研究

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QIAN Lu, HE Zhenying, HE Gang, JI Xiaohong, OUYANG Jianping, CHEN Ye, SUN Qibiao. Bacteria isolated from the ectomycorrhizosphere of *Castanea henryi* weather insoluble inorganic phosphates and simultaneously form carbonates[J]. Acta Microbiologica Sinica, 2024, 64(6): 2042-2056.

**摘　要：**【目的】外生菌根真菌在菌根际塑造特异的细菌群落是从土壤中活化和获取不溶性矿质元素的一个重要途径。本研究旨在探究锥栗外生菌根际解磷细菌的组成及其解磷特性。【方法】通过溶磷圈法从锥栗菌根际分离解磷细菌，并运用 16S rRNA 基因测序进行菌株鉴定，采用液体发酵方式比较分析解磷菌对磷酸三钙和磷灰石的降解能力，利用扫描电镜和 X 射线衍射仪对风化降解产物微观形貌和晶体结构进行分析。【结果】分离获得 5 株高效解磷菌，经 16S rRNA 基因测序和比对分析，LSCh1、LSCh2 和 LSCh5 菌株被鉴定为拉塔伯克霍尔德菌(*Burkholderia lata*)，LSCh3 和 LSCh4 被鉴定为副伯克霍尔德菌属(*Paraburkholderia* sp.)。解磷实验结果表明不同菌株对磷酸三钙的降解能力依次为 LSCh3 (556.94 mg/L)>LSCh2 (206.91 mg/L)>LSCh1 (170.83 mg/L)>LSCh5 (55.16 mg/L)>LSCh4 (14.21 mg/L)，对磷灰石的降解能力依次为 LSCh2 (51.33 mg/L)>LSCh1 (43.51 mg/L)>LSCh3 (40.99 mg/L)>LSCh5 (1.11 mg/L)>LSCh4 (1.00 mg/L)。【结论】LSCh3 菌株不仅对磷酸三钙和磷灰石都具有较好的降解能力，而且能够诱导碳酸盐的形成。该菌株是一株具有重要潜在应用价值的

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植物促生菌，可用于促进植物磷营养和改善土壤质量。

**关键词：**锥栗；外生菌根；解磷细菌；磷灰石；碳酸盐

# Bacteria isolated from the ectomycorrhizosphere of *Castanea henryi* weather insoluble inorganic phosphates and simultaneously form carbonates

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**Abstract: [Objective]** Ectomycorrhizal fungi can shape the bacterial community in the ectomycorrhizosphere, which represents an important way to mobilize and acquire insoluble mineral elements from the soil. This study aims to investigate the composition and phosphate-solubilizing characteristics of the dominant phosphate-solubilizing bacteria (PSB) in the ectomycorrhizosphere of *Castanea henryi*. **[Methods]** The transparent halo method was employed to isolate PSB from the ectomycorrhizosphere of *C. henryi*. The strains were identified by 16S rRNA gene sequencing. The degradation abilities of the strains for tricalcium phosphate (TCP) and apatite were studied by liquid fermentation. A scanning electron microscope and a X-ray diffractometer were used to observe the appearance and crystal structures of the degradation products. **[Results]** Five highly efficient strains of PSB were isolated from the ectomycorrhizosphere of *C. henryi*. Strains LSCh1, LSCh2, and LSCh5 were identified as *Burkholderia lata*, and strains LSCh3 and LSCh4 were identified as *Paraburkholderia* sp. The degradation abilities of the strains for TCP followed the trend of LSCh3 (556.94 mg/L)>LSCh2 (206.91 mg/L)>LSCh1 (170.83 mg/L)>LSCh5 (55.16 mg/L)> LSCh4 (14.21 mg/L). The degradation abilities of the strains for apatite were in a descending order of LSCh2 (51.33 mg/L)>LSCh1 (43.51 mg/L)>LSCh3 (40.99 mg/L)>LSCh5 (1.11 mg/L)> LSCh4 (1.00 mg/L). **[Conclusion]** Strain LSCh3 showed good performance in degrading both TCP and apatite and induced the formation of carbonates. It is a plant growth-promoting bacterium with potential application values in improving the phosphorus nutrients of plants and the quality of soil.

**Keywords:** *Castanea henryi*; ectomycorrhiza; phosphate-solubilizing bacteria; apatite; carbonate

Phosphorus (P) is one of the essential macro-mineral elements necessary for the growth and development of plants. It is an important component of cell membranes and nucleic acids and is involved in almost all metabolic processes of plants, such as photosynthesis, cell division, signal transmission, respiration, energy production, and macromolecular biosynthesis[1-2]. Although the total amount of P in the soil is abundant, the content of bioavailable P is often in a deficiency state. Only about 0.1% of P (such as $HPO_4^{2-}$ and $H_2PO_4^-$) can be absorbed and utilized directly by plants. Most P is easily passivated by $Ca^{2+}$, $Fe^{3+}$, $Al^{3+}$, and soil aggregates or is in the form of organophosphorus and P-containing minerals[3-6].

More than 80% of terrestrial plants can form mycorrhizal associations with soil fungi, and the fungal symbionts play an extremely important role in promoting plan uptake of mineral nutrients, particularly P, nitrogen, and potassium[7]. As one of the most important types of mycorrhizal fungi, ectomycorrhizal (ECM) fungi play a crucial role in the growth of forest trees. The reciprocal symbiosis between forest trees and ECM fungi is one of the most widespread and successful interactions in terrestrial ecosystems. The fundamental groups and dominant tree species (accounting for about 2% of plant species) in forest ecosystems, including *Pinaceae*, *Fagaceae*, *Betulaceae*, *Salicyaceae*, and *Dipteraceae*, can form ectomycorrhizas[8]. ECM fungi obtain mineral nutrients through mycelial absorption, enzymatic hydrolysis, and bioweathering and then transport them to symbiotic plants. For example, more than 15% of P required for plant growth is provided by ECM fungi[9-10]. Bioweathering is one of the important ways for ECM fungi to obtain soil P, mainly through the secretion of protons and low molecular weight organic acids to trigger acidification or complexation to weather soil minerals[11-13]. Smits et al.[14] studied the weathering efficiency of *Paxillus involutus* on apatite under P deficiency using the microcosmic method. The result showed that the colonization of *P. involutus* increased the release of P from apatite by three times compared to the control. Wallander[15] studied the weathering effect of three ECM fungi on apatite and the growth-promoting effect on *Pinus sylvestris* through pot experiments. It showed that the weathering rate of apatite by fungi was 0.3%–0.9% after 6 months of cultivation. However, many studies have found significant differences in the mineral weathering capacity among different ECM fungi[16-20]. Under the background of increasing atmospheric $CO_2$ concentration, nutrient deficiency stress caused by the intensification of plant photosynthesis has increased[21], resulting in increased demand for ECM fungi. Furthermore, in the process of long-term symbiotic evolution, the ability of ECM fungi to use complex organic matter in the soil is constantly weakened due to the loss of genes related to the degradation of complex organic substances[22-23]. Due to these factors, it is essential for ECM fungi to improve the nutrient supply to plants in exchange for the carbon source they themselves require. Therefore, it is particularly important to explore the way ECM fungi acquire soil mineral elements (e.g. P).

