土壌リン動態とトウモロコシ苗のリン吸収におけるリン溶解細菌の役割

The role of phosphate solubilizing bacteria in soil phosphorus dynamics and phosphorus accumulation of maize seedlings

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Abstract

Phosphorus (P) is a macronutrient element with low availability in soil, which restricts plant growth. Several studies have indicated that phosphate-solubilizing bacteria (PSB) can help for converting unavailable P in the soil and promote plant growth. However, few reports have been found on the differences in P release by PSB strains in red soil (La) and cinnamon soil (Ci), nor have there been reports on the differential effects of PSB on P accumulation in maize grown in these two soils. These two soil types are highly important as arable land types in China and possess different P fractionation and physicochemical properties. This study aims to investigate the role of PSB in soil P dynamics and maize seedling P accumulation, and to determine the similarities and differences in P fractionation changes caused by PSB and P accumulation in maize seedlings in these two soils.

This study revealed that while different PSB strains might cause variations in P fractionation, the patterns of change within the same soil remained consistent. For La, which had a pH of 5.0-5.5 and high iron and aluminum content, the P fractionation was predominantly influenced by moderately labile P (M labile P). Through PSB inoculation, regardless of the provision of tri-calcium phosphate (TCP), the released P was primarily used to increase the content of M labile P, with only a small amount entering the labile P fraction. On the other hand, for Ci with a pH of 8.0 and high calcium phosphate content, the P released by PSB mostly entered the labile P fraction, enhancing the soil's P availability to plants. Therefore, it can be concluded that the effects of PSB strains on soil P release and P fractionation were primarily controlled by soil type. Although the five strains used

in the experiment exhibited different abilities to release phosphates, the trends of change induced by all these strains remained consistent within the same soil. In other words, in Ci, regardless of the strain, none of them caused an increase in M labile P beyond the increase in labile P. Similarly, in La, none of the strains caused an increase in labile P beyond the increase in M labile P, as further confirmed by the results of Pearson correlation analysis.

PSB strains significantly enhanced maize seedling P accumulation in both sterilized La and Ci soils. When PSB was combinedly utilized with TCP in La, it significantly increased maize seedling P accumulation compared to PSB alone. However, in Ci, PSB alone showed promising results in P accumulation, and the combination with TCP did not result in higher P accumulation than PSB alone. Based on the results of Pearson correlation analysis, PSB inoculation was directly and positively correlated with maize seedling P accumulation in Ci without the supply of TCP. In La, although no direct correlation was found between the two, a certain indirect positive correlation, mediated by soil indicators, was observed. In addition, in chapter 3, when PSB strains were inoculated into sterilized soils, PSB inoculation was positively correlated with M labile P in La and with labile P in Ci, while in this sterilized co-culture, PSB inoculation was positively correlated with labile P in all cases except Ci with TCP.

PSB strains did not enhance maize seedling P accumulation in natural soil (non-sterilized La) as significantly as soluble P did. In addition, strain A did not exhibit the same promoting effect on maize seedling P accumulation in natural La as it did in sterilized La (chapter 4). The investigation of PSB's effect on the

rhizobacterial community of maize seedlings revealed that the inoculated strains did not become dominant species in the rhizobacterial community. Nevertheless, PSB inoculation still altered the structure of the rhizobacterial community. Canonical Correspondence Analysis found a positive correlation between the abundance of *Pseudomonas*, to which strain G belongs, and maize seedling P accumulation, and the abundance of dominant species in all three groups (A, F, and G) was positively correlated with PSB inoculation. When data of P treatment was excluded, Pearson correlation analysis revealed an indirect positive correlation between PSB inoculation and maize seedling P accumulation, which was mediated by soil acid phosphatase activity.

Chapter I

Introduction

1.1 Background

Phosphorus (P) is a macroelement for plants and is one of the limiting factors restricting their growth (Aerts and Chapin, 1999; Raghothama, 2005). Although it widely exists in the soil (Larsen, 1967), only a small part can be absorbed by plants directly. P is mainly present in unavailable forms such as insoluble and organic forms. Insoluble P in soils can be mobilized by plant and microbial functions. Since scientists found that some bacteria can dissolve insoluble natural raw rock phosphate in the early 20th century (Sackett *et al.*, 1908), research on phosphate-solubilizing bacteria (PSB) has been widely carried out in various aspects (Khan *et al.*, 2009; Ingle and Padole, 2017). It has been shown that a wide range of PSB isolates contributes to transforming unavailable P in soils and supporting plant growth. Some studies have focused on PSB themselves, such as the strains' function and mechanism of degrading phosphates (Amy *et al.*, 2022; Ding *et al.*, 2021), as well as PSB population and its dynamics (Djuuna *et al.*, 2022). Studies involving crop productivity have also been conducted, including the application to transform soil unavailable P (Alam *et al.*, 2021; Liu, X. *et al.*, 2021) and supply P to crops (Khan *et al.*, 2022; Alam *et al.*, 2022), and even to improve soil quality (Dasila *et al.*, 2022; Pathak *et al.*, 2021).

1.1.1 Phosphorus (P)

1.1.1.1 What is P

Phosphorus (P) is a geochemical element with atomic number 15, represented by the symbol P. P ranks approximately tenth in the Earth's elemental reserves, with a content of around 0.12% (Rudnick *et al.*, 2003). It primarily exists in the natural environment of the Earth in the form of phosphate minerals such as apatite and calcite, which are commonly found in rocks (Li *et al.*, 2019; Decrée *et al.*, 2020; Dong *et al.*, 2020). As rocks containing these minerals undergo weathering and erosion processes, phosphates are slowly released into the soil and water, making them available in other forms or more easily accessible to organisms (Sun *et al.*, 2005). Alongside the nutrients brought about by rock weathering, the decomposition of feces and animal and plant carcasses also serves as an important source of soil P nutrition (Ito *et al.*, 2010; Kear, 1963) (Fig. 1.1.1.1).

P is one of the major chemical elements of life, forming the backbone of DNA and RNA molecules and serving as the primary source of energy in cells. It participates in various metabolic pathways such as phosphorylation, signal transduction, and fatty acid metabolism. Plants absorb P in the form of $H_2PO_4^-$ and HPO_4^{2-} and utilize it for synthesizing nucleic acids, phospholipids, and coenzymes. They regulate the activity of diverse enzymes through phosphorylation and use P to synthesize phosphoric esters with sucrose, facilitating the transport of sucrose within plants. Insufficient P in plants leads to metabolic blockages, resulting in stunted growth, reduced tillering, delayed maturity, and decreased reproductive ability. The presence of a purple-red coloration in the stems or leaves often

indicates P deficiency (Pan, 2004; Marschner, 2011).

A study conducted by Mollier *et al.* (1999) found that when maize plants are deficient in P, leaf area expansion and root development are significantly hindered.

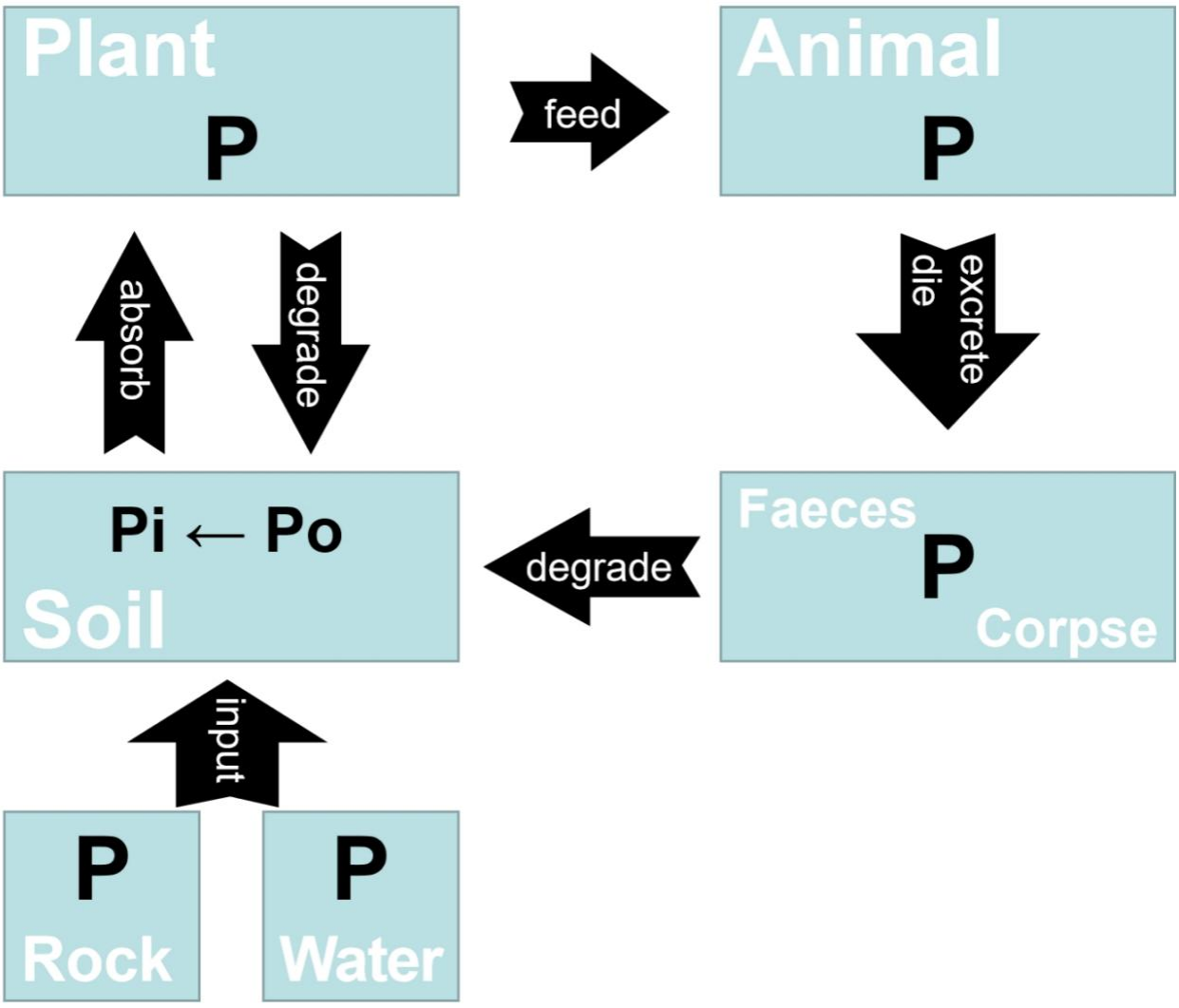


Fig. 1.1.1.1 Phosphorus cycling in soils

Although root development is slightly stimulated in the first few days of P starvation, it ultimately cannot grow well due to the inhibition of leaf development caused by P deficiency, leading to a decrease in photosynthesis. According to the findings of Zhao *et al.* (2013), the deficiency of P is a factor that contributes to the manifestation of symptoms in citrus huanglongbing disease, and supplying P to diseased trees helps alleviate HLB symptoms. Lovelock (2006) discovered through

