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ORIGINAL ARTICLE

# Biochar promotes $FePO_4$ solubilization through modulating organic acids excreted by *Talaromyces pinophilus*

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**Abstract**

Inoculating phosphate-solubilizing microbes (PSMs) is an effective approach to increasing the soluble phosphorus content in soil for plant growth. While biochar has been shown to promote microbe growth, it can also lead to phosphate precipitation. Therefore, the specific benefits of biochar application for phosphate activation remain unclear. In this study, biochars with varying physicochemical properties were utilized as carriers for microbes. PSMs of *Talaromyces pinophilus* (*T. pinophilus*) strains were isolated from the rhizosphere soil of corn, radish, and wheat plants, with $FePO_4$ as the sole phosphorus source. To understand how biochar modulates the phosphate-solubilizing capacity of *T. pinophilus*, we measured the dissolved phosphate content, strain biomass, and the concentrations of various organic acids excreted by the strains. Our findings suggested that biochar primarily enhanced the phosphate-solubilizing ability of *T. pinophilus* by stimulating the secretion of organic acids, rather than by increasing strain biomass. The phosphate-solubilizing ability increased by 356% after adding corn straw biochar to the strains isolated from wheat rhizosphere soil. Notably, citric acid showed the most significant correlation with the phosphate-solubilizing ability of the strains. Laboratory-simulated experiments using individual organic acids indicated that the abundant carboxyl groups in citric acid may be a primary property contributing to phosphate release from $FePO_4$. This study highlights the essential role of organic acids excreted by PSMs, which are stimulated by biochar, in phosphate solubilization. These findings not only provide valuable insights for optimizing phosphate utilization in the environment but also contribute to the development of biochar-based biofertilizers, advancing sustainable agricultural practices.

## Highlights

- Biochar effectively enhances the phosphate-solubilizing ability of *T. pinophilus*.
- The dissolved P is associated with organic acids, not the biomass of *T. pinophilus*.
- Citric acid shows the highest phosphate-solubilizing ability among the organic acids.
- Citric acid solubilizes phosphate due to its abundant carboxyl groups.

**Keywords** Rhizosphere microorganisms, Biofertilizers, Phosphate-solubilizing ability, Organic acid, Bio-carriers

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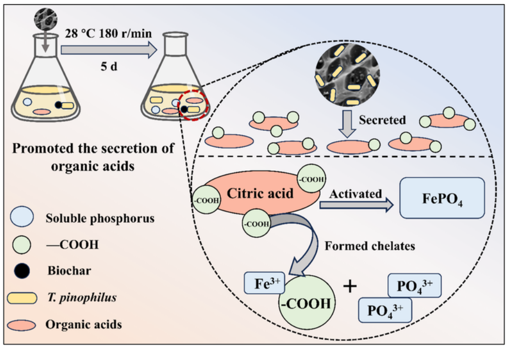
## Graphical Abstract



## 1 Introduction

Phosphorus (P) is the second most critical nutrient for plant growth, following nitrogen (Ben Zineb et al. 2020; Xiao et al. 2020). It plays an important role in various physiological processes such as photosynthesis, glycolysis, respiration, nucleic acid synthesis, cell membrane formation, and enzyme activity regulation in plant growth (Manzoor et al. 2017). Phosphate fertilizers (the most soluble form of P) can fulfill the short-term P nutritional requirements of crops in agricultural production (Khan et al. 2017), while they are susceptible to binding with metal ions (such as Al$^{3+}$, Fe$^{3+}$, and Ca$^{2+}$), transforming into the form of deposited P (Rodríguez and Fraga. 1999; Zhang et al. 2023). On the other hand, excessive application of phosphate fertilizers in agricultural activities not only greatly enhances the cost of crop production but also triggers a series of environmental issues, such as the eutrophication of water bodies (Harris et al. 2006).

The activation of the deposited P using microbes has great potential to be applied in agricultural activities due to its green and cost-effective advantages. Researches indicate that phosphate-solubilizing microbes (PSMs) can convert deposited P into soluble forms by secreting phosphatase, phytase, and/or organic acids (Xiao et al. 2020). However, it is reported that intensive agriculture activities may significantly alter the community structure of PSMs, most likely resulting in a decrease in the diversity and abundance of the strains (Ma et al. 2016). Adding exogenous PSMs is always challenged by the decreased survival ratios over time because of the great pressure to survive through the indigenous animals and microorganisms, or the lack of suitable habitat and nutrients (Sun et al. 2016; Wang et al. 2022).