The interaction between ECM fungi and bacteria is a common phenomenon in natural forest ecosystems, and its important role in promoting soil mineral weathering and plant nutrition is receiving increasing attention[24-26]. Nicolitch et al.[27] compared and analyzed the mineral weathering capacity of bacteria isolated from the ectomycorrhizosphere of *Fagus sylvatica* and the adjacent bulk soil. The results showed that the ectomycorrhizosphere has a significant enrichment effect on bacteria (mainly *Burkholderia* and *Collimonas*) with high mineral weathering ability. This shows that the lower the soil bioavailable P content, the more specific bacterial taxa related to inorganic nutrient mobilization are largely and exclusively enriched in the ectomycorrhizosphere[27]. Fontaine et al.[28] obtained similar results that there are large number of phosphate-solubilizing bacteria (PSB) in the ectomycorrhizosphere of *Picea glauca*, and

*Burkholderia* showed a particularly prominent ability to weather fluorapatite. Sun et al.[29] also concluded that ECM fungi can enrich bacteria with high mineral weathering potential and promote apatite weathering through the mesh bag *in situ* culture method. These suggest that the formation of specific PSB could be one of the essential ways for ECM fungi to promote P nutrition of plants under P-deficient conditions.

The taxa of rhizospheric PSB formed by different plant ectomycorrhiza are diverse. Furthermore, it remains unclear which specific group of species is enriched with highly effective mineral weathering bacteria by ECM fungi. Therefore, studying the composition and ability of PSB in the ectomycorrhizosphere is helpful to understand the interaction between ECM fungi and bacteria and the use of functional bacterial resources to promote plant growth. In this study, *Castanea henryi* (*Fagaceae*) in Lushan National Nature Reserve was selected as the research object. The main soil type in this reserve is acidic red soil with a very low content of bioavailable P. The PSB of the ectomycorrhizosphere of *C. henryi* were isolated and their phosphate- solubilization ability was analyzed. This will provide a theoretical basis for solving the problem of low P and soil fertility in acidic-red soils.

# 1 Materials and Methods

## 1.1 Isolation and identification of PSB

The ectomycorrhizospheric soil of *C. henryi* was collected from Lushan National Nature Reserve, and PSB were isolated by the phosphate-solubilizing halo method. After repeated rescreening, five strains with high phosphate-solubilizing ability were finally obtained and used for this study. The obtained PSB strains were inoculated in Luria-Bertani (LB) liquid medium, and cultured at 30 ℃ and 180 r/min for 2 days. Then the fermentation broth was centrifuged at 12 000 r/min for 2 min to collect bacteria. DNA (deoxyribonucleic acid) was extracted using the Bacterial Genomic DNA Rapid Extraction Kit (Sangon Biotech Co., Ltd., Shanghai, Chian), and 16S rRNA gene was amplified using primers 27F (5′-AGAGTTTGA TCMTGTGGCTCAG-3′) and 1492R (5′-CGGTT ACCTTGTTACGACTT-3′)[30]. The PCR reaction system (25 μL): 2×Phanta Max Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China) 12.5 μL, primer 27F (10 μmol/L) 1 μL, primer 1492R (10 μmol/L) 1 μL, DNA template 0.1 μL, $ddH_2O$ 10.4 μL. Reaction procedure: predenaturation at 95 ℃ for 3 min, denaturation at 95 ℃ for 15 s, annealing at 56 ℃ for 15 s, extension at 72 ℃ for 90 s, 30 cycles; at 72 ℃ for 5 min. The PCR products were detected by 1% agarose gel electrophoresis and sent to Sangon Biotech Co., Ltd. for sequencing. DNA base quality obtained by sequencing was assessed using SnapGene Viewer 6.2.1 software, pairwise sequencing results were spliced using the SeqMan tool in Lasergene 7.1 software to obtain a complete 16S rRNA gene sequence, and BLAST (Basic Local Alignment Search Tool) program was performed in GenBank, and finally MEGA 11.0 software was used for sequence alignment and phylogenetic tree construction.

The NBRIP medium was use as the isolation medium (g/L): glucose 10.00, $(NH_4)_2SO_4$ 0.50, $MgSO_4·7H_2O$ 0.30, $MnSO_4$ 0.03, KCl 0.30, $FeSO_4$ 0.03, NaCl 0.30, $Ca_3(PO_4)_2$ (tricalcium phosphate, TCP) 5.00, agar 20.00, pH 7.0–7.2[31].

## 1.2 Assay of the capacity of bacteria to degrade TCP and apatite

Five bacterial strains were each inoculated onto solid LB medium and cultured at 37 ℃ for 48 h. Subsequently, a ring of bacterial thalli was picked and inoculated in NBRIP and modified NBRIP (TCP was replaced with the equivalent apatite powder) liquid media (without soluble phosphate) and cultured at 30 ℃, 150 r/min for 10 days. Each treatment was carried out in triplicate. The pH and soluble P content of the culture solution were measured every 2 days using the molybdenum-antimony resistance colorimetry method[32].

Analytical pure TCP was purchased from Sinopharm Chemical Reagent Co., Ltd. Apatite

powder (75–150 μm) consists mainly of fluoropapatite, hydroxyapatite and a small amount of phlogopite. X-ray fluorescence analysis showed that the main elements were O 77.35%, Ca 11.10%, P 6.09%, F 1.58%, Mg 1.36%, Si 1.33%, Al 0.35%, Fe 0.29%, Sr 0.23%, K 0.19%, S 0.06%, I 0.06% and Y 0.01%.

### 1.3 Detection of mineral components in precipitates

After cultivation, the precipitates were collected and purified three times each with deionized water and anhydrous ethanol. Finally, the precipitates were dried in an oven at 60 ℃. A scanning electron microscope (SEM) VEGA II LSU (TESCAN, Czech Republic) equipped with an energy dispersive X-ray spectrometer (EDS) Inca X-act (Oxford, UK) was used to observe and analyze the micromorphology and elemental composition of the precipitates. The structure and composition of the precipitates were determined with an X-ray diffractometer (XRD) D8 ADVANCE (Bruker, Germany) using Cu-Kα radiation with a voltage of 35 kV.

### 1.4 Data processing

The data are expressed in the form: mean ± standard deviation (bars) using the three replicates. Pearson correlation analysis between pH and P concentration was performed in R version 4.1.3 using the ggplot2 package.