the study of mangrove physiological processes that insufficient P inhibits normal physiological development and photosynthesis of mangrove plants.

Plants absorb phosphorus (P) from the soil through their roots, but due to various factors, the amount of P absorbed by plants is limited (Bieleski, 1973; Raghothama, 2005; Smil, 2000; Sohrt *et al.*, 2017). In fact, plant productivity in terrestrial ecosystems, including natural ecosystems and farmland, is primarily constrained by P (Du *et al.*, 2020; Hou *et al.*, 2020; Vitousek *et al.*, 2010).

1.1.1.2 P in soils

Plants mainly absorb P from the soil through their roots, and the absorbed P is in the form of water-soluble inorganic compounds ($H_2PO_4^-$ and a small amount of HPO_4^{2-}) that exist in the soil solution. The concentration of these P compounds is usually around 1 ppm or lower (Mullins, 2009). However, the total amount of P in the soil is not disappointingly low. For example, the total P content in Frigid desert soils (Great group in Chinese soil taxonomy) in China reaches 19.1×10^2 g/m^3, Fluvi-aquic soils are 17.7×10^2 g/m^3, and volcanic soils also reach 16.0×10^2 g/m^3 (Zhang *et al.*, 2005). The common concentration of total P in global soils is about 200-800 mg/kg of soil (Tiessen, 2008).

Soil P availability is influenced by many factors, such as soil pH, organic matter, free iron or aluminum, calcium carbonate, and temperature (Muindi, 2019). Once P enters the soil, it is fixed in various ways: in acidic soils, P is quickly immobilized with iron and aluminum, while in alkaline soils, it is immobilized with calcium ions to form calcium phosphate or calcium magnesium phosphate

(Hemwall, 1957; Hsu, 1965; Bear and Toth, 1942; Cho and Caldwell, 1959). Chang and Chu (1961) reported that in soils with a pH range of 5.3 to 7.5, most of the P was fixed to form Al-phosphate, a portion was fixed by Fe-phosphate, and the form of Ca-phosphate was retained the least. Mackenzie and Amer (1964) found that in soils such as Haldimand clay, Vasey, Oneida, Honeywood, Guelph, and Minesing (pH: 5.3-7.6), the increase of Ca-P exceeded A1-P or Fe-P in Minesing soil at pH 7.6. The potential of the soil to remove phosphate from the soil solution is called soil Phosphorus Retention Potential (PRP) (Wild, 1950), which is generally classified as Low, Moderate, High, and Very High. Soils with Very High PRP are mainly concentrated in Central and South America, Southwestern North America, Central Africa, Northern Europe, and South Asia. Soils with Low PRP are mainly concentrated in Central North America, Central Europe, Central Asia, West Asia, Southeast Asia, and some parts of Australia (Reich, 1998). PRP is also influenced by soil mineralogy, clay content, soil pH, as well as climate factors such as temperature and humidity (Batjes, 2011).

In order to study the distribution of phosphorus (P) in soil more effectively and support practical applications such as P mobilization, scientists have been exploring increasingly rational schemes for soil P fractionation extraction and determination for decades.

In 1957, Chang and Jackson proposed a relatively complete soil fractionation extraction method based on the different solubilities of various phosphate compounds. The main steps are as follows:

1）P in the soil solution, physical adsorption P and exchangeable calcium,

obtained via 1 N NH_4Cl;

2）Al-P, P combined with aluminum, obtained via leaching with 0.5 N NH_4F (forms of P extractable by this solution: Al-P completely, Fe-P slightly);

3）Fe-P, P combined with iron, obtained via leaching with 0.1 N NaOH (forms of P extractable by this solution: Al-P, Fe-P, organic-P);

4）Ca-P, P combined with calcium, obtained via leaching with 0.5 N H_2SO_4 (forms of P extractable by this solution: Ca-P completely, Al-P and Fe-P considerably);

5）O-P, reductant soluble Fe-phosphate (iron oxide occluded), occluded Al-P and occluded Al-Fe-P.

Although this method is widely used in acidic and neutral soils, NH_4F is not very effective in distinguishing between Al-P and Fe-P. In addition, NH_4F reacts with calcite in calcareous soils to form CaF_2, which strongly adsorbs P and affects the actual measurement of Ca-P. To overcome this limitation, Chinese scientists Jiang Baifan and Gu Yichu (1989) proposed a fractionation extraction method specifically for calcareous soils. The main steps are as follows:

1) Ca_2-P, obtained via 0.25 M sodium bicarbonate solution at pH 7.5;

2) Ca_8-P, obtained via leaching with 1 M NH_4Ac solution at pH 4.2;

3) Al-P, obtained via leaching with 0.5 M NH_4F solution at pH 8.2;

4) Fe-P, obtained via leaching with 0.1 M NaOH-Na_2CO_3 solution;

5) O-P (occluded-P), obtained via leaching 0.3 M sodium citrate - 0.5 g sodium dithionite - 0.5 M sodium hydroxide solution;

6) Ca10-P, obtained via leaching with 0.5 M H_2SO_4 solution.

7) This method subdivided calcium bound P into different types as dicalcium phosphate (Ca_2-P), octacalcium phosphate (Ca_8-P) and decacalcium phosphate (Ca_{10}-P). it is widely used in the study of P fractionation in calcareous soils in the central and northern regions.

Today, the soil P fractionation measuring method that was widely used is the method proposed by Hedley in 1982. Hedley method combines the determination of inorganic and organic P, and extracts soil P with five solutions, measuring seven P fractions:

1) Resin exchangeable P (Resin-P) mainly contains inorganic P. This part of inorganic P can be exchanged via anion exchange resin, plants can absorb this part directly;

2) $NaHCO_3$ extracted P ($NaHCO_3$-P), including inorganic P ($NaHCO_3$-Pi) and organic P ($NaHCO_3$-Po). The organic P part contains the organic P dissolved in the soil solution, which is easy to be mineralized and absorbed by plants, with high effectiveness. Inorganic P mainly refers to the inorganic P adsorbed on the soil surface, which is easy to be absorbed and utilized by plants, with high effectiveness;

3) NaOH extracted P (NaOH-P), including inorganic P (NaOH-Pi) and organic P (NaOH-Po). Sodium hydroxide extracted P mainly refers to the chemically adsorbed P on the surface of iron aluminum oxides and clay minerals, which is very rich in soil;

4) HCl extracted P (HCl-P), including inorganic P; roughly composed of calcium phosphate, but also including some inorganic P which is occluded within

sesquioxides and released on the partial dissolution of these oxides.

5) Residual P, including inorganic P and organic P. Residual P mainly refers to P that cannot be extracted in the previous steps and is extracted by high-temperature digestion, include occluded phosphates and most stable organic phosphates.

Based on Hedley method, Yang and Post (2011) investigated the relation between the distribution of different forms of P and the stage of soil development. Hou *et al.* (2018) provide a global dataset of soil P fractions separated using the Hedley method. Xu *et al.* (2018) used the Hedley method to study the nonadditive effects of biochar amendments on soil P composition in acidic and alkaline soil. Hedley method was also used to analyze P fractions in Andisol and Ultisol (Delfim *et al.*, 2020) and plantation soil in Jiangxi, China (Liu *et al.*, 2022).

In the Hedley method, the P extracted by resin and $NaHCO_3$, which can be directly absorbed and utilized by plants, is referred to as available P. The most widely used direct method for measuring available P in soil is the $NaHCO_3$ extraction method proposed by Olsen in 1954. Using the Olsen method, researchers have reported the latest global surface soil available P content (McDowell *et al.*, 2023). The report indicates that the content of available P in soils is below 2 mg/kg soil in some parts of northern Africa, central and western Asia, central Australia, and southern North America. In some areas of South America, South Africa, southern Asia, and central Asia, it ranges from 2-5 mg/kg soil. The proportion of land with available P greater than 40 mg is relatively low and includes only small areas of Europe, eastern Asia, eastern North America, and

the western coast of South America. However, for cultivated land, due to human intervention, the accumulation of P has reached a high level. Li (2011) reported that from 1980 to 2007, the average P accumulation in arable land in China was 242 kg per hectare. Meanwhile, Olsen-P increased from 7.4 mg/kg soil in 1980 to 24.7 mg/kg soil in 2007. Furthermore, 9.3% of cultivated land has exceeded a level of 40 mg/kg soil (Li *et al.*, 2011), which poses a risk of P leaching (Zhong *et al.*, 2004). George also mentioned in their article that many cultivated soils worldwide have accumulated large amounts of P that are sufficient for decades of food production (George *et al.*, 2016) but also pose significant risks to the environment through loss and harm (Elser and Bennett, 2011). The fundamental cause of the accumulation of P in cultivated land is the huge input of P fertilizer in agricultural production.