Biochar is a product of biomass pyrolysis under limited oxygen circumstances and has a significant practical value (Zhang et al. 2016; Rafique et al. 2017). Previous studies have reported that biochar application may be beneficial in alleviating phosphate deficient problems (Yuan et al. 2024). For example, the applied phosphate fertilizers may be adsorbed or deposited on a biochar surface. This process could prevent the leaching of excessive phosphate (Peng et al. 2012; Streubel et al. 2012; Yao et al. 2013). Moreover, the dissolvable organic and inorganic nutrients in biochar could serve as a food source for microbes, while the porous structure provides a favorable habitat for these strains (Sun et al. 2016). Studies have demonstrated that biochar can effectively enhance the phosphate-solubilizing ability of PSMs (Cantrell et al. 2012; Wang et al. 2022). However, no explicit conclusion was reached regarding biochar properties in controlling the phosphate-solubilizing ability of PSMs. This may be understood from the following two reasons: (1) Measuring the dissolved phosphate is essential to quantify the phosphate-solubilizing ability. Most studies measured phosphate concentration following filtration of the supernatant (Nautiyal 1999; Oliveira et al. 2009). It should be noted that during the growth of microbes, the dissolved phosphate may be quickly uptake and become microbe cell compositions. However, this portion of phosphate activation was not taken into consideration in most studies, which may have resulted in significant deviation; (2) Most studies quantified the biomass of the strains to support the conclusion of the enhanced phosphate-solubilizing ability (Wei et al. 2016; Liu et al. 2017). However, it should be noted that the microbes in the biochar system may be highly diverse, and different strains may have dramatically different phosphate-solubilizing abilities. A more fundamental parameter closely relating to the process that solubilizes phosphate should be incorporated to better understand this key process.

Therefore, this study is designed to reveal the mechanism of biochar in enhancing the phosphate-solubilizing ability of PSMs. Chicken feather biochar and corn straw biochar produced at various pyrolysis temperatures (300 °C, 450 °C, and 700 °C) were individually applied to PSMs isolated from the rhizosphere soils of different plant species (corn, radish, and wheat). Our study aimed to investigate how biochar modulates the phosphate-solubilizing capacity of PSMs, by analyzing the dissolved phosphate content, strain biomass, and the concentrations of various organic acids excreted by the strains. The phosphate-solubilizing ability of the strains was evaluated considering both organic and inorganic phosphorus. This study will provide fundamental theoretic support for biochar application to modulate phosphate bioavailability in soil systems.

## 2 Materials and methods

### 2.1 Biochar preparation and characterizations

The collected chicken feathers and corn straws were air-dried at room temperature for 10 d, crushed into 1 cm pieces, and pyrolyzed in a muffle furnace (OTF-1200X, Hefei Kejing, China). The pyrolysis temperature was 300 °C, 450 °C, or 700 °C with $N_2$ gas flow to maintain an oxygen-free environment. The temperature was increased at the rate of 10 °C/min from room temperature to the target temperature, and maintained for 120 min, followed by cooling to room temperature under $N_2$ gas flow. The obtained biochar was milled, sieved through a 100-mesh sieve, and stored in a desiccator.

The specific surface area and pore size of biochars were determined using a surface area and porosity analyzer (JW-BK132F, JWGB SCI. & TECH., China). Their total organic and inorganic carbon contents were analyzed on a TOC analyzer (vario TOC select, elementar, Germany). An elemental analyzer (Unicube, elementar, Germany) was used to determine the C, H, and N contents of the biochar. The C/H values were calculated based on the molar ratios.

### 2.2 Purification of PSMs and the determination of their phosphate-solubilizing ability

Samples of PSMs were isolated from the rhizosphere soil of corn, radish, and wheat plants in farmlands located in Kunming, Yunnan Province (24° 49′34.76″ N, 102° 43′29.29″ E). Each soil sample (0.01 g) was mixed with 100 mL of phosphate buffer solution (0.01 mol/L) and then diluted 10 times. Sequentially, 1 mL of each soil suspension was inoculated onto a National Botanical Research Institute's phosphate growth medium (NBRIP) for strain screening using the streak plate method (Pikovskaya 1948). The genes of three identified strains were sequenced by Personalbio company in Shanghai, revealing that all strains belonged to the same phosphate-solubilizing microbes genus: *Talaromyces pinophilus* (*T. pinophilus*; Gene sequences are shown in Table S1).

In this experiment, an improved method was employed to quantify the phosphate-solubilizing ability of the strains. It was noted that the activated phosphate may exist as freely dissolved phosphate or be absorbed or even be utilized becoming a portion of the microbe cells (Zhao et al. 2001; Turner et al. 2003). Therefore, when determining the phosphate-solubilizing ability of the strains, both the released P and the stored P should be considered. *T. pinophilus* cultures were incubated until the logarithmic stage. To determine absorbed soluble P and the converted organic P in the strains (Zhao et al. 2001), 25 mL of the cultures were homogenated using a cell crusher and digested with 18.40 mol/L sulfuric acid. After centrifugation, the supernatants were separated and the pH values were adjusted to 5 ~ 6. The molybdate colorimetric method was then used to determine the soluble P content (A) (Murphy and Riley 1962). Simultaneously, 25 mL logarithmic stage strain cultures were added to 25 mL NBRIP and incubated on a shaker for 5 d. After filtering out the residual $FePO_4$ particles, the strain cultures were treated using the same method, and the overall soluble P content (B) was measured. The difference between the two soluble P contents (B-A) was used to represent the phosphate-solubilizing ability of *T. pinophilus.*