## 2 Results and Discussion

### 2.1 Molecular identification of PSB

Secretion of gluconic acid is one of the essential ways for bacteria to dissolve insoluble phosphates. Therefore, it is an effective mean to identify PSB by identifying the key genes of its synthesis. For example, pyrroloquinoline quinine is an important cofactor of glucose dehydrogenase in the production of gluconic acid, and its synthetic genes (*pqqA*, *pqqB*, *pqqC*, *pqqD*, and *pqqE*) are widely used for PSB detection[33]. However, determining the formation of phosphate-solubilizing halo is still the most direct and effective way for screening PSB. PSB tend to weaken or even lose phosphate solubilization ability during the cultivation process[34]. Therefore, in this study, five PSB strains (LSCh1, LSCh2, LSCh3, LSCh4, and LSCh5) with strong and stable phosphate solubilization ability were isolated from the ectomycorrhizosphere of *C. henryi* by multiple rescreening. Subsequently, their 16S rRNA gene sequences were obtained by sequencing and submitted to GenBank (accession numbers: OQ519686, OQ519687, OQ519694, OQ519695, and OQ519688, respectively). Phylogenetic analysis showed that strains LSCh1, LSCh2 and LSCh5 belonged to *Burkholderia lata*, while LSCh3 and LSCh4 belonged to *Paraburkholderia* sp. (Figure 1). To date, *Burkholderia* which belongs to *Pseudomonadaceae* are considered to be the most efficient mineral weathering bacteria in the ectomycorrhizosphere[27-28,35-37]. In this study, the high-efficiency phosphate-solubilizing strains isolated from the ectomycorrhizosphere of *C. henryi* belonged to *Burkholderia* and *Paraburkholderia*. It further indicated that bacteria in these taxonomic groups play an important role in mobilizing insoluble phosphates in the ectomycorrhizosphere. *Paraburkholderia* was newly separated from the genus *Burkholderia* based on molecular signatures and phylogenomic analysis by Sawana et al.[38], and is primarily an environmental species with plant growth promotion effects. Therefore, a large proportion of previously reported *Burkholderia* strains with high weathering potential actually belong to *Paraberkholderia*, indicating that as bacterial taxonomy continues to evolves, the understanding of highly efficient mineral weathering bacteria in the ectomycorrhizosphere will also bring new changes.

### 2.2 Changes in pH during phosphate solubilization

Acidification is one of the most important ways for PSB to promote the dissolution of insoluble inorganic phosphates, such as secreted gluconic acid, acetic acid, lactic acid, oxalic acid, malic acid, and other low molecular weight organic acids, which not only acts directly to

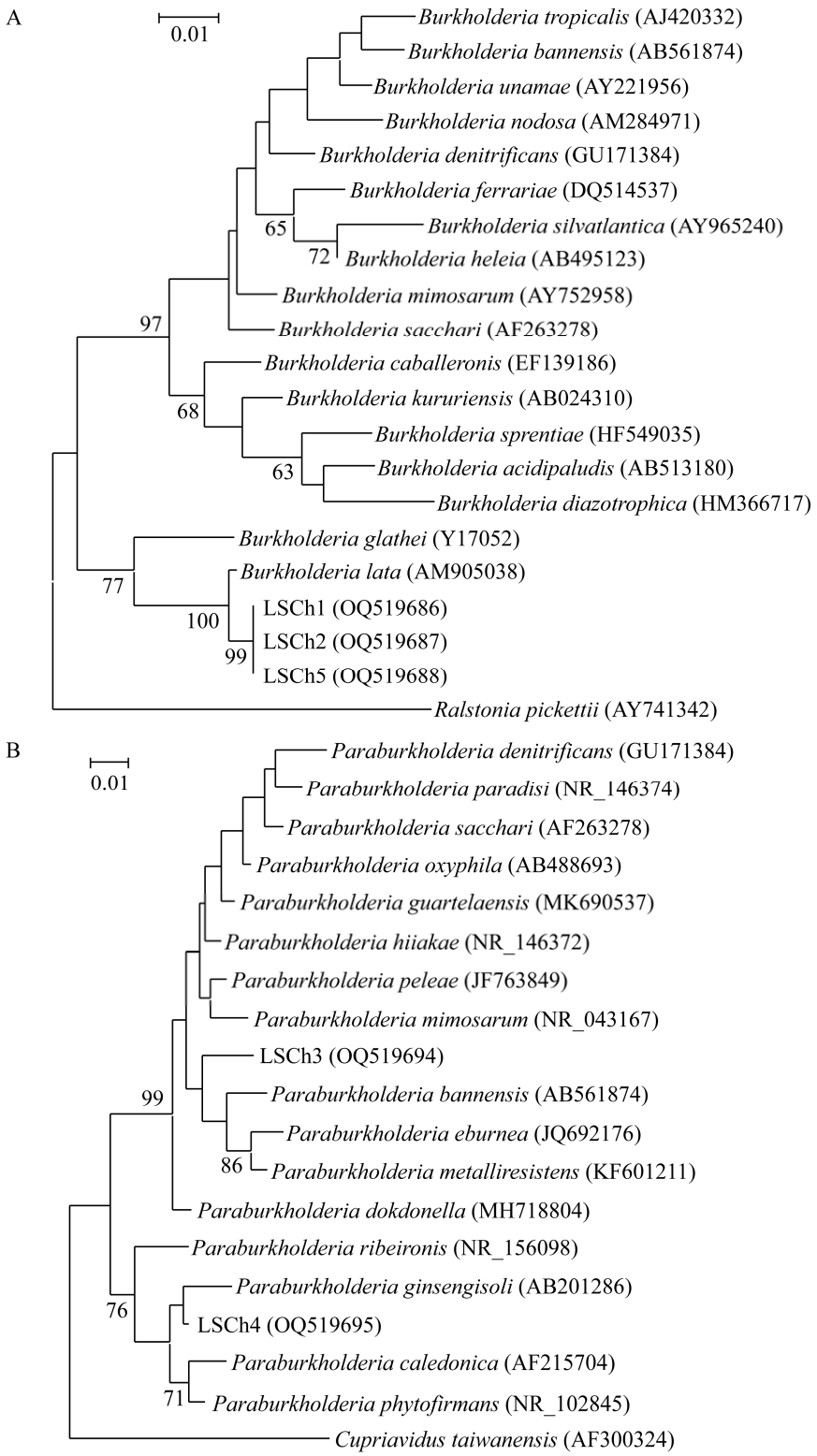


Figure 1 Phylogenetic tree constructed by maximum likelihood method using MEGA 11.0 software based on 16S rRNA gene sequences of isolated strains. A: Phylogenetic tree of *Burkholderia*. B: Phylogenetic tree of *Paraburkholderia*.

dissolve the fixed phosphates, but can also hinder the reimmobilization of P by $Fe^{3+}$, $Al^{3+}$ and other ions[39-41]. Figure 2 shows the change in pH of liquid media during different strains degrading phosphates. In the initial degradation phase of TCP, the pH of the medium decreased significantly. The pH of the medium fluctuated between 4.27 and 7.96 during cultivation, with the pH trending to initially decrease and then increase again. During apatite degradation, the pH of the medium was between 3.31 and 6.92. These further indicate that acidification is an important way for PSB to mobilize insoluble phosphorus sources.

## 2.3 Different ability of strains to degrade different insoluble phosphates

Bashan et al.[42] suggested that TCP as a single insoluble phosphate is not suitable for screening PSB and that the determination should be combined with the characteristics of the isolation environment and other corresponding insoluble P sources. In this study, TCP was used as the insoluble phosphate for the first screening and apatite as the second phosphate for the re-screening and conformation. However, five PSB strains isolated by double screening showed different solubilization abilities toward different phosphates (Figure 3). The total P released from