1.1.1.3 Supply of Soil P - Phosphate Fertilizer

The P provided by natural soil can hardly reach the P nutritional level required for high-density agricultural cultivation (Breman *et al.*, 2001; Fageria and Oliveira, 2014). If farming want to get a good yield in planting, the P concentration of the soil solution should reach 1.5 μmol-P/L in wheat and rapeseed, 5 μmol-P/L in tomato and soybean, and 7 μmol-P/L in onion (Föhse *et al.*, 1988). In order to make up for the shortage of P supply in natural soil, in agricultural production, P was added to cultivated land by applying P fertilizer, but in fact, the P fertilizer applied in agriculture is often much higher than the actual P demand of crops. Taking vegetable cultivation in China as an example, greenhouse vegetables

harvested 44 kg-P per hectare per season, while fertilization was as high as 541 kg-P per hectare; open-air cultivated land harvests 25 kg-P per hectare per season, and the input of phosphate fertilizer reached 117 kg-P per hectare (Yan *et al.*, 2013). In Japan in 1997, the amount of P applied to farmland soil was 6.3 times the amount absorbed by crops (Mishima *et al.*, 2003). A small part of the P put into the soil was used by crops, and most of it was bound to the soil and retained in the soil (Mishima *et al.*, 2003). The survey found that the annual accumulation of P in agricultural land in China exceeded 39 kg per hectare (Zhang *et al.*, 2019). A study on the continuous application of P fertilizer in paddy soil in southern South Korea for 31 years found that the application of chemical fertilizers did not increase the inorganic P content, but increased the organic P content, and it significantly increased the total P of the soil (Lee *et al.*, 2004), this research indicated that during 31 years of continuous cultivation, approximately 37% of P was contributed to crop accumulation, and approximately 50% of P was accumulated in the soil.

Although the yield of agricultural products benefits from the application of P fertilizer, the utilization rate of P fertilizer rapidly decreases uncontrollably with the increase of P fertilizer application amount. In the past 10 years until 2014, the use of P fertilizer in China has been continuously increasing, with an average annual increase of 3.5 kg per hectare. However, as the use of P fertilizer increases, the utilization rate of P fertilizer (crop P yield ÷ P input ×%) Decreased by 48 percentage points, and by 2014, the utilization rate of agricultural P fertilizer could only remain at 20% (Zhang *et al.*, 2019). Many studies have reported on the critical value (The soil available P level required when the plant output reaches

95% of the maximum output) of Olsen-P in soil. For example, the critical value for British grasslands was 23-25 mg Olsen-P kg^{-1} soil (Johnston *et al.*, 2013); The critical value of Australian grassland was 9-15 mg Olsen-P kg^{-1} soil (Sandral *et al.*, 2019); The critical value of Spanish ryegrass soil was 24 mg Olsen-P kg^{-1} soil (Sánchez *et al.*, 2015). Researches suggested that exceeded the critical value, no amount of P input could significantly increase plant output. Studies have shown that applying P fertilizer in low P soil (Olsen-P $\leqslant$ 5 mg kg^{-1} soil) could effectively promote an increase in absolute yield; In soils with higher P content (Olsen-P > 10 mg kg^{-1} soil), even applying a large amount of P cannot achieve high P utilization efficiency (Ros *et al.*, 2020).

In addition, numerous researchers have discovered that P, which was not absorbed by crops or fixed in the soil, was carried away from the land through rainwater erosion and surface runoff. The greater the application of P fertilizer, the higher the magnitude of the loss (Cao and Zhang, 2004; Xia *et al.*, 2008; Zhang *et al.*, 2003). In 31 years of continuous fertilization and cultivation in South Korea, when 37% of the P input was absorbed by plants, 13% of P might be lost through leaching (Lee *et al.*, 2004). In Japan, when the P utilization rate in farmland reached 16% of the P application rate, the P loss caused by leaching from farmland was 0.6% of the P application rate (Mishima *et al.*, 2003). Such agricultural spills have become a major cause of P pollution in the Earth's water system (Chowdhury *et al.*, 2017).

In general, P fertilizer had become one of the factors that modern agricultural production depends on, but the utilization rate was very low. It has resulted in a

low ratio of production input and return, raising production costs, and these high inputs have also brought about serious environmental pollution problems such as water eutrophication (Sims and Sharpley, 2005; Sharpley *et al.*, 2013).

In addition, the increasing inputs were depleting the Earth's P reserves, which is becoming a serious problem as such production methods cannot support long-term human agricultural production. Fortunately, only a small portion of the P input in the early stages of agriculture leached out of the soil, with the majority being retained in the cultivated soil (Lu and Tian, 2017). Whether P fertilizer input can be combined with the development and mobilization of these retained P in the soil to reduce the amount of P fertilizer input in the future is a question worth exploring. In this regard, researchers have proposed a series of methods to reduce P fertilizer application and increase P efficiency. Among them, the utilization of phosphate solubilizing bacteria (PSB) as biological phosphate fertilizer to mobilize soil P is an environmental and sustainable way to partially substituted chemical phosphate fertilizer (Zhang *et al.*, 2019; Walpola and Yoon, 2012; Dandessa and Bacha, 2018; Sharma *et al.*, 2013).

1.1.2 Phosphate solubilizing bacteria (PSB)

1.1.2.1 What is PSB

Since scientists found that some bacteria can dissolve insoluble natural raw rock phosphate in the early 20th century (Sackett *et al.*, 1908), research elucidating PSB has been pursued widely (Khan *et al.*, 2009; Ingle and Padole, 2017).

PSB can accelerate soil P cycling (Hafeez *et al.*, 2019; Liu *et al.*, 2022) and

can counteract the antagonistic effects of soil calcification on bioavailable P (Adnan *et al.*, 2017). The PSB number can be related closely to the P fraction (He and Wang, 2022). It has also been also shown that PSB can increase the available P by reducing the intensity of soil P retention (Halder *et al.*, 1990; Chen *et al.*, 2006; Ku *et al.*, 2018; Tian *et al.*, 2021),

Reportedly, PSB shows different effects on P transformation in different soils. Hafeez *et al.* (2019) found that PSB raised the labile P fraction considerably in an alkaline (pH 8.1) sandy loam soil after adding TCP. Delfim *et al.* (2020) found that PSB exerted strong effects on NaOH-P in Andisol and Ultisol, which are both acidic soils with similar P fractions. Furthermore, PSB showed an ability to slash "P-fixation capacity" (Biswas *et al.*, 2022). The reports described above are informative, but few comparative studies have examined effects of the same PSB strains on P fractions in Lateritic red earths (La) and Cinnamon soils (Ci) with different P fractions and PRP.

PSB can be divided into two categories according to their substrates: one is inorganic P bacteria that can convert insoluble inorganic P (such as inorganic phosphate, phosphorous lime, etc.) into soluble P; One is organic P bacteria that can mineralize P containing organic substances such as phospholipids and organic P pesticides. However, most P bacteria can not only dissolve inorganic P, but also degrade organic P, so it is difficult to accurately distinguish between organic P bacteria and inorganic P bacteria. There are many bacteria that have been isolated and confirmed to have phosphate solubilizing ability: Wani *et al.* (2005) isolated *Serratia*, *Pseudomonas* and *Bacillus* with P-solubilizing ability from different

rhizosphere soils; Chen *et al.* (2006) identified *Serratia*, PSB of genera *Bacillus*, *Rhodococcus*, *Arthrobacter*, *Chryseobacterium*, *Delftia*, *Gordonia* and *Phyllobacterium*; There are also *Xanthomonas* , *Enterobacter*, *Pantoea*, *Klebsiella*, *Vibrio proteolyticus*, *Xanthobacter agilis*, *Burkholderia*, *Paraburkholderia*, *Novosphingobium*, and *Ochrobactrum* and so on (Chen *et al.*, 2021; Chung *et al.* 2005; De Freitas *et al.* 1997; Vazquez *et al.* 2000).

PSB are widely distributed in the soil. PSB exist in normal soil environments such as forests, farmlands, and orchards, and in adverse environments such as mining areas, saline alkali lands, heavy metal contaminated areas and low-temperature soils. The species and quantity of PSB in different habitats are also different, and their distribution is affected by many factors, such as soil properties, tillage measures and methods, rhizosphere effects, etc. The species and diversity of PSB in the rhizosphere soil of natural forests are generally higher than those of artificial forests. The number and population abundance of PSB in the soil of phosphate mining areas are generally higher than those of heavy metal pollution areas and saline areas. The abundance of PSB affected by soil organic components and available P content.

The distribution of PSB is related to tillage measures and methods. The physiological metabolic activities of different plant roots are different, so the community structure and dominant structure of P bacteria in rhizosphere are also different. In addition, cultivated crop intercropping, reasonable rotation and other tillage methods will affect the microbial community structure of soil P bacteria, which will increase the number and abundance of P bacteria. Besides, the

distribution of PSB also shows obvious rhizosphere effect.

1.1.2.2 Mechanism of PSB release P

The P release mechanism of PSB to insoluble inorganic P is relatively complex. At present, the generally recognized mechanisms mainly include the secretion of organic acids, the production of inorganic acids, the assimilation of NH^{4+}, the secretion of iron carriers and extracellular polysaccharides, etc.