### 2.3 Effects of biochars on the phosphate-solubilizing ability of PSMs

When *T. pinophilus* cultures reached the logarithmic stage, they were centrifuged, and the supernatants were discarded. The strains were then washed three times to prepare the cell suspension. Aliquots of sterile biochar (0.05 g) were added to 25 mL of cell suspension along with 25 mL of sterile NBRIP (Fig. 1). The mixtures containing biochars were labeled as C/R/W/F + J/Y + 300/450/700 (C, R, W, and F representing *T. pinophilus* strains from the rhizosphere soil of corn, radish, wheat, and composite strains from the three plants, respectively; J and Y representing chicken feather biochar or corn straw biochar respectively; and 300, 450, and 700 representing biochar pyrolysis temperatures). Following incubation on a shaker for 5 d, the mixtures were centrifuged to remove deposited biochar and unactivated $FePO_4$. The strain cultures underwent cell crushing, acid digestion, and pH adjustment to determine their phosphate-solubilizing ability after biochar addition (A). Simultaneously, an equal volume of strain cultures without biochar was added into NBRIP and incubated under the same condition. To account for the effect of adsorption and desorption of biochar on soluble P content, the soluble P

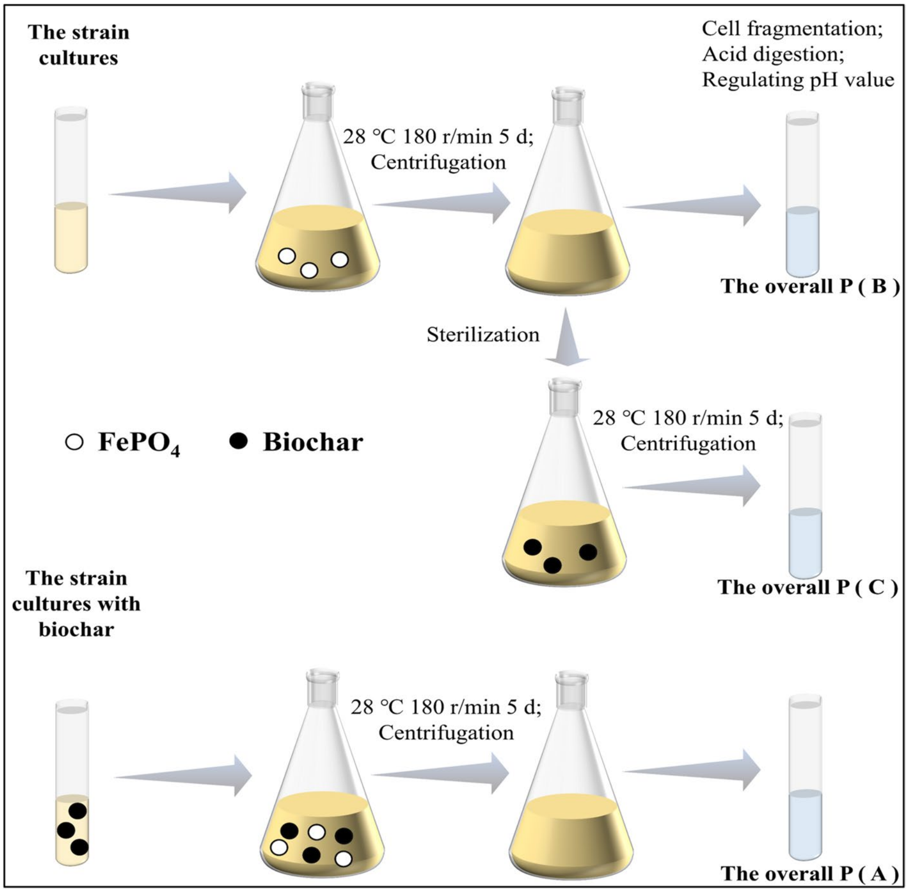


**Fig. 1** Experiment procedure for determining the effect of biochars on the phosphate-solubilizing ability of PSMs

content (B) activated by the strains in the system without biochar was determined. After removing unactivated $FePO_4$, the strain cultures were sterilized, and aliquots of biochars (0.05 g) were added before incubating on a shaker for 5 d. The overall soluble P content (C) was then determined. The adsorption of phosphate on biochar was calculated as B-C. In summary, the phosphate-solubilizing ability of the strains after the addition of biochar (D) was expressed numerically as D = A + (B-C).

### 2.4 Determination of PSM biomass and organic acids secreted by PSMs

The biomass of *T. pinophilus* was determined by measuring the absorbance of the strain cultures at 600 nm using a UV spectrophotometer (Evolution pro, Thermo Fisher Scientific, USA). A liquid chromatograph (SIL-40, SHIMADZU, Japan) was employed to identify the types and quantities of organic acids secreted by *T. pinophilus*. Individual solutions of oxalic acid, malic acid, tartaric acid, lactic acid, citric acid, and malonic acid with 99.99% purity were used to prepare standard solutions. The types and contents of organic acids secreted by *T. pinophilus* were then determined based on the peak time and peak area in the standard curve.

### 2.5 Statistical analysis

Origin 2023b software was utilized for graph drawing and principal component analysis to examine the controlling factors in strains' phosphate-solubilizing ability. Additionally, correlation analysis was conducted between strains' phosphate-solubilizing ability and the overall or individual content of different organic acids. The one-way analysis of variance (ANOVA) was carried out using IBM SPSS Statistics 27 to identify statistically significant differences in the bar graph data.