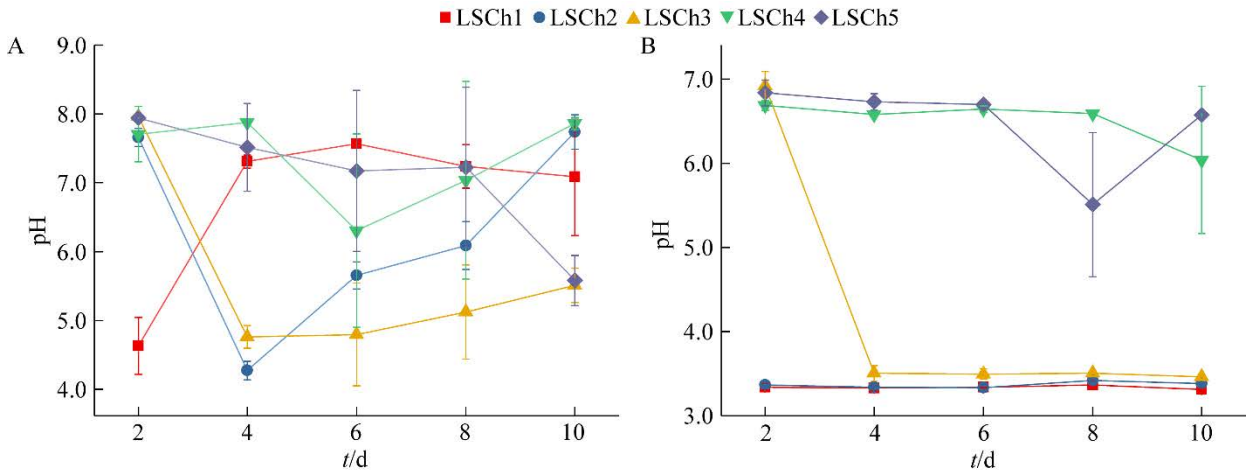


Figure 2 Changes in medium pH during degradation of TCP (A) and apatite (B) by isolated strains.

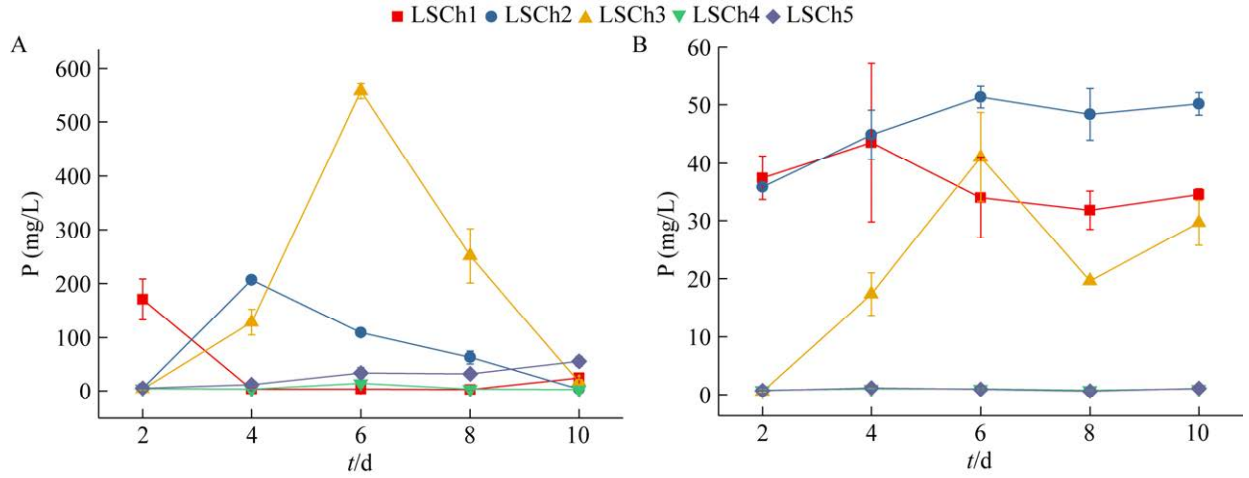


Figure 3 The content of P released by PSB cultured with TCP (A) and apatite (B) at different time.

TCP peaked after 2 days of culture with strain LSCh1 ((170.83±37.40) mg/L), while the released P peaked on the 4th day ((206.91±1.91) mg/L) and 10th day ((55.16±9.33) mg/L) when cultivated with the strains LSCh2 or LSCh5, respectively. *B. lata* PN1 isolated from the rhizosphere of bamboo by Yang et al.[43] showed a peak of P dissolution (117.50 mg/L) on the 6th day of cultivation, which was lower than that of strains LSCh1 and LSCh2 in this study. Different strains have different phosphate solubilization abilities and the time at which the highest P-release level is reached also varies. For example, the highest P-releasing content (315.72 mg/L) was found in *B. cepacia* 71-2 isolated from tobacco rhizosphere by Liu et al.[44], while that of *B. cepacia* P6 isolated from the rhizosphere of *Castanea mollissima* by Chen et al.[45] was only 78.84 mg/L. In this study, *Paraburkholderia* sp. LSCh3 had the strongest phosphate-solubilization ability among the isolated five strains and the highest P-release capacity can reach (556.94±13.83) mg/L, while *Paraburkholderia* sp. LSCh4 had the weakest phosphate solubilization ability, with the maximum dissolved P content being only (14.21±1.63) mg/L.

Weathering of apatite, which accounts for more than 95% of the total P in the Earth's crust, is the main source of P in natural ecosystems[46-47]. However, so far there are only a few studies on the quantitative analysis of *Burkholderia* and *Paraburkholderia* for weathering apatite[48-49]. In this study, the apatite solubilization ability of five strains isolated from the ectomycorrhizosphere of *C. henryi* was compared and analyzed. Compared to TCP, the apatite solubilization ability of this PSB was significantly lower, and the P-releasing amount was between 0.49 mg/L and 51.33 mg/L (Figure 3B). Among them, strains LSCh1, LSCh2, and LSCh3 had strong ability to dissolve apatite, and the highest P-releasing content was ((51.33±1.85) mg/L) (LSCh2). Sun et al.[49] isolated a strain of *B. gladioli* MEL01 from the soil of a rice-wheat rotation field and found that it could dissolve TCP and apatite well. The liquid fermentation results showed that the release of P from apatite reached the peak value (107.69 mg/L) after 2 days of culture. Silva et al.[48] isolated a phosphate-solubilizing strain of *Parburkholderia* 149H from the root of the medicinal plant *Aloe vera*, which can release 45.70 mg/L P from the apatite culture after 3 days.

These studies showed that there were significant differences in phosphate-solubility among different strains, even which the same species. Furthermore, the time required for different strains to show the highest P-releasing content was also inconsistent, which may be related to the growth characteristics and adaptability of PSB to P stress. Species of *Burkholderia* and *Paraburkholderia* typically have a variety of plant growth-promoting effects, such as nitrogen fixation, production of IAA and iron carriers as well as antibacterial effects[45,48,50-51]. It may have some influence on the strains' ability to solubilize phosphate. In addition, by comparing the correlation between the amount of released P and the pH of the fermentation solution, it was found that the phosphate dissolving ability of PSB had a significant negative correlation with the pH of the medium in the dissolution process of dissolving TCP and apatite (Figure 4). This further suggested that acidification is an important mechanism for PSB to solubilize insoluble inorganic phosphate.