P release by organic acids is a mechanism widely recognized by PSB. Organic acids can not only reduce the pH in the environment, but also chelate with metal cations in the environment to dissolve insoluble phosphates (Wang *et al.*, 2009). Different kinds of organic acids have different dissolution effects on insoluble phosphates (Lu *et al.*, 1998). PSBs isolated from the rhizosphere of *Trifolium repens* can secrete various organic acids at different level, such as malic acid, oxalic acid detected in the process of P release (Li *et al.*, 2018).

The P release of most PSB is closely related to the low molecular weight organic acids produced by their metabolism. On the one hand, these organic acids can bind with cations such as Ca^{2+}, Mg^{2+}, Al^{3+}, Fe^{3+}, and release phosphate ions; On the other hand, it can also reduce the environmental pH, thus promoting the dissolution of insoluble phosphate (Seshachala and Tallapragada, 2012). Many studies have shown that the pH of the strain culture medium is significantly negatively correlated with the total organic acid content, and the P release ability is significantly correlated with the organic acid concentration and pH (Yang *et al.*, 2021). The kind and content of organic acids secreted by different PSB are also

different. As mentioned above, such as gluconic acid, malic acid and oxalic acid were detected.

In addition, gluconic acid is the main organic acid synthesized by PSB in the process of P release (Lin *et al.*, 2006; Lee *et al.*, 2012; Stephen and Jisha, 2011), and gluconic acid is oxidized by glucose under the joint action of glucose dehydrogenase (GDH) and coenzyme pyrroloquinoline quinone (PQQ) produced by bacteria. Therefore, GDH or PQQ synthesis related genes of PSB are crucial to their P release function. PQQ biosynthesis involves many genes, often in the form of gene clusters (Wagh *et al.*, 2014; Kim *et al.*, 2003; Gliese *et al.*, 2010).

Some PSB can produce HCl, HN_3, H_2SO_4 and other inorganic acids to release insoluble phosphates. Some bacteria of the genera *Nitrosomonas* spp. and *Thiobacillus* spp. can produce nitric acid and sulfuric acid to dissolve phosphate compounds. However, compared with organic acids, the P release efficiency of inorganic acids is relatively low. In addition, H_2S released by acidophilic sulfur oxidizing bacteria can react with iron phosphate to produce ferrous sulfate, thus releasing phosphate ions. Roy and Roy (2019) found that *Delftia* spp. can oxidize thiosulfate and elemental sulfur to sulfate, and the accumulation of P in plants increased significantly after inoculation of the strain.

PSB release proton hydrogen through the assimilation of NH^{4+}, reducing the environmental pH, thus causing the dissolution of phosphate. Illmer and Schinner (1995) found that the pH of *Pseudomonas spp.* culture solution decreased significantly, and the effective P content increased, but no organic acid was detected. Therefore, they believe that the release of H^+ accompanied by respiration

or assimilation of NH^{4+} is another important P release mechanism.

The iron carrier secreted by PSB is a small molecule iron ion chelate, which can combine with heavy metal ions in the soil to activate insoluble inorganic P. In addition, the formed metal iron carrier complex can combine with the iron carrier receptor protein on the cell membrane to enter cells, improve the iron absorption of plants and promote plant growth. Similar to the chelation effect of iron carrier, the exopolysaccharide secreted by the PSB has abundant hydroxyl and carboxylic acid groups on its surface, which can react with the Al^{3+}, Zn^{2+}, Fe^{3+}, Mg^{2+} in the soil to release phosphate ions. The strain secreting exopolysaccharide has stronger ability to degrade P than the strain not secreting exopolysaccharide, and the strain secreting exopolysaccharide and organic acid has stronger ability to degrade phosphates.

It is generally believed that enzymatic hydrolysis is the main mechanism of organic P degradation. Common enzymes include phosphatase, C-P lyase, phytase, etc. Phosphatase can remove the phosphate group in phosphate ester, which can be generally divided into acid phosphatase and alkaline phosphatase. The content of acid phosphatase was higher in acid soil; Alkaline phosphatase is common in neutral and alkaline soils. C-P lyase can catalyze the cleavage of C-P bond, thereby degrading organic P. Phytin is the main component of organic P in soil, it can’t be absorbed by plants directly; phytase can degrade phytin into inositol and inorganic phosphate. Therefore, P bacteria planted in plant rhizosphere can improve P absorption of plants by secreting phytase.

1.1.2.3 What can PSB do for agriculture

The transformation of soil P by PSB can be described as that PSB promotes the redistribution of P in soil through its own dissolution of insoluble phosphates. The reason why it is called "redistribution" here is that: under the action of PSB, the transformation result of soil P is not only the increase of soluble P, but also the content change of each extractable fractions. These changes combined the transformation of insoluble phosphate into soluble phosphate and the re fixation or re adsorption of soluble phosphate by each extracted fraction. The relationships are plotted in the following diagram (Fig.1.1.2.3). PSB released its metabolites (organic acids, phosphatases, polysaccharides, etc.) into the soil solution, where insoluble phosphates are dissolved or mineralized, releasing dissolved P into the soil solution. Some of the dissolved P released into the soil solution is used by the PSB for its own biomass synthesis, some is reabsorbed or immobilized by the soil, and the remainder is retained in the soil solution as available P which can be used directly by plant.

PSB converts non-available P into effective P from the soil through mineralization (secretion of phosphatase, phytase, etc.) or dissolution (secretion of organic acids, protons, etc.), and there are many reports on PSB improving crop yield and increasing P accumulation in crops (Alori *et al.*, 2017), regarding the ability to function under environmental stress There are many reports of PSBs that dissolve P to promote growth (Dey *et al.*, 2021). Therefore, using PSB to inoculate soil is an effective strategy for soil P release, and it is a good way to reduce chemical P fertilizer input and alleviate environmental pressure.

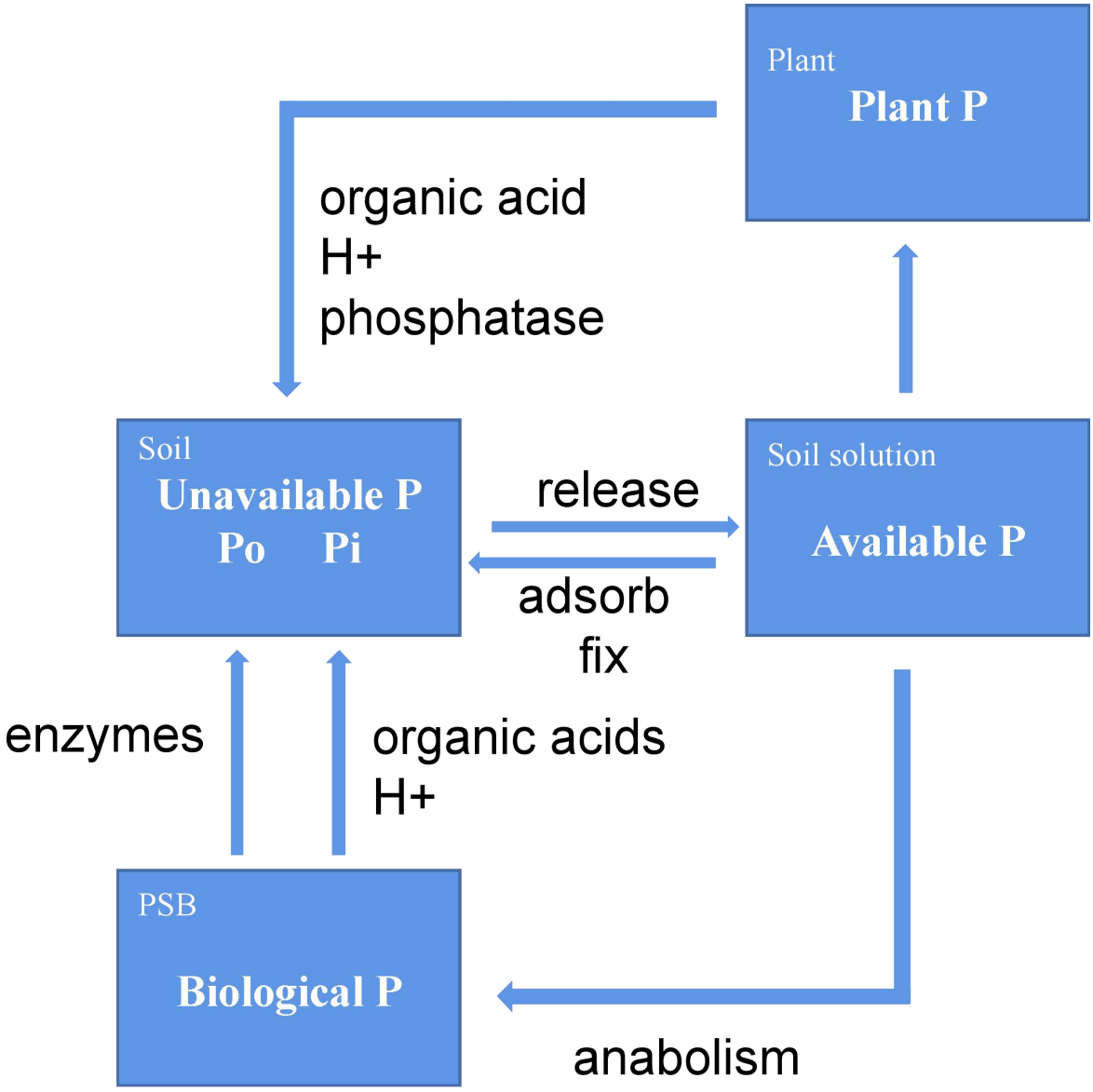


Fig. 1.1.2.3 Soil phosphorus translocation under the influence of PSB

1.2 Research purpose

The purpose of this study was to study the P stratification, enzyme activity and organic acid dynamics induced by PSB in soil and rhizosphere soil, so as to show the role of P solubilizing bacteria in the dynamic change of soil P structure, and to analyze the effect of PSB on P accumulation in maize seedlings based on this. The effects of PSB on the bacterial community in the rhizosphere of maize seedlings were studied by detecting and analyzing the changes of bacterial community structure and diversity in the rhizosphere of maize seedlings caused by PSB. In the soil inoculation and co culture experiments, this study used two kinds of soils with large differences in P grade structure to test, aiming to prompt the physiological and biochemical differences of PSB in different soil environments (mainly different P composition environments), so as to evaluate the ability of PSB as a P supply means under environmental conditions. By studying the influence of PSB inoculation on the composition and diversity of rhizosphere bacterial community of maize seedlings, it is analyzed that PSB affects plant growth by affecting the structure or diversity of rhizosphere bacterial community, so as to evaluate the enthusiasm of PSB inoculation. The innovation of this study is to explore for the first time the different effects of the same PSB strain on the P classification of laterite and cinnamon soil. In addition, this study also discussed the difference of the degradation ability of PSB in the culture medium and soil, and revealed the relationship between the soil type, P component change and PSB strain.