## 3 Results

### 3.1 Characterization of biochars

Table 1 presents the physicochemical properties of six biochars utilized in the study, including pH value, dissolved organic carbon (DOC), total inorganic carbon (TIC), ash content, specific surface area, pore size, C/H ratio, and N%. Biochars derived from corn straw exhibited higher pH value, DOC, TIC, pore size, and

**Table 1** Physiochemical properties of biochars prepared at different temperatures

| Material | Temperature (°C) | pH value | DOC (mgC/L) | TIC (mg/L) | Ash content % | Specific surface area ($m^2$/g) | Pore size (nm) | C/H | N% |
|---|---|---|---|---|---|---|---|---|---|
| Corn straw | 300 | 7.18 | 168.66 | 2.70 | 2.97 | 3.17 | 8.06 | 1.49 | 0.14 |
| | 450 | 10.24 | 45.22 | 33.31 | 4.96 | 5.14 | 7.99 | 2.42 | 0.10 |
| | 700 | 10.49 | 20.60 | 49.85 | 5.89 | 257.64 | 1.96 | 5.19 | 0.08 |
| Chicken feather | 300 | 6.86 | 26.09 | 0.27 | 8.45 | 6.61 | 6.17 | 1.03 | 1.04 |
| | 450 | 6.90 | 1.77 | 0.99 | 11.57 | 8.72 | 5.49 | 2.04 | 1.07 |
| | 700 | 7.23 | 1.45 | 2.31 | 14.68 | 213.52 | 1.72 | 4.87 | 0.79 |

C/H ratio compared to chicken feather biochars at the same pyrolysis temperatures. Specifically, corn straw biochars had the highest DOC concentration of 168.66 and 45.22 mg C/L at 300 °C and 450 °C, respectively. Moreover, a decrease in pore size was observed with increasing pyrolysis temperature, with Y300 demonstrating the largest pore size. Conversely, ash content increased with rising pyrolysis temperature. Additionally, nitrogen content was found to be higher in chicken feather biochar than in corn straw biochar produced at the same pyrolysis temperatures.

### 3.2 The phosphate-solubilizing ability and biomass of PSMs after adding biochars

To investigate the role of biochars in regulating the phosphate-solubilizing ability of PSMs, six biochars were individually introduced to PSMs-$FePO_4$ systems. The phosphate-solubilizing ability was improved to different extents depending on the strain and biochar types as depicted in Fig. 2a. For example, the phosphate-solubilizing ability was increased by 356% after adding Y450 and 40% after adding Y300 to the strains isolated from wheat rhizosphere soil. According to the principal component analysis, Y300 and Y450 generally enhanced the phosphate-solubilizing ability of PSMs (Fig. 2b).

Moreover, $OD_{600}$ value was used to characterize the biomass of PSMs after adding biochars as illustrated in Fig. 2a. The Corn straw biochar was more effective in enhancing the biomass of strains compared to the chicken feather biochar under the same pyrolysis temperature. The highest increase in biomass of the strains was observed after the addition of Y700, with a 19.2-fold increase in the biomass of the strain isolated from corn rhizosphere soil. Interestingly, although the addition of Y700 resulted in the highest strain biomass, it did not optimize their phosphate-solubilizing ability. No significant correlation was observed between the phosphate-solubilizing ability and biomass of strains (Fig. 2c).

### 3.3 Organic acids excreted by PSMs after adding biochars

As shown in Fig. 3, the investigated biochars exhibited various effects on the overall content of organic acids secreted by the strains. The largest increase in the overall organic acid content was generally observed after adding Y450. Specifically, the total organic acids content increased by 22.0%, 47.0%, 10.8%, and 5.26% after adding Y450 for the strains of C, R, W, and F, respectively. Y300 was found to promote the secretion of organic acids by the strains less effectively than Y450. In addition, among the chicken feather biochars prepared under three pyrolysis temperatures, J300 was identified as the most effective in promoting the secretion of total organic acids by the strains.

More importantly, further investigation into the relationship between different components of organic acids and the phosphate-solubilizing ability had been conducted.

As shown in Table 2, the phosphate-solubilizing ability was strongly related to the total organic acids content. Among the various organic acids, citric acid content exhibited the strongest positive correlation with the phosphate-solubilizing ability compared with oxalic acid, malic acid, tartaric acid, malonic acid and lactic acid content, which may play an important role in the phosphate-solubilizing process.

### 3.4 The ability of different kinds of organic acids to activate $FePO_4$

The observed six organic acids were individually added in $FePO_4$ to investigate phosphate dissolution. When the equal molar concentration of organic acids was applied in the system, the soluble P content was obviously higher after adding citric acid than adding other organic acids (Fig. 4a). In addition, we conducted another experiment with organic acids containing the same molar concentration of carboxyl groups. The result showed much less variation among different organic acids (Fig. 4b). Especially, the ratio of phosphate-solubilizing ability to organic acids concentration (Fig. 4c) was higher when organic acids containing the same molar concentration of carboxyl