## 2.4 Changes in crystal structure and secondary product formation during phosphate solubilization

Little attention is currently paid to the changes in crystal structure and morphology of phosphate minerals and the formation of secondary precipitates during phosphate solubilization by PSB. The study by Sun and Lian[52] showed that *Aspergillus nidus* can weather wollastonite (Ca-bearing mineral) with the formation of carbonate minerals. TCP and apatite are calcium-rich minerals. After treatment with PSB, the crystal morphology and structure may change and even form secondary minerals such as carbonate minerals. By analyzing the microscopic

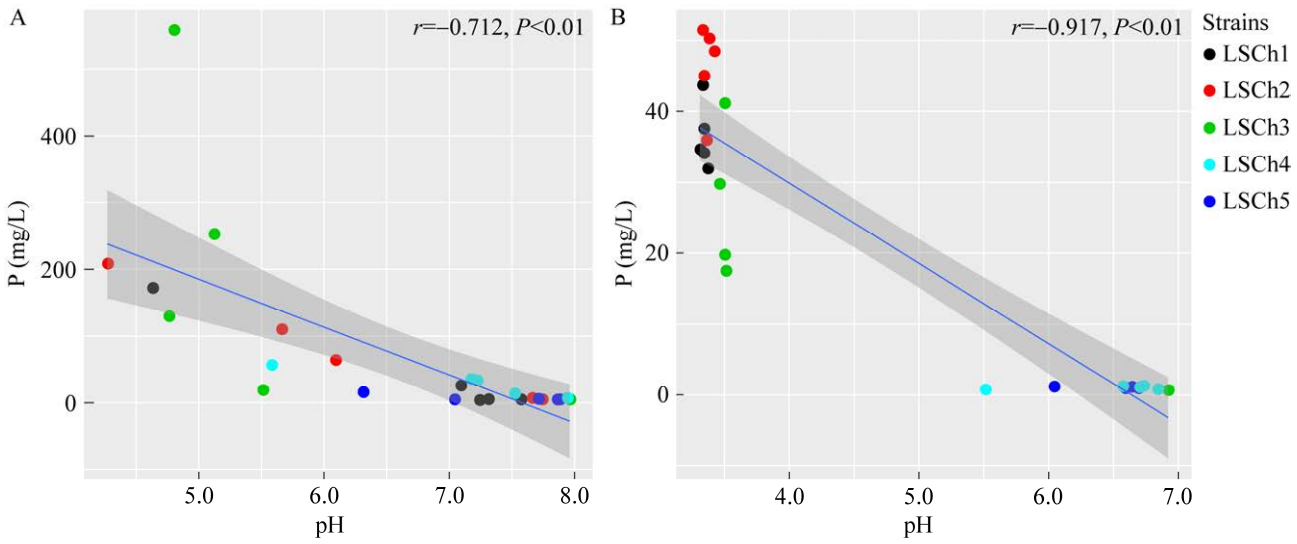


Figure 4 Correlation between pH value of fermentation solution and P-releasing content during the weathering of TCP (A) and apatite (B) by different strains.

morphology and mineral composition of the precipitates after 10 days of cultivation, the results showed that precipitates of different sizes were formed in the TCP medium after bacterial exposure, and EDS analysis showed that these precipitates had high carbon content (Figure 5). In addition, the XRD results showed that the crystal planes (1 0 4) and (0 0 6) of the carbonate mineral calcite were detected in the precipitates after cultivation with the five strains (Figure 5), suggesting that these bacteria were able to induce the formation of carbonate in the process of mobilization of insoluble phosphate. The secondary precipitates produced by strain LSCh3, a strain with strong TCP dissolution ability, contained higher carbon content (45.01%, Figure 5D). At the same time, the crystal planes (0 2 0), (0 4 0), and (1 1 2) of the secondary mineral brushite were also detected in the precipitates after cultivation with LSCh3. Sun et al.[53] also found that bacteria have the ability to simultaneously induce the formation of bruschite and calcite. All strains also had an obvious weathering effect on apatite. The SEM results showed that the surface of apatite had obvious corrosion, and the XRD results also showed that the crystal structure of apatite also changed to different extents, such as that the crystal plane (1 2 1) of fluorapatite/hydroxyapatite disappeared after exposure to LSCh1 and LSCh5, and the strength of the crystal plane (1 1 3) of phlogopite was weakened or even disappeared (Figure 6). In addition, EDS analysis showed that high C content was detected on the surface of precipitates after bacterial exposure. For example, the secondary precipitates produced by strains LSCh4 and LSCh5 contained C element, but no carbonate minerals were detected by XRD. There may be amorphous carbonates or the content of carbonate minerals caused by bacteria is too low to be detected. The results of this study showed that bacteria can still induce carbonate precipitation even under weakly acidic conditions. The studies of Liu and Lian[54-55] showed that the microbially induced carbonate has robust stability under acidic conditions and has a good heavy metal adsorption effect. This indicates that the carbonate induced by PSB in the process of phosphate dissolution has a certain potential to buffer soil acidity and passivate heavy metals in acidic-red soil environment, thereby improving plant growth.

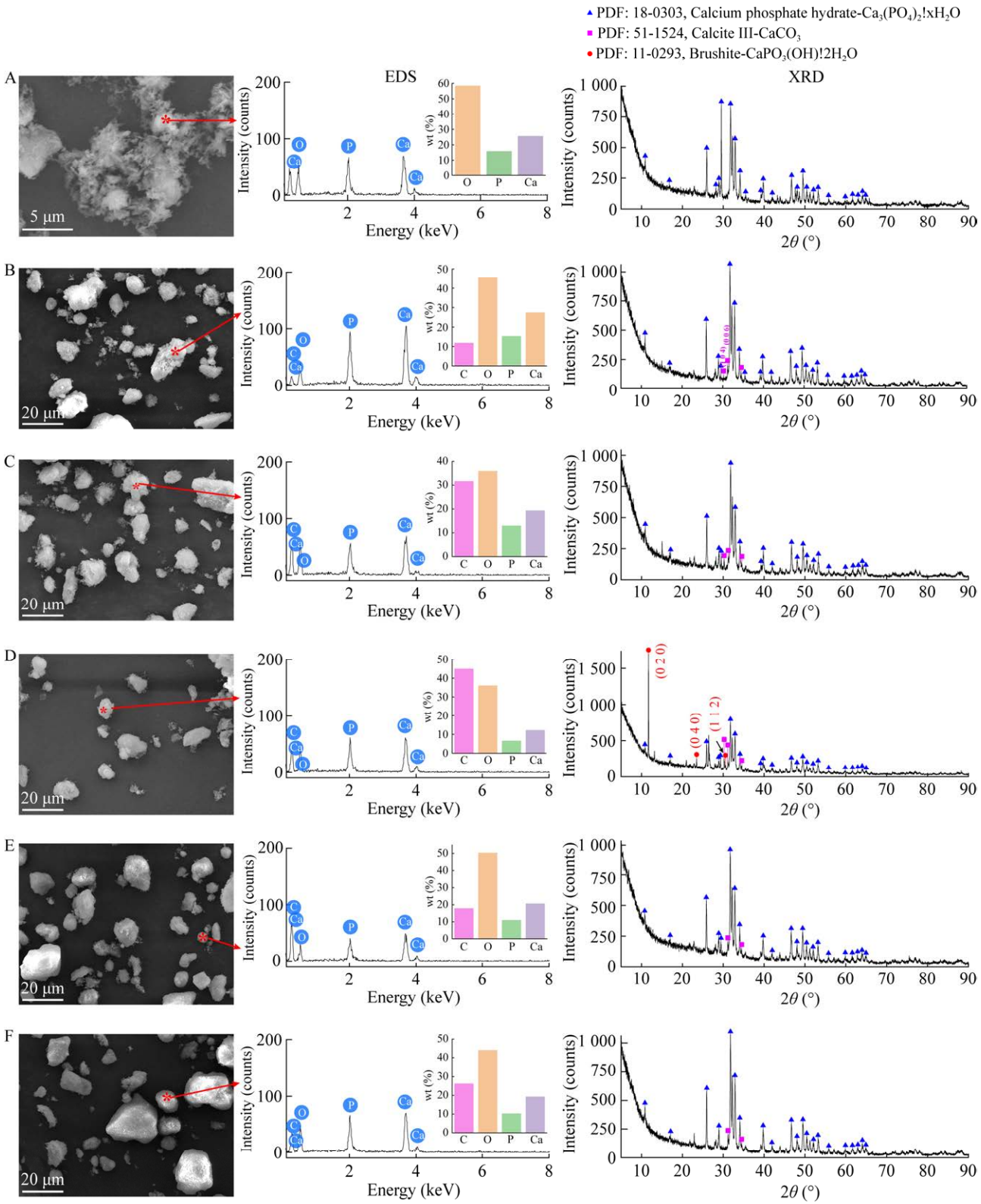


Figure 5 Results of SEM-EDS and XRD of precipitates after bacterial action. A: TCP without bacterial action. B–F: After the action of LSCh1, LSCh2, LSCh3, LSCh4, and LSCh5, respectively.