1.3 Research frame

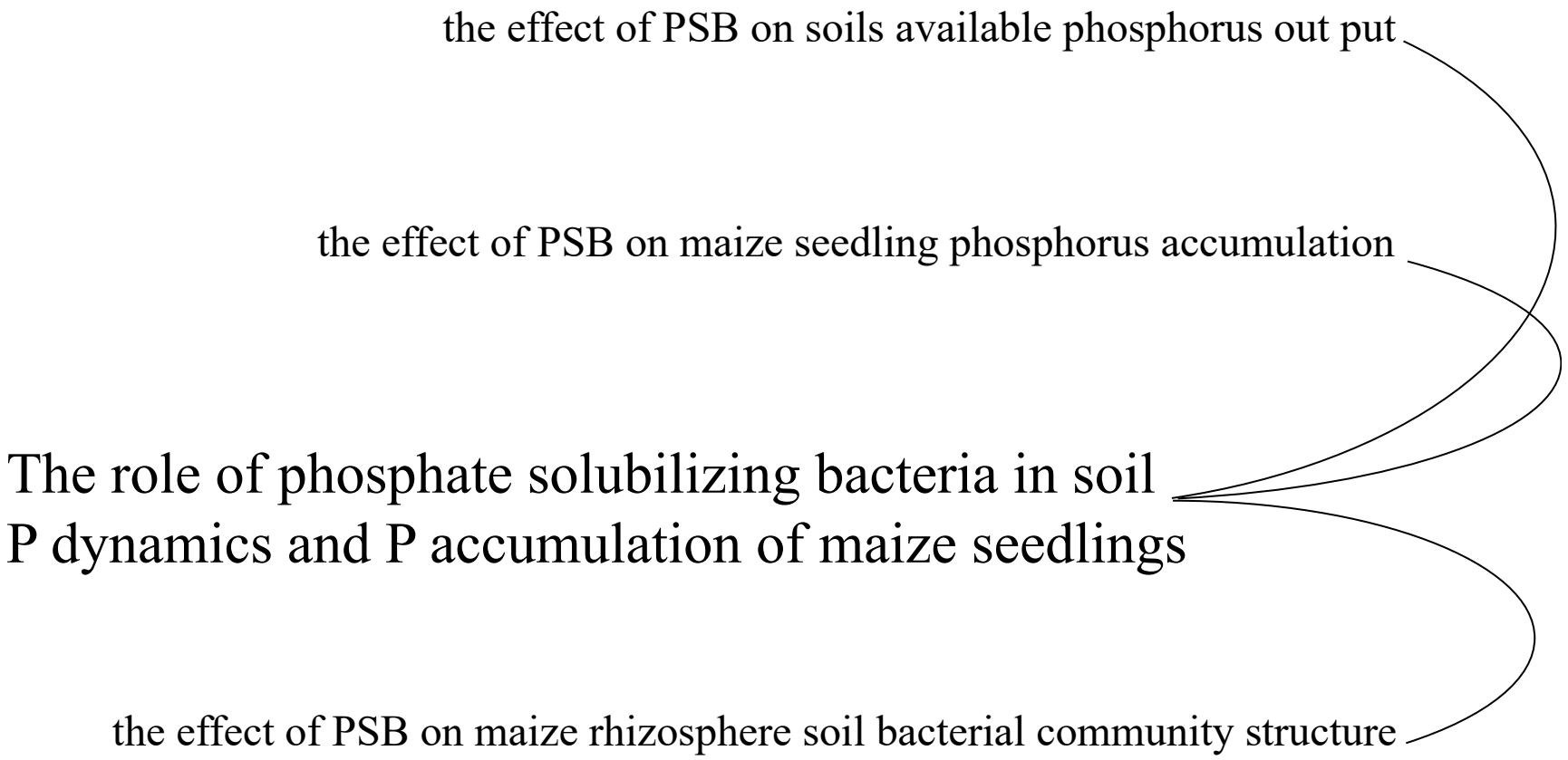


Fig. 1.3.1. Research frame of my study

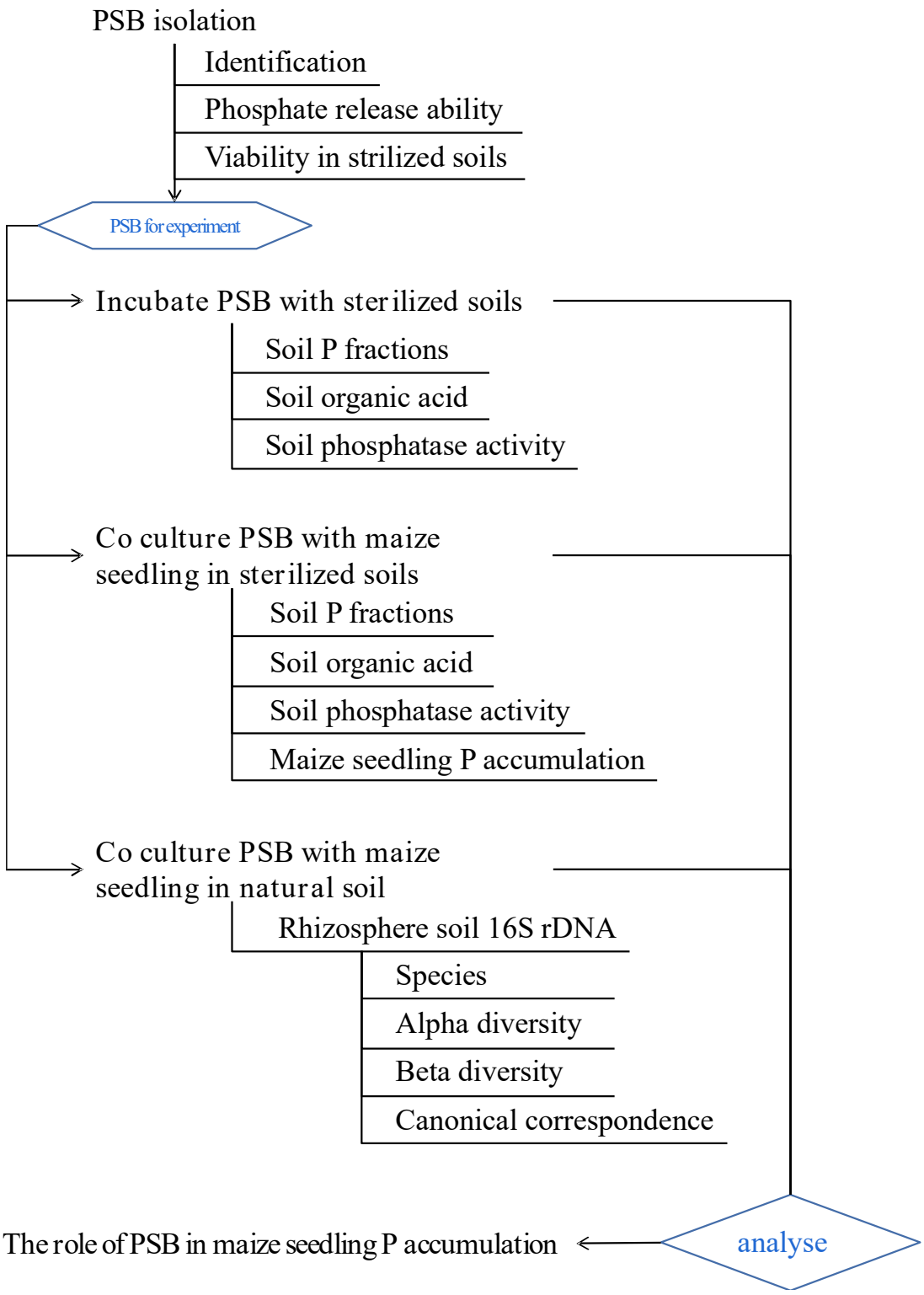


Fig. 1.3.2. Research flow of my study

The frame of the study was indicated in Fig.1.3.1. And the flow oht the study was shown in Fig. 1.3.2. In the short term, this research started with the isolation and identification of PSB, determining the identified PSB strains' P releasing ability to 5 phosphates in shake flasks, and their viability in sterilized soils. PSB strains that exhibited a greater P release capacity and had viability in sterilized soils were used to investigate the effect of PSB on soil P release and maize seedling P accumulation.