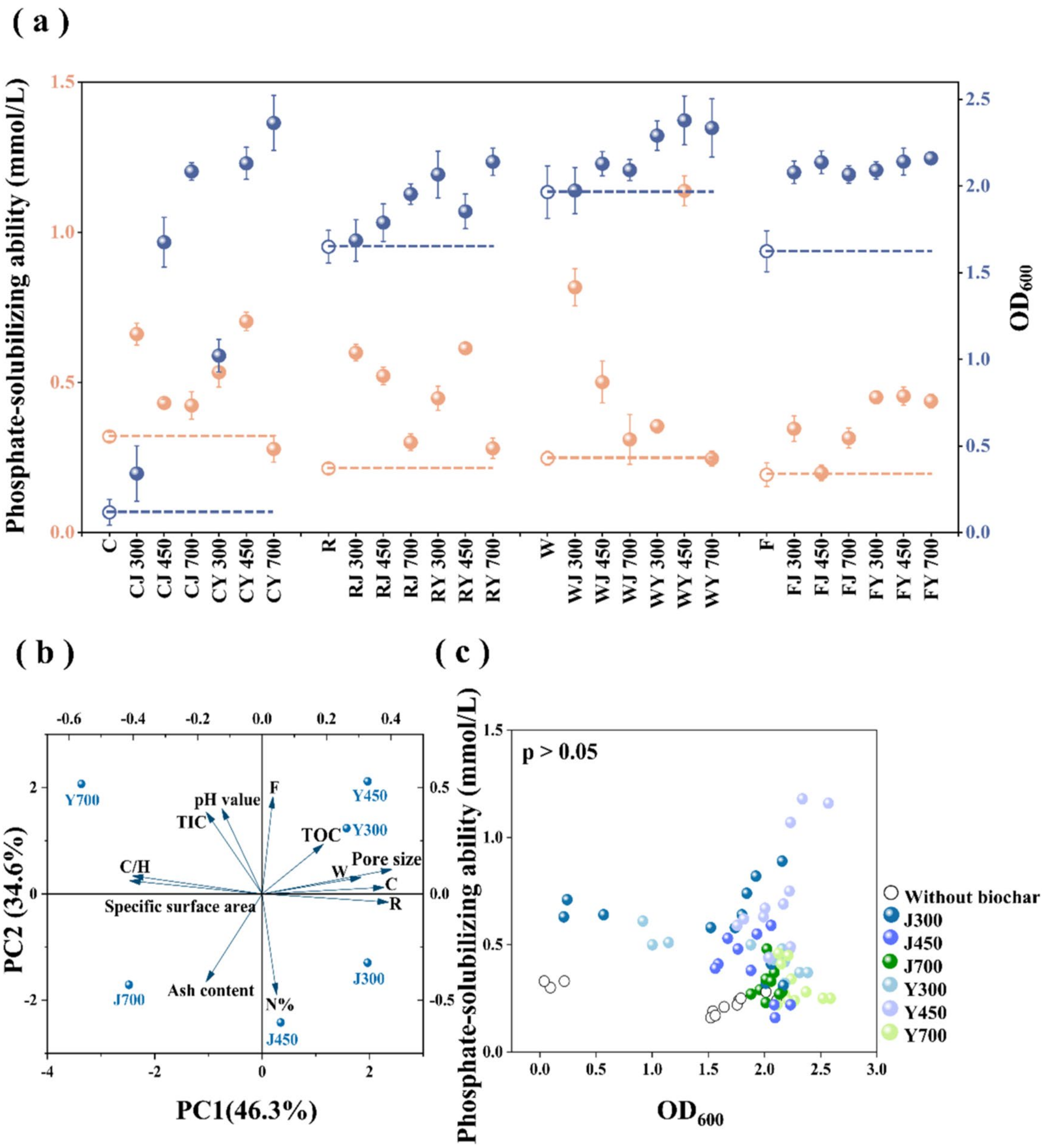


**Fig. 2** **a** The phosphate-solubilizing ability and biomass of strains after adding biochars. **b** Principal component analysis of the physicochemical properties of biochars and the phosphate-solubilizing ability of different strains. **c** The correlation analysis between phosphate-solubilizing ability and biomass of strains after adding biochars

groups were added, compared to adding organic acids of equal molar concentration.

## 4 Discussion

### 4.1 Biochars enhanced the phosphate-solubilizing ability of PSMs

The phosphate-solubilizing ability was improved to different extents after adding biochars as depicted in Fig. 2a. Corn straw biochars were more effective in enhancing the ability compared to the chicken feather biochars. Principal component analysis revealed that Y300 and Y450 generally enhanced the phosphate-solubilizing ability of PSMs (Fig. 2b). These two biochars showed the comparatively higher DOC content and larger pore size, which seemed to correlate with the activity of the strains. For example, DOC could serve as a crucial carbon source for rapid strain growth, and the larger pore size may facilitate strains colonization (Dai et al. 2021). The addition of biochar can enhance the phosphate-solubilizing ability of PSMs, by promoting