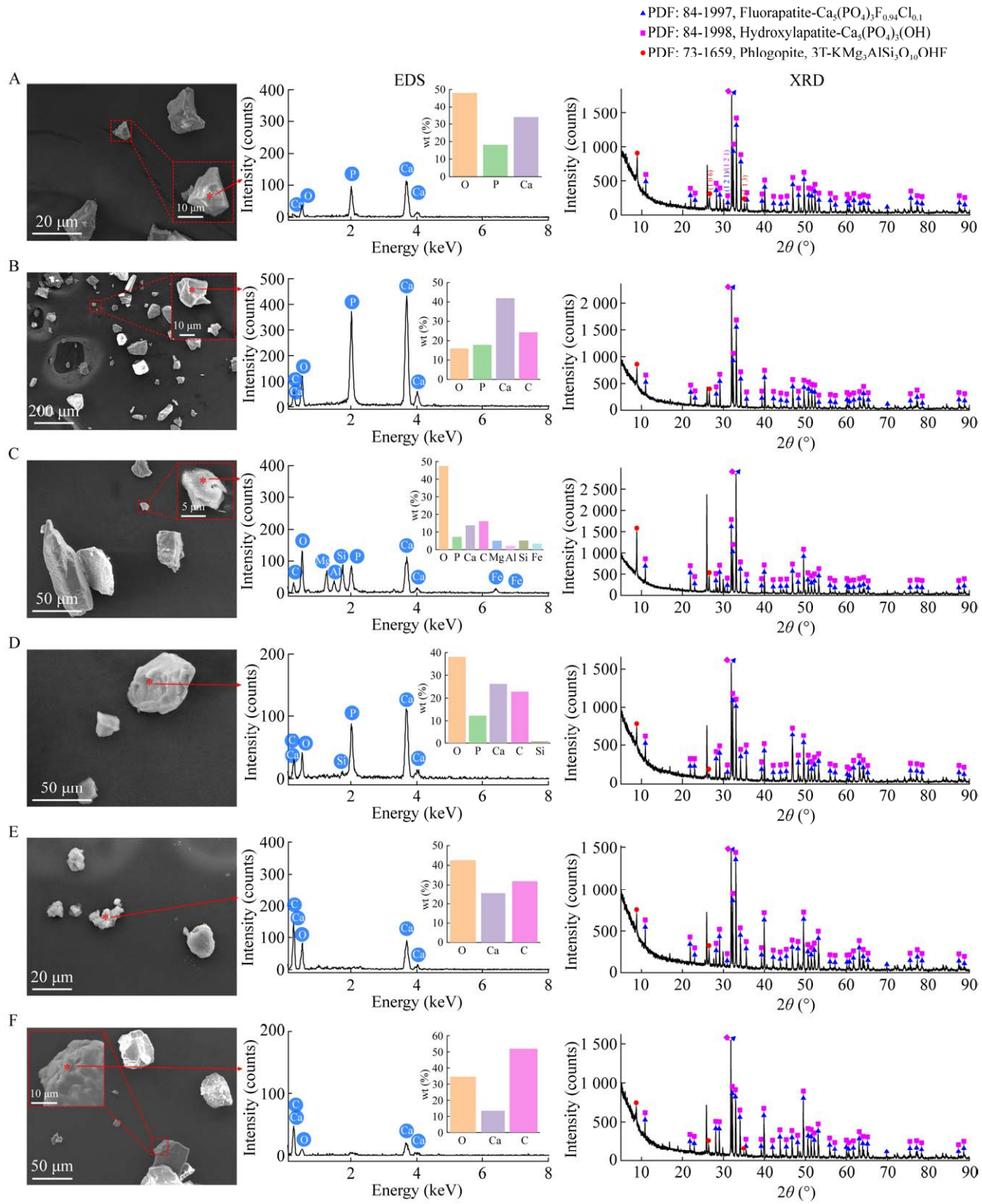


Figure 6 Results of SEM-EDS and XRD of apatite after bacterial action. A: Apatite without bacterial action. B–F: After the action of LSCh1, LSCh2, LSCh3, LSCh4, and LSCh5, respectively.

# 3 Conclusion

In this study, a total of five PSB strains were isolated from the ectomycorrhizosphere of *C. henryi*, belonging to *Burkholderia* and *Paraburkholderia* sp., suggesting that they are widespread phosphate-solubilizing microorganisms in soil. By analyzing the solubility of these strains toward TCP and apatite, it was found that strain LSCh3 (*Paraburkholderia* sp.) had the strongest degradation ability for TCP, with the highest P-releasing amount of 556.94 mg/L, while strain LSCh2 (*B. lata*) had the strongest weathering ability for apatite, with the highest P-releasing amount of 51.33 mg/L. In general, strain LSCh3 had a good solubilization effect on both TCP and apatite and can induce the formation of carbonate, suggesting that it is a plant growth-promoting bacterium with potential application value for future use to improve P nutrition of plants and soil quality.

## 参考文献


[1] ULRICH AE, FROSSARD E. On the history of a reoccurring concept: phosphorus scarcity[J]. Science of the Total Environment, 2014, 490: 694-707.

[2] KHAN MS, ZAIDI A, AHEMAD M, OVES M, WANI PA. Plant growth promotion by phosphate solubilizing fungi-current perspective[J]. Archives of Agronomy and Soil Science, 2010, 56(1): 73-98.

[3] MILLER SH, BROWNE P, PRIGENT-COMBARET C, COMBES-MEYNET E, MORRISSEY JP, O'GARA F. Biochemical and genomic comparison of inorganic phosphate solubilization in *Pseudomonas* species[J]. Environmental Microbiology Reports, 2010, 2(3): 403-411.

[4] SATI SC, PANT P. Evaluation of phosphate solubilization by root endophytic aquatic hyphomycete *Tetracladium setigerum*[J]. Symbiosis, 2019, 77: 141-145.

[5] RAGHOTHAMA KG, KARTHIKEYAN AS. Phosphate acquisition[J]. Plant and Soil, 2005, 274: 37-49.

[6] VANCE CP, UHDE-STONE C, ALLAN DL. Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource[J]. New Phytologist, 2003, 157(3): 423-447.

[7] SMITH SE, READ DJ. Mycorrhizal symbiosis[M]. 3rd Edition. Amsterdam: Academic Press, 2008.