Chapter II

Isolation and Screening of PSB Strains

This chapter presents the procedure for isolating phosphate solubilizing bacteria (PSB) from soil and subsequently screening them for further experiments. The processes involved in this study encompass the isolation of PSB from freshly collected natural soils, identification of the isolated PSB strains, selection of different bacterial strains for phosphate release experiments using shake flasks, and testing the survival capability of strains exhibiting high phosphate release ability in soil. Following these experiments, five strains demonstrating both high phosphate release ability and the capacity to survive in sterilized La and Ci soils were chosen for subsequent investigations.

2.1 Materials and Methods

2.1.1 PSB separation and identification

To isolate PSB, non-cultivated La and rhizosphere soil in which Chinese cabbage (*Brassica rapa* L. var. *pekinensis Rupr*, Fig.2.1.1) had been grown were used.

Fig.2.1.1 *Brassica rapa* L. var. *pekinensis Rupr*

2.1.1.1 PSB separation

One gram of each soil was suspended with 99 mL 0.85% NaCl sterilized solution and was gradient diluted (Fig.2.1.2).

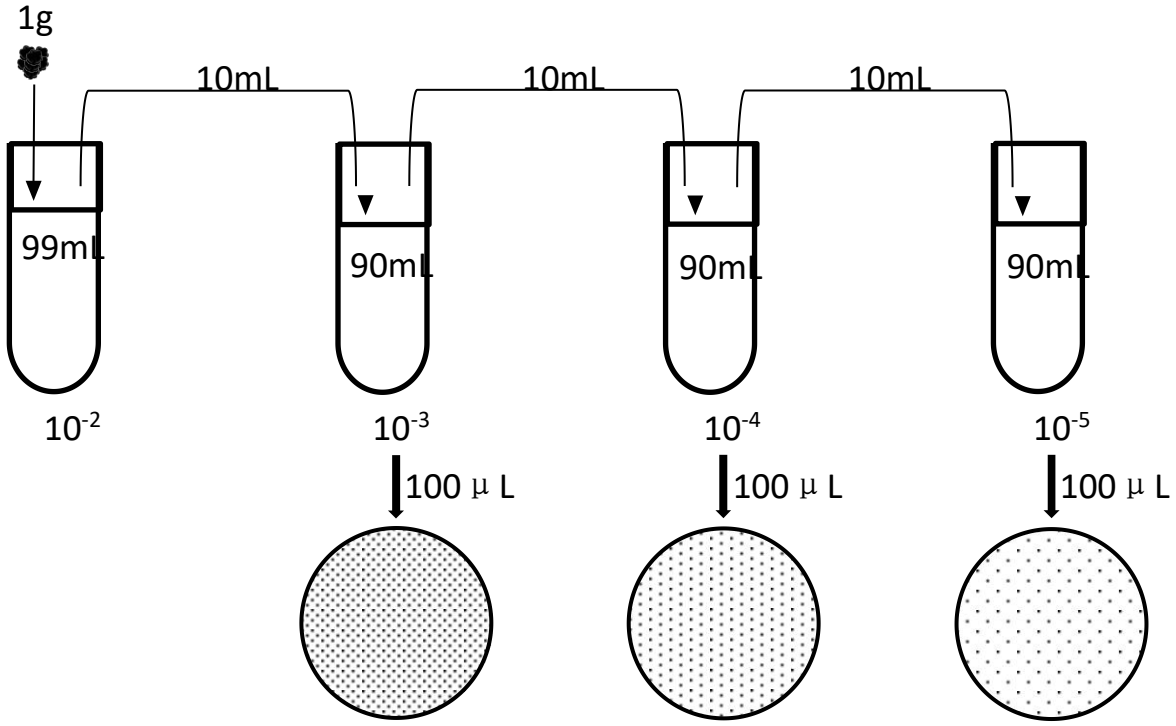
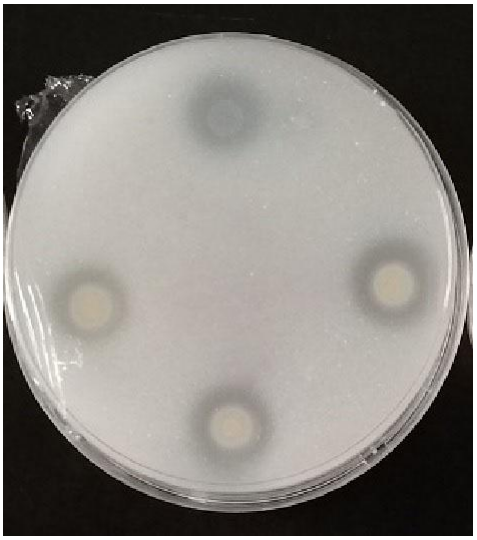


Fig.2.1.2 Image of dilution scheme of soil suspension

After 100 μL quantities of 10^{-3}, 10^{-4}, and 10^{-5} dilutions were cultured on NBRIP agar medium (Nautiyal, 1999), they were incubated at 28°C for 5 days.



Select those colonies with earlier transparent ring formation (Fig.2.1.3), wider ring width and higher ring transparency for purification. The selected colonies were purified on NBRIP (TCP source) until monoclonal colonies were obtained.

Fig.2.1.3 Appearance of transparent rings

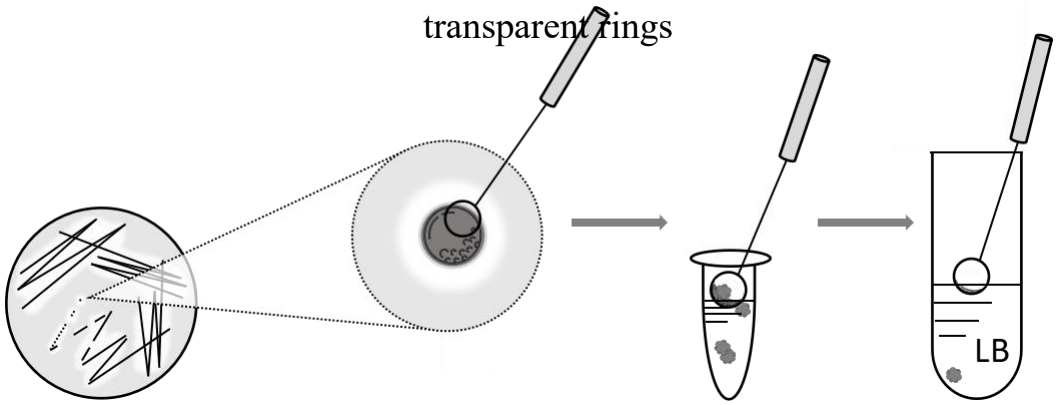


Fig.2.1.4 Image of PSB cultivation in LB

Each monoclonal colony was partly collected in 1.5 mL EP tubes contained with 1 mL sterile pure water for DNA extraction for identification and partly inoculated into 5 mL LB* which were contained in 15 mL culture tube with dual position cap to amplify cells for expanded cultivation (Fig.2.1.4).

2.1.1.2 PSB DNA extraction and identification

For the preliminary identification of 35 strains, DNA were extracted via Chelex100.

Add 2 grams of Chelex100 into a 50 mL tube, filled pure water to 20 mL, and then put a magnetic stir-bar into the tube. Sterilized the tube by autoclave at 121℃ for 15 min. After cooling, stirred the Chelex100 suspension on the magnetic stirrer. Aliquot 200 μL suspension into sterile 0.5 mL EP tubes while keeping the suspension well mixed. Remove 20 μL colony suspension from 1.5 mL EP tubes into 0.5 mL EP tube which contained with chelex100 suspension, and then vortex for about 10 seconds for mixing. Centrifuge the sample tubes quickly for about one second so that all the samples were at bottom together. Put the samples tubes into heat block at 95℃ for 15 min. Vortex and centrifuge again. DNA were in the supernatant.

The kit was used to extract DNA of 5 selected PSB which were used for the following experiments: Purified strains were enrichment with LB at 28℃ for 24 hours, then used to extract DNA with TIANamp Bacteria DNA Kit (Tiangen biotech Co., Ltd, Beijing, China). Extracted DNA were used as a temple for PCR amplification with universal bacterial primers targeting the 16S rRNA gene which are 27F (Lane, 1991) and 1492R (Turner *et al.*, 1999).

PCR was performed with 1μg template DNA, 1 μL (10 μM) of each primer, 12.5 μL 2×Taq PRC Mix (KT210, Tiangen biotech Co., Ltd, Beijing, China), 9.5 μL double distilled H_2O, and followed the step blow: initial denaturation (94°C, 3 min), denaturation (94°C, 30 sec), annealing (55℃, 30 sec), extension (72°C, 1 min). Final extension (72℃, 5 min) was performed after 30 cycles between denaturation and extension.

PCR products were used to agarose gel electrophoresis, then stained the gels with GeneGreen Nucleic Acid Dye (RT210, Tiangen biotech) and visualized by Gel imaging system (WD-9413B, Beijing Liuyi Biotechnology Co., Ltd, China). Purified PRC products with TIANgel Midi Purification Kit (DP209, Tiangen biotech). Purified PCR-amplified 16S rDNA fragments were sequenced by AuGCT Co., Ltd, Wuhan, China. The obtained 16S rDNA sequences of isolated strains were compared and upload to apply the NCBI number in NCBI GenBank.

2.1.1.3 Strain Preservation

Incubated LB with PSB inoculation at 28℃ for 24 hr with rotary shaking at 120 g. Transfer 200 micro liter culture and 300 micro liter 50% glycerol* to a 1.8 mL sterile cryo tube together. Mixed well then stored in -20℃ for short time (half a year) storage and -80℃ for long

term storage.