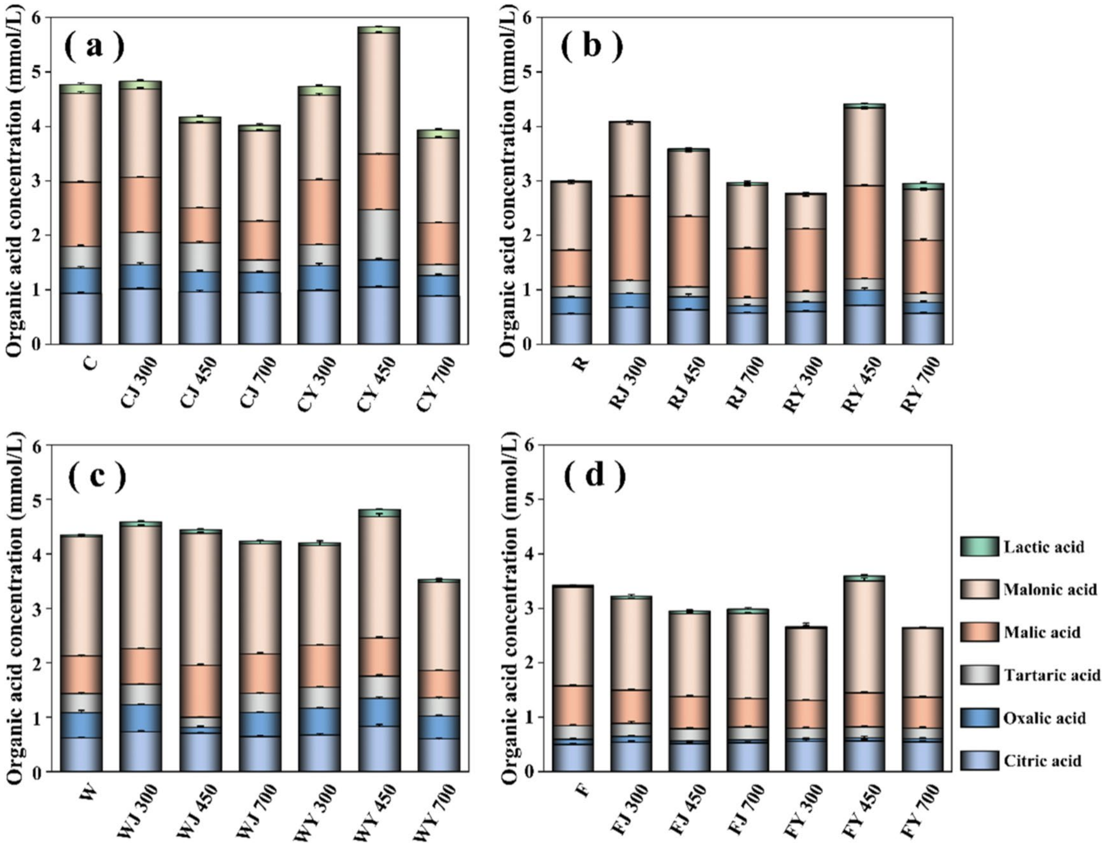


**Fig. 3** Different organic acid concentrations secreted by *T. pinophilus* from the rhizosphere soil of corn (**a**), radish (**b**), wheat (**c**), and the composite strains (**d**) after adding biochars

**Table 2** The correlation analysis between the phosphate-solubilizing ability and the concentration of overall or individual organic acids

| | PSA | TOA | CA | OA | $MA_1$ | TA | $MA_2$ | LA |
|---|---|---|---|---|---|---|---|---|
| PSA | | | | | | | | |
| TOA | 0.56** | | | | | | | |
| CA | 0.47* | 0.80*** | | | | | | |
| OA | 0.38* | 0.78*** | 0.70*** | | | | | |
| $MA_1$ | 0.28 | 0.35 | 0.29 | 0.17 | | | | |
| TA | 0.40* | 0.76*** | 0.70*** | 0.65*** | 0.025 | | | |
| $MA_2$ | 0.34 | 0.66*** | 0.28 | 0.39* | −0.27 | 0.46* | | |
| LA | 0.32 | 0.59** | 0.77*** | 0.47* | 0.11 | 0.43* | 0.26 | |

*PSA* Phosphate-solubilizing ability, *TOA* Total organic acids, *CA* Citric acid, *OA* Oxalic acid, $MA_1$ Malic acid, *TA* Tartaric acid, $MA_2$ Malonic acid, *LA* Lactic acid

$^{***}$: $p \leq 0.001$

$^{**}$: $p \leq 0.01$

$^{*}$: $p \leq 0.05$

their growth and reproduction, thus enhancing their biomass (Hill et al. 2019).

The OD600 value with corn straw biochar was higher than that with chicken feather biochar. Combined with the results of DOC content in Table 1, this might be related to the fact that corn straw biochar can provide more carbon sources for PSMs (Dai et al. 2021). Despite the high nitrogen content in chicken feather biochar, it did not show significant benefits in promoting strain biomass. Therefore, the nitrogen content of biochar did