[8] van der HEIJDEN MGA, MARTIN FM, SELOSSE MA, SANDERS IR. Mycorrhizal ecology and evolution: the past, the present, and the future[J]. New Phytologist, 2015, 205(4): 1406-1423.

[9] LANDEWEERT R, HOFFLAND E, FINLAY RD, KUYPER TW, van BREEMEN N. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals[J]. Trends in Ecology & Evolution, 2001, 16(5): 248-254.

[10] READ DJ, PEREZ-MORENO J. Mycorrhizas and nutrient cycling in ecosystems-a journey towards relevance?[J]. New Phytologist, 2003, 157(3): 475-492.

[11] BONNEVILLE S, BRAY AW, BENNING LG. Structural Fe(II) oxidation in biotite by an ectomycorrhizal fungi drives mechanical forcing[J]. Environmental Science & Technology, 2016, 50(11): 5589-5596.

[12] PINZARI F, CUADROS J, JUNGBLUT AD, NAJORKA J, HUMPHREYS-WILLIAMS E. Fungal strategies of potassium extraction from silicates of different resistance as manifested in differential weathering and gene expression[J]. Geochimica et Cosmochimica Acta, 2022, 316: 168-200.

[13] SMITS MM, JOHANSSON L, WALLANDER H. Soil fungi appear to have a retarding rather than a stimulating role on soil apatite weathering[J]. Plant and Soil, 2014, 385(1): 217-228.

[14] SMITS MM, BONNEVILLE S, BENNING LG, BANWART SA, LEAKE JR. Plant-driven weathering of apatite-the role of an ectomycorrhizal fungus[J]. Geobiology, 2012, 10(5): 445-456.

[15] WALLANDER H. Uptake of P from apatite by *Pinus sylvestris* seedlings colonised by different ectomycorrhizal fungi[J]. Plant and Soil, 2000, 218: 249-256.

[16] van SCHÖLL L, SMITS MM, HOFFLAND E. Ectomycorrhizal weathering of the soil minerals muscovite and hornblende[J]. New Phytologist, 2006, 171(4): 805-814.

[17] SUN QB, FU ZY, FINLAY R, LIAN B. Transcriptome analysis provides novel insights into the capacity of the ectomycorrhizal fungus *Amanita pantherina* to weather

K-containing feldspar and apatite[J]. Applied and Environmental Microbiology, 2019, 85(15): e00719-e00719.

[18] ADELEKE RA, CLOETE TE, BERTRAND A, KHASA DP. Mobilisation of potassium and phosphorus from iron ore by ectomycorrhizal fungi[J]. World Journal of Microbiology and Biotechnology, 2010, 26(10): 1901-1913.

[19] STUART EK, CASTAÑEDA-GÓMEZ L, MACDONALD CA, WONG-BAJRACHARYA J, ANDERSON IC, CARRILLO Y, PLETT JM, PLETT KL. Species-level identity of *Pisolithus* influences soil phosphorus availability for host plants and is moderated by nitrogen status, but not $CO_2$[J]. Soil Biology and Biochemistry, 2022, 165: 108520.

[20] 江盈, 邹锋, 黄建, 戴伟红, 左荣花, 田诗义, 熊欢. 六个外生菌根真菌菌株在不同难溶性磷源下的溶磷特性[J]. 菌物学报, 2023, 42(6): 1311-1329.
JIANG Y, ZOU F, HUANG J, DAI WH, ZUO RH, TIAN SY, XIONG H. Phosphorus dissolving characteristics of six ectomycorrhizal fungal strains under different insoluble phosphorus sources[J]. Mycosystema, 2023, 42(6): 1311-1329 (in Chinese).

[21] OLIVER RJ, BLYTH E, TAYLOR G, FINCH JW. Water use and yield of bioenergy poplar in future climates: modelling the interactive effects of elevated atmospheric $CO_2$ and climate on productivity and water use[J]. GCB Bioenergy, 2015, 7(5): 958-973.

[22] KOHLER A, KUO AL, NAGY LG, MORIN E, BARRY KW, BUSCOT F, CANBÄCK B, CHOI C, CICHOCKI N, CLUM A, COLPAERT J, COPELAND A, COSTA MD, DORÉ J, FLOUDAS D, GAY G, GIRLANDA M, HENRISSAT B, HERRMANN S, HESS J, et al. Convergent losses of decay mechanisms and rapid turnover of symbiosis genes in mycorrhizal mutualists[J]. Nature Genetics, 2015, 47(4): 410-415.

[23] MIYAUCHI S, KISS E, KUO AL, DRULA E, KOHLER A, SÁNCHEZ-GARCÍA M, MORIN E, ANDREOPOULOS B, BARRY KW, BONITO G, BUÉE M, CARVER A, CHEN C, CICHOCKI N, CLUM A, CULLEY D, CROUS PW, FAUCHERY L, GIRLANDA M, HAYES RD, et al. Large-scale genome sequencing of mycorrhizal fungi provides insights into the early evolution of symbiotic traits[J]. Nature Communications, 2020, 11: 5125.

[24] BONFANTE P, ANCA IA. Plants, mycorrhizal fungi, and bacteria: a network of interactions[J]. Annual Review of Microbiology, 2009, 63: 363-383.

[25] FREY B, RIEDER SR, BRUNNER I, PLÖTZE M, KOETZSCH S, LAPANJE A, BRANDL H, FURRER G. Weathering-associated bacteria from the Damma glacier forefield: physiological capabilities and impact on granite dissolution[J]. Applied and Environmental Microbiology, 2010, 76(14): 4788-4796.

[26] JOHANSSON JF, PAUL LR, FINLAY RD. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture[J]. FEMS Microbiology Ecology, 2004, 48(1): 1-13.

[27] NICOLITCH O, COLIN Y, TURPAULT MP, UROZ S. Soil type determines the distribution of nutrient mobilizing bacterial communities in the rhizosphere of beech trees[J]. Soil Biology and Biochemistry, 2016, 103: 429-445.

[28] FONTAINE L, THIFFAULT N, PARÉ D, FORTIN JA, PICHÉ Y. Phosphate-solubilizing bacteria isolated from ectomycorrhizal mycelium of *Picea glauca*are highly efficient at fluorapatite weathering[J]. Botany, 2016, 94(12): 1183-1193.

[29] SUN QB, LIU XM, WANG SJ, LIAN B. Effects of mineral substrate on ectomycorrhizal fungal colonization and bacterial community structure[J]. Science of the Total Environment, 2020, 721: 137663.

[30] HEUER H, KRSEK M, BAKER P, SMALLA K, WELLINGTON EM. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients[J]. Applied and Environmental Microbiology, 1997, 63(8): 3233-3241.

[31] NAUTIYAL CS. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms[J]. FEMS Microbiology Letters, 1999, 170(1): 265-270.

[32] 于淼, 吴红艳, 冯健, 方新, 王智学. 解磷菌 623-3 的鉴定、培养条件优化及应用[J]. 四川农业大学学报, 2020, 38(5): 572-579.
YU M, WU HY, FENG J, FANG X, WANG ZX. Identification, optimization of culture conditions and application of dephosphorizing bacteria 623-3[J]. Journal of Sichuan Agricultural University, 2020, 38(5): 572-579 (in Chinese).

[33] RODRÍGUEZ H, FRAGA R, GONZALEZ T, BASHAN Y. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria[J]. Plant and Soil, 2006, 287(1): 15-21.