2.1.2 PSB pre screen

2.1.2.1 Shake flask culture

PSB strains were cultured respectively in LB liquid media at 28°C for 24 hr. Culture solutions were centrifuged and then washed three times with 0.85% NaCl sterilized solution to collect the bacterial cells. To resuspend the cells, P free NBRIP medium was used. The OD_{600} was adjusted to 0.1 to obtain PSB suspensions for use in additional experiments.

To test phosphate releasing ability, $Ca_3(PO_4)_2$ (tricalcium phosphate, TCP), $FePO_4$, $AlPO_4$, Phytin (inositol hexakisphosphate, Mg and Ca salt) and lecithin (Yuanye, Shanghai, China) with the same P contents were added, respectively, to NBRIP as the P source. Adjusted the pH with 0.1 M NaOH and HCl to ensure that the pH reaches 7.0 ± 0.2 after autoclave. Then the NBRIP was autoclaved at 115°C for 20 min. The PSB suspension was inoculated into the sterilized NBRIP at a ratio of 1% (v/v) of the culture medium. Later, PSB was cultured with incubation at 28°C for 5 days. The rotation speed of 150 g min^{-1} was used with a rotary shaker. After culture, the soluble inorganic P in NBRIP solution content was measured.

2.1.2.2 P concentration of culture solution measure

Soluble inorganic P concentrations of all extracts and the digested solution were quantified using Molybdenum blue method* (Murphy and Riley, 1962).

2.1.2.3 PSB soil viability test

For this step, the soils were autoclaved twice at 121°C for 60 min. The viability of PSB in La and Ci was tested. After the PSB suspension (2.5 mL) was inoculated into 50 g soil, 12.5 mL sterile distilled water was used to maintain the moisture level. Soils were incubated at 28°C in the dark for 7 days.

After incubation, about 1 g soil was suspended with 99 mL sterilized 0.85% NaCl solution. Furthermore, 50 μL of soil suspension was spread on NBRIP agar medium and was cultured at 28°C for 5 days. Strains that formed colonies with transparent circles on NBRIP were considered to have soil viability.

2.1.3 PSB release P from phosphates in shake flask

2.1.3.1 Shake flask culture

PSB suspension was prepared as that in 2.1.2.1 whereas the culture method also as same as that in 2.1.2.1. Each phosphate has a control without PSB inoculation. Each culture and control had three replicates.

2.1.3.2 P concentration of culture solution measure

Same as 2.1.2.2.

2.1.3.3 pH of culture solution measure

The pH of culture solution was measured by pH meter*.

2.1.3.4 Organic acid of culture solution detection

Filtered the culture solution by a 0.45 μm filter membrane. Use the filtered solution to measure the organic acid. This measurement was carried out on the LC-100 HPLC instrument made by WuFeng Shanghai China and using C18-AQ as the separation column.

Eight organic acids are used as the standard. They are oxalic acid, tartaric acid, malic acid, malonate acid, acetic acid, maleic acid, citric acid and succinic acid. Use 0.02M KH_2PO_4 solution of pH2.3 as the mobile relative sample for analysis. Keep the flow rate at 1ml/min, and control the column temperature of the separation column at 28 ℃. At this time, the pressure in the column is relatively maintained at 11 MPa.

The organic acid mixture with gradient concentration is configured for measurement, and the separation diagram is displayed in the results. Make corresponding standard curve for organic acid.

2.1.4 Data analysis

Software (SPSS Statistics, Ver. 21.0.0.0; IBM Corp., Chicago, USA) was used to analyze the data.

2.2 Results

2.2.1 PSB identification

Thirty five PSB strains were initially isolated and identified as 6 genera (including *Enterobacter, Klebsiella, Phytobacter, Erwinia, Pantoea*, and *Pseudomonas*) and 14 Top-hit taxon. They are *Enterobacter sichuanensis*, *LECZ_s*, *Klebsiella huaxiensis*, *Phytobacter ursingii*, *Erwinia persicina*, *Pantoea dispersa*, *Pantoea endophytica*, *Pantoea rodasii*, *NEIG_s*, *Pseudomonas alloputida*, *Pseudomonas lactis*, *Pseudomonas monteilii*, *Pseudomonas plecoglossicida*, and *Pseudomonas protegens* (Table 2.2.1).

The list shows the similarity and completeness of the comparison results. As the preliminary sequencing is one-way sequencing, the completeness is about 50%.

Fourteen PSB strains (A~N) which has the highest similarity of each top-hit strain were used for pre screen.

Table 2.2.1 Preliminary Identification

Name	Top-hit taxon	Top-hit strain	Similarity (%)	Completeness (%)	Top-hit taxonomy
A	*Enterobacter sichuanensis*	WCHECl1597	99.65	59.3	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Enterobacter
Z0	*Enterobacter sichuanensis*	WCHECl1597	99.54	59.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Enterobacter
H2	*Enterobacter sichuanensis*	WCHECl1597	99.39	56.4	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Enterobacter
N	*LECZ_s*	GN03164	99.32	50.1	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Enterobacter
F	*Klebsiella huaxiensis*	WCHKl090001(T)	96.44	44.5	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Klebsiella
C	*Phytobacter ursingii*	ATCC 27989	96.32	47.9	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Phytobacter
B50	*Phytobacter ursingii*	ATCC 27989	97.13	41.6	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Phytobacter
D	*Erwinia persicina*	NBRC 102418	99.63	55.8	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B16	*Erwinia persicina*	NBRC 102418	99.64	57.5	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B15	*Erwinia persicina*	NBRC 102418	99.55	45.5	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B14	*Erwinia persicina*	NBRC 102418	99.7	45.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B12	*Erwinia persicina*	NBRC 102418	99.54	44.4	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B11	*Erwinia persicina*	NBRC 102418	99.65	59.1	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B8	*Erwinia persicina*	NBRC 102418	99.86	48.1	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B7	*Erwinia persicina*	NBRC 102418	99.65	59.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B4	*Erwinia persicina*	NBRC 102418	98.15	59.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B3	*Erwinia persicina*	NBRC 102418	99.30	58.4	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia

B2	*Erwinia persicina*	NBRC 102418	98.95	58.8	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
B1	*Erwinia persicina*	NBRC 102418	98.98	67.3	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Erwinia
M	*Pantoea dispersa*	LMG 2603	99.58	52.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
W7	*Pantoea dispersa*	LMG 2603	100	55.8	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
W6	*Pantoea dispersa*	LMG 2603	100	61.3	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
W4	*Pantoea dispersa*	LMG 2603	100	57.9	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
W3	*Pantoea dispersa*	LMG 2603	100	59.2	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
W2	*Pantoea dispersa*	LMG 2603	100	48.4	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
E	*Pantoea endophytica*	596	99.38	55.1	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
B	*Pantoea rodasii*	SHQLI9-1	99.72	58.9	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea
G	*NEIG_s*	R17(2017)	99.71	47.3	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
L	*Pseudomonas alloputida*	Kh7	100	53.6	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
K	*Pseudomonas lactis*	DSM 29167	99.85	46.7	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
B22	*Pseudomonas lactis*	DSM 29167	99.88	56.3	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
B21	*Pseudomonas lactis*	DSM 29167	99.59	66.2	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
J	*Pseudomonas monteilii*	NBRC 103158	99.89	63.4	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
I	*Pseudomonas plecoglossicida*	NBRC 103162	100	51.3	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas
H	*Pseudomonas protegens*	CHA0	96.00	64.9	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas

2.2.2 PSB pre screen

The first column in the Table 2.2.2 is the name of the strain (named by author), and the second to sixth columns are marked with the name of phosphate respectively. The data in the column indicated the soluble inorganic P concentration of the shake flask solution after the PSB (in the leftmost column) was inoculated and cultured for 5 days. The seventh column is the results of PSB survival tests in soils.

According to the measurement results of soluble inorganic P in shake flask, it was found that strains F and G had released more P than other strains from TCP; strains A and H released more P from $FePO_4$; F, H and A released more P from $AlPO_4$; and B and M released more P from Phytin. All of six PSB had almost not released P from Lecithin.

These 6 strains were incubated in sterilized La and Ci to test their survival ability in sterilized soil environment. After one week incubating, the incubated soil was made into suspension and spread onto NBRIP agar plates. These plates, coated with soil suspension, were incubated at 28 ℃. After 5 days, colonies with transparent circles grew on the NBRIP agar plates of soil samples inoculated with strains A, B, F, G, and H. No colonies grew on the plates of soil samples inoculated with strain M. This indicated that strains A, B, F, G, and H could survived and maintained TCP solubility in sterilized La and Ci, while strain M may not have this ability or may require different nutrients to grow.

Table 2.2.2 PSB strains pre screen

Strain	TCP	$FePO_4$	$AlPO_4$	Phytin	Lecithin	Viability
A	0.257	0.015	0.013	0.306	0.001	○
B	0.265	0.002	0.010	0.448	0.000	○
C	0.286	0.001	0.000	0.303	0.000	
D	0.188	0.010	0.005	0.113	0.000	
E	0.212	0.003	0.008	0.162	0.000	
F	0.483	0.005	0.015	0.389	0.000	○
G	0.594	0.003	0.012	0.381	0.000	○
H	0.181	0.018	0.013	0.096	0.000	○
I	0.220	0.006	0.005	0.313	0.001	
J	0.162	0.012	0.003	0.223	0.000	
K	0.405	0.005	0.002	0.137	0.000	
L	0.162	0.002	0.011	0.159	0.000	
M	0.383	0.003	0.001	0.392	0.000	×
N	0.441	0.002	0.003	0.228	0.000	

The data is the content of soluble inorganic P in the shake flask solution, the unit is g/L; ○：after 7 days of inoculation in the soil, it is found that it survives and maintains the P release ability through testing, × indicates no viable bacteria are detected.