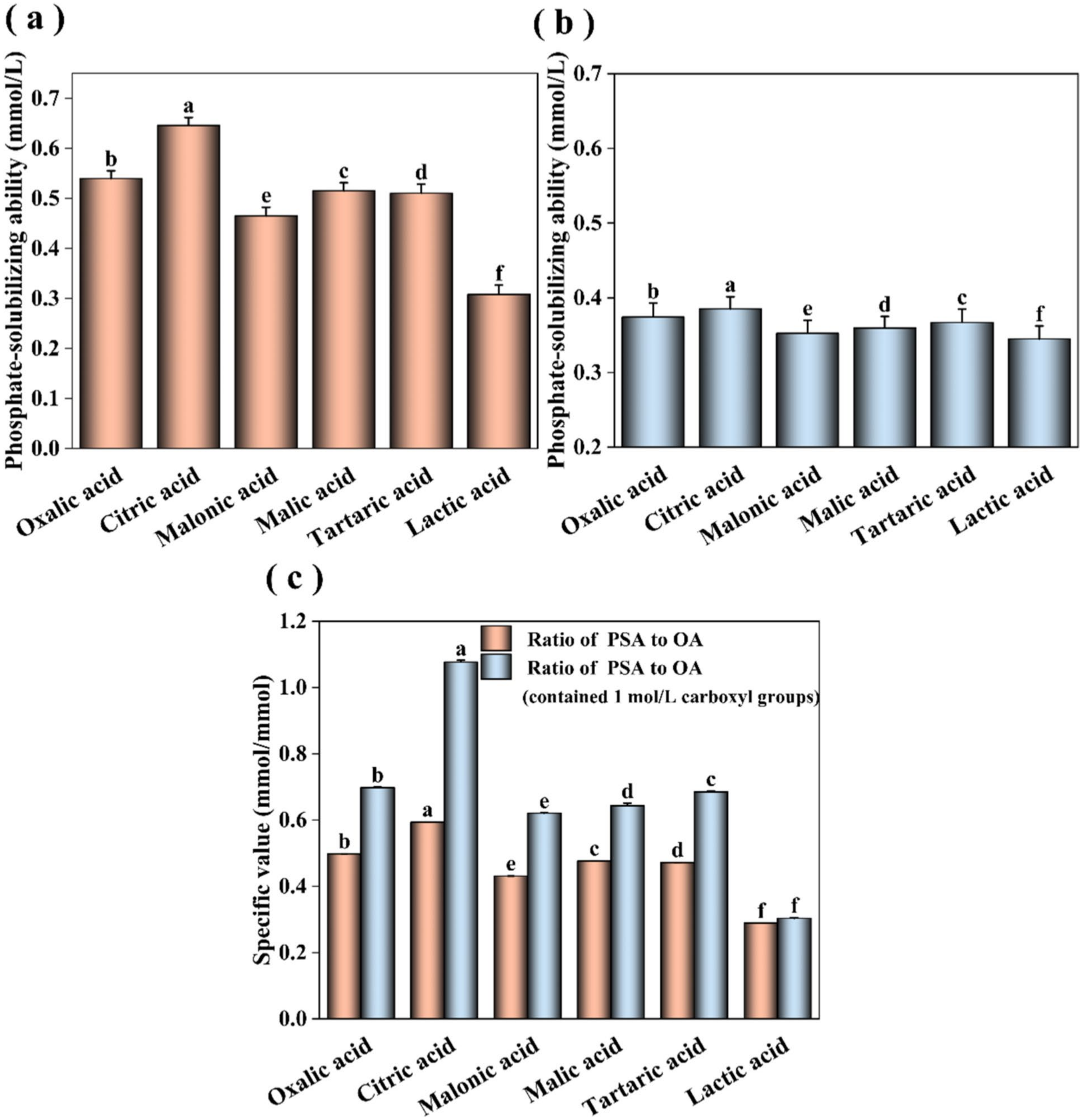


**Fig. 4** **a** Phosphate-solubilizing ability after adding 1 mL of 0.1 mol/L solution of different organic acids to a 10 mL $FePO_4$ mixture. **b** Phosphate-solubilizing ability after adding 1 mL of different organic acid solutions containing 0.1 mol/L carboxyl groups to a 10 mL $FePO_4$ mixture. **c** The ratio of phosphate-solubilizing ability to organic acid concentration (PSA: phosphate-solubilizing ability; OA: organic acid concentration). Bars with different letters (a, b, c, d, e, and f) indicate a significant difference at $p < 0.05$ level

not play a vital role in improving strain biomass or the phosphate-solubilizing ability of PSMs. Meanwhile, the correlation analysis (Fig. 2c) showed no significant correlation between the phosphate-solubilizing ability and biomass of strains. It could be initially concluded that the increased biomass may not directly enhance the phosphate-solubilizing ability. Subsequent analysis will explore the impact of biochar on the activities of *T. pinophilus,* which is crucial in solubilizing P (Ding et al. 2005).

### 4.2 Biochar promoted the secretion of organic acids by PSMs

Previous studies have suggested that the activity of PSMs could be described by their excreted phosphatase and/

or organic acids (Xiao et al. 2020). The concentrations of phosphatase generally increased after biochar application and different strains showed dramatically different phosphatase excretion (Fig. S1). However, no general correlation was observed between phosphatase concentration and phosphate-solubilizing ability. It has been reported that phosphatase mostly interacts with organic P forms, while this study applied inorganic $FePO_4$ as the sole P source (Rawat et al. 2020). Thus, this study focused on the discussion of the impacts of the excreted organic acids on the phosphate-solubilizing ability of PSMs. Particularly, strain Y450 demonstrated a notable advantage in promoting organic acid secretion, aligning with the observed enhancement of phosphate-solubilizing ability. This observation highlights the importance of organic acids secreted by PSM in facilitating phosphate solubilization.