[34] 柯春亮, 陈宇丰, 周登博, 黄绵佳, 张锡炎, 高祝芬. 香蕉根际土壤解磷细菌的筛选、鉴定及解磷能力[J]. 微生物学通报, 2015, 42(6): 1032-1042.
KE CL, CHEN YF, ZHOU DB, HUANG MJ, ZHANG XY, GAO ZF. Isolation, identification and phosphate solubilization analysis of phosphate-solubilizing bacteria derived from banana rhizosphere soil[J]. Microbiology China, 2015, 42(6): 1032-1042 (in Chinese).

[35] UROZ S, CALVARUSO C, TURPAULT MP, PIERRAT JC, MUSTIN C, FREY-KLETT P. Effect of the mycorrhizosphere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil[J]. Applied and Environmental Microbiology, 2007, 73(9): 3019-3027.

[36] VISHAL V, MUNDA SS, SINGH G, LAL S. Cataloguing the bacterial diversity in the active ectomycorrhizal zone of *Astraeus* from a dry deciduous forest of *Shorea*[J]. Biodiversity Data Journal, 2021, 9: e63086.

[37] OUAHMANE L, REVEL JC, HAFIDI M, THIOULOUSE J, PRIN Y, GALIANA A, DREYFUS B, DUPONNOIS R. Responses of *Pinus halepensis* growth, soil microbial catabolic functions and phosphate-solubilizing bacteria after rock phosphate amendment and ectomycorrhizal inoculation[J]. Plant and Soil, 2009, 320: 169-179.

[38] SAWANA A, ADEOLU M, GUPTA RS. Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species[J]. Frontiers in Genetics, 2014, 5: 429.

[39] BEN FARHAT M, FARHAT A, BEJAR W, KAMMOUN R, BOUCHAALA K, FOURATI A, ANTOUN H, BEJAR S, CHOUAYEKH H. Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of *Gafsa*[J]. Archives of Microbiology, 2009, 191(11): 815-824.

[40] LI XL, ZHAO XQ, DONG XY, MA JF, SHEN RF. Secretion of gluconic acid from *Nguyenibacter* sp. L1 is responsible for solubilization of aluminum phosphate[J]. Frontiers in Microbiology, 2021, 12: 784025.

[41] LIN TF, HUANG HI, SHEN FT, YOUNG CC. The protons of gluconic acid are the major factor responsible for the dissolution of tricalcium phosphate by *Burkholderia cepacia* CC-Al74[J]. Bioresource Technology, 2006, 97(7): 957-960.

[42] BASHAN Y, KAMNEV AA, DE-BASHAN LE. Tricalcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate-solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure[J]. Biology and Fertility of Soils, 2013, 49(4): 465-479.

[43] 杨豆, 李广强, 吕永财, 李冬, 万松泽, 张扬. 毛竹根系解磷细菌的解磷条件和解磷特性[J]. 经济林研究, 2021, 39(2): 115-122.
YANG D, LI GQ, LÜ YC, LI D, WAN SZ, ZHANG Y. Phosphate-solubilizing characteristic and conditions of a phosphate solubilizing bacteria *Burkholderia lata* from *Phyllostachys edulis* root[J]. Non-wood Forest Research, 2021, 39(2): 115-122 (in Chinese).

[44] LIU CJ, MOU L, YI JL, WANG J, LIU AX, YU JF. The *eno* gene of *Burkholderia cenocepacia* strain 71-2 is involved in phosphate solubilization[J]. Current Microbiology, 2019, 76(4): 495-502.

[45] 陈岩岩, 叶项宇, 常肖锐, 徐碧林, 吕锐玲, 郑永良. 板栗根际高效解磷菌的筛选[J]. 经济林研究, 2021, 39(2): 132-139.
CHEN YY, YE XY, CHANG XR, XU BL, LYU RL, ZHENG YL. Screening of highly efficient phosphate-solubilizing bacteria of *Castanea mollissima* rhizosphere[J]. Non-wood Forest Research, 2021, 39(2): 132-139 (in Chinese).

[46] GUIDRY MW, MACKENZIE FT. Apatite weathering and the Phanerozoic phosphorus cycle[J]. Geology, 2000, 28(7): 631.

[47] PELTZER D, WARDLE D, ALLISON V, BAISDEN W, BARDGETT R, CHADWICK O, CONDRON L, PARFITT R, PORDER S, RICHARDSON S, TURNER B, VITOUSEK P, WALKER J, WALKER L. Understanding ecosystem retrogression[J]. Ecological Monographs, 2010: 100415133813094.

[48] SILVA CF, VITORINO LC, MENDONÇA MAC, ARAÚJO WL, DOURADO MN, ALBUQUERQUE LC, SOARES MA, SOUCHIE EL. Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant *Aloe vera*[J]. South African Journal of Botany, 2020, 134: 3-16.

[49] SUN XW, SHAO C, CHEN L, JIN XM, NI H. Plant growth-promoting effect of the chitosanolytic

phosphate-solubilizing bacterium *Burkholderia gladioli* MEL01 after fermentation with chitosan and fertilization with rock phosphate[J]. Journal of Plant Growth Regulation, 2021, 40(4): 1674-1686.

[50] 宫安东, 朱梓钰, 路亚南, 万海燕, 吴楠楠, Cheelo Dimuna, 龚双军, 文淑婷, 侯晓. 吡咯伯克霍尔德菌 WY6-5 的溶磷、抑菌与促玉米生长作用研究[J]. 中国农业科学, 2019, 52(9): 1574-1586.
GONG AD, ZHU ZY, LU YN, WAN HY, WU NN, DIMUNA C, GONG SJ, WEN ST, HOU X. Functional analysis of *Burkholderia pyrrocinia* WY6-5 on phosphate solubilizing, antifungal and growth-promoting activity of maize[J]. Scientia Agricultura Sinica, 2019, 52(9): 1574-1586 (in Chinese).

[51] 朱德旋, 杜春梅, 董锡文, 薛春梅, 王瑞. 一株寒地高效解无机磷细菌的分离鉴定及拮抗作用[J]. 微生物学报, 2020, 60(8): 1672-1682.
ZHU DX, DU CM, DONG XW, XUE CM, WANG R. Identification and antagonism activity of an inorganic phosphorus-dissolving bacterial strain isolated from cold region[J]. Acta Microbiologica Sinica, 2020, 60(8): 1672-1682 (in Chinese).

[52] SUN QB, LIAN B. The different roles of *Aspergillus nidulans* carbonic anhydrases in wollastonite weathering accompanied by carbonation[J]. Geochimica et Cosmochimica Acta, 2019, 244: 437-450.

[53] SUN QB, LI J, FINLAY RD, LIAN B. Oxalotrophic bacterial assemblages in the ectomycorrhizosphere of forest trees and their effects on oxalate degradation and carbon fixation potential[J]. Chemical Geology, 2019, 514: 54-64.

[54] LIU RL, LIAN B. Non-competitive and competitive adsorption of $Cd^{2+}$, $Ni^{2+}$, and $Cu^{2+}$ by biogenic vaterite[J]. Science of the Total Environment, 2019, 659: 122-130.

[55] LIU RL, LIAN B. Immobilisation of Cd(II) on biogenic and abiotic calcium carbonate[J]. Journal of Hazardous Materials, 2019, 378: 120707.