Table 2.2.3 Strain information of PSB used for following experiments

Strain	Source	Closest relatives	Similarity	Classification	NCBI number
A	Lateritic red earths	*Enterobacter chuandaensis*	99.44	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Enterobacter	ON778739
B	rhizosphere soil of Chinese cabbage	*Pantoea rodasii*	99.72	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Erwiniaceae;Pantoea	ON778745
F	rhizosphere soil of Chinese cabbage	*Klebsiella aerogenes*	92.96	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacterales;Enterobacteriaceae;Klebsiella	ON7078779
G	rhizosphere soil of Chinese cabbage	*Pseudomonas hunanensis*	99.86	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	ON778780
H	Lateritic red earths	*Pseudomonas protegens*	98.38	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	ON778778

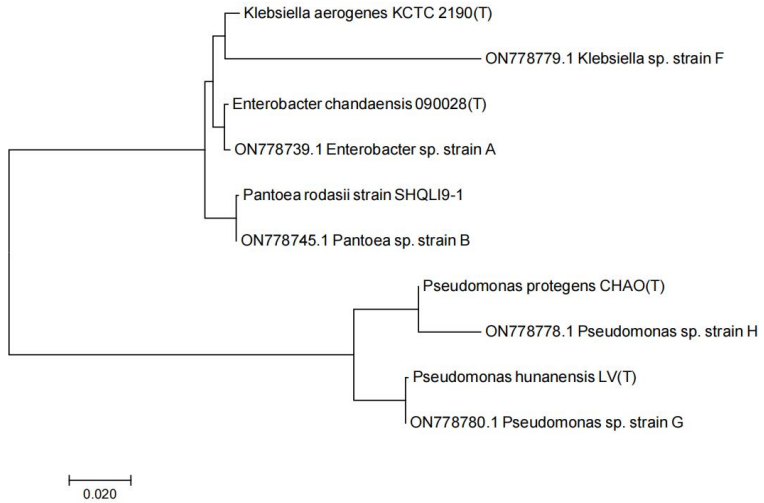


Fig. 2.2.3 Phylogenetic tree

The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 0.34542664 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [2] and are in the units of the number of base substitutions per site. The analysis involved 10 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1361 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [3].

1. Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.

2. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

3. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution 33:1870-1874.

Finally, 5 PSB strains (A, B, F, G and H) were selected for following experiments.

2.2.3 Five PSB strains' information

The 5 PSB that selected for the following experiments were sequenced with two-way sequencing. Their NCBI number showed in Table 2.2.3. Compare the sequence of bacteria species with that of similar species to build the phylogenetic tree (Fig. 2.2.3).

2.2.4 PSB strains release P from phosphates in shake flask

2.2.4.1 Strain A release P from phosphates in shake flask

After 5 days, the P released from TCP by A is 0.27 g/L, and the pH was 4.59, which is 2.44 lower than that of control 7.03 (Table 2.2.4.1a). Compared with the dissolution of TCP, the dissolution of $FePO_4$ by A was very weak. After 5 days, it was only 0.013 g/L. The pH dropped to 4.00, 1.30 lower than that of control 5.30, and less than that of TCP dissolution.

The ability of A to release P from $AlPO_4$ was similar to that from $FePO_4$ (Table 2.2.4.1a). It released 0.012 g/L of P in 5 days, which was the smallest release compared to the release of TCP and $FePO_4$. However, the pH dropped from 6.40 to 3.37, which is the largest pH drop compared to the release of TCP and $FePO_4$.

Table 2.2.4.1a Inorganic phosphate release in shake flask with strain A inoculation

	TCP	$FePO_4$	$AlPO_4$
solution P concentration (mg/L)	0.270	0.013	0.012
pH	4.59	4.00	3.37

The determination of TCP release and pH change of strain A for 5 days showed that the pH decreased significantly on the first day and the second day of the 5 days, and then became flat on the third day (Fig.2.2.4.1a). The concentration of P in the solution rose rapidly on the first 4 days and became flat on the fifth day. It can be seen that when strain A releases TCP, pH was negatively related to the amount released.

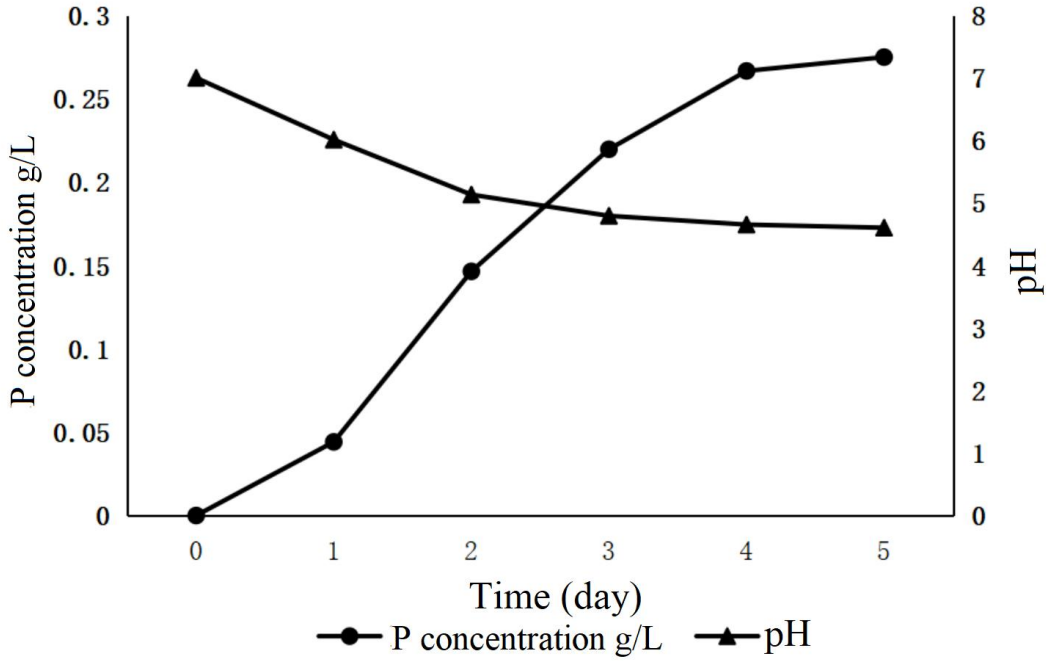


Fig. 2.2.4.1a Strain A release P from TCP in 5 days

The release of organic phosphate by strain A in phytin shows good release power (Table 2.2.4.1b). The amount of P released in 5 days is

higher than that in TCP, reaching 325 mg/L, and the pH drops to 4.24. Strain A seems to have weak release effect on lecithin. 1 mg/L release is detected, and the pH drops to 3.68.

Table 2.2.4.1b Organic phosphate release in shake flask with strain A inoculation

	phytin	lecithin
solution P concentration (mg/L)	325	1.01
pH	4.24	3.68

The determination of the release of phytin and the change of pH in strain A for 5 days showed that the trend of pH decrease was similar to that of dissolved TCP, and it tended to be flat after the third day, while the release of phytin showed a sharp increase in the first two days, and tended to be flat after the second day (Fig. 2.2.4.1b).

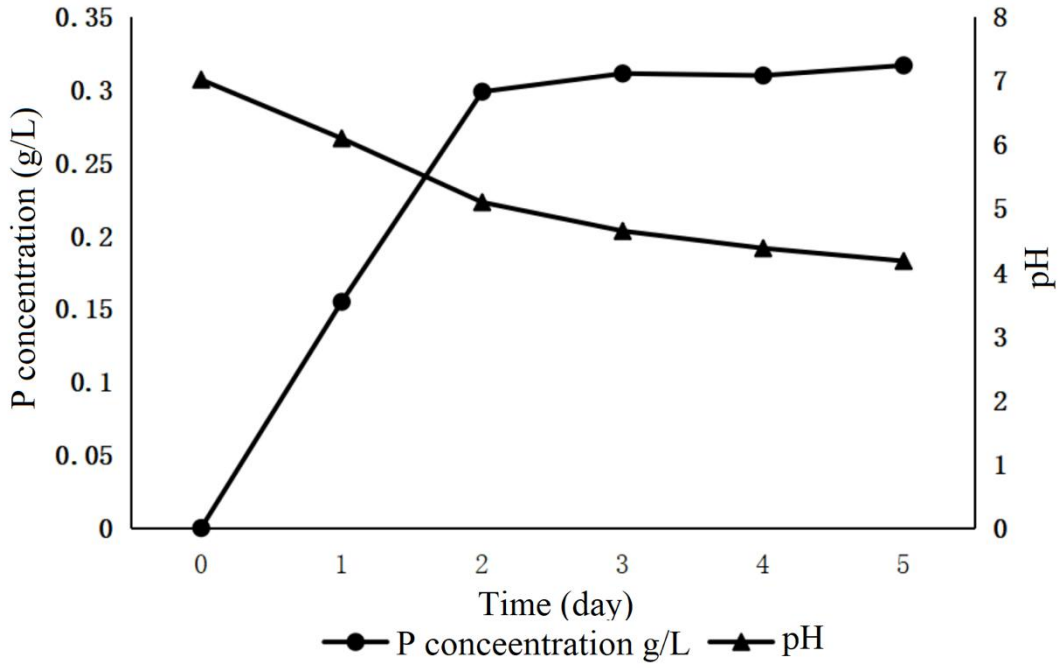


Fig. 2.2.4.1b Strain A release P from Phytin in 5 days

The organic acid analysis of the culture medium 5 days after shaking the flask showed that the output of organic acids is high in the phytin-source culture solution with acetic acid, malic acid, oxalic acid, and also trace succinic acid (Table 2.2.4). No known acid was found in lecithin-source culture solution.

Table 