However, there were instances where the phosphate-solubilizing ability improved with biochar addition (Fig. 2a), yet the total organic acids decreased (Fig. 3). For example, the strains isolated from corn rhizosphere soil showed enhanced phosphate-solubilizing ability in J450 and J700 systems, but this was accompanied by a decreased total organic acid content. Similar results were observed in strains from radish and wheat rhizosphere soils. Thus, the increase in total organic acid content alone does not fully explain the enhanced phosphate-solubilizing ability. Further investigation into the relationship between different components of organic acids and the phosphate-solubilizing ability should be conducted.

The enhanced phosphate-solubilizing ability is thus better explained when incorporating the organic acid components. For instance, in the systems of J450 and J700 to the strains from corn rhizosphere soil, Y300 to the strains from radish rhizosphere soil, and J700 and Y300 to the strains from wheat rhizosphere soil, although the total organic acids decreased, the increased content of citric acid could well explain the improved phosphate-solubilizing ability. Combined with the correlation analysis (Table 2), it showed that citric acid may play an important role in phosphate-solubilizing process. Therefore, we emphasize that the improved phosphate-solubilizing ability of the strains after biochar addition could not be understood from the abundance of the biomass, but closely related to the type of organic acids. However, our correlation analysis also suggested that citric acid content is positively related to other organic acids, such as oxalic acid and tartaric acid. The following analysis was conducted to reveal if phosphate activation resulted from the inherent properties of citric acid.

### 4.3 The phosphate activation of different organic acids

Citric acid demonstrated the strongest activating ability to $FePO_4$ among the tested organic acids, providing support for the earlier supposition that citric acid may play a primary role in the phosphate-solubilizing process (Fig. 4a). More importantly, it has been well documented that organic acids can increase the solubility of phosphate by lowering the pH of the environment or chelating the metals associated with phosphate (Pereira and Castro 2014). These properties are both related to the carboxyl group in the organic acid structures (Jiang et al. 2020). Citric acid contains three carboxyl groups in its molecular structure, which is the highest among the investigated organic acids. The experiment with the same molar concentration of carboxyl groups (Fig. 4b) also proved the important role of carboxyl groups in activating phosphate.

Some variations of phosphate solubilization could still be noted in Fig. 4b. For example, (1) citric acid still exhibited the highest phosphate solubilization, which may be related to its relatively stronger complexation with Fe (Table S2); (2) it is easy to understand that Fig. 4b presents organic acid concentrations lower than those in Fig. 4a, except lactic acid. When phosphate solubilization was normalized by organic acid concentrations, it could be noted that the effectiveness of phosphate solubilization was higher at lower organic acid concentrations (Fig. 4c). We have previously reported that homo-conjugation among organic acids may result in decreased complexation with metals (Zhao et al. 2018). The increased organic acid concentration, or the coexistence of various organic acids may decrease the effectiveness of carboxyl groups in activating phosphate. These proposed processes need to be investigated in an independently designed experiment for the purpose of maximizing the role of organic acids in phosphate activation.

## 5 Conclusions

This study investigated the effects of biochar on the phosphate-solubilizing ability of PSMs isolated from the rhizosphere soil of various plants. The application of biochar generally enhanced the phosphate-solubilizing ability of the PSM strains. This study emphasized the importance of secreted organic acids by PSMs, rather than an increase in strain biomass, in activating deposited phosphate sources. Particularly, citric acid showed the most significant correlation with the phosphate-solubilizing ability of the strains, highlighting the crucial role of carboxyl groups in this process. Therefore, enhancing the availability of these carboxyl groups could be a valuable technique to efficiently activate inorganic phosphate. However, it is crucial to consider factors such as the homo-conjugation of organic acids, their adsorption onto mineral particles, and the presence of other multivalent metals, all of which could limit the availability of organic acids for phosphate activation. Further research in these areas could contribute to the development of phosphate-activating techniques and sustainable agricultural practices.

### Abbreviations

| | |
|---|---|
| DOC | Dissolved organic carbon |
| NBRIP | National Botanical Research Institute's phosphate growth medium |
| P | Phosphorus |
| PSMs | Phosphate-solubilizing microbes |
| *T. pinophilus* | *Talaromyces pinophilus* |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44246-025-00193-w.

Supplementary Material 1

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### Authors' contributions

All authors contributed to the study conception and design. Investigation, data curation and writing-original draft were performed by Lijia Lu. Investigation and validation, editing the draft were performed by Weiheng Qin. Writing-review & editing, funding acquisition, and project administration were completed by Min Wu. Investigation, methodology, and preparing visualization were performed by Quan Chen. Writing-review & editing, funding acquisition were completed by Bo Pan. Writing-review and editing were performed by Baoshan Xing. All authors read and approved the final manuscript.

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### Data availability

The data proving the results of this study are available within the paper and related supporting information.

## Declarations

### Competing interests

Bo Pan and Baoshan Xing are editors for *Carbon Research* and were not involved in the editorial review, or the decision to publish this article. All authors declare that there are no competing interests.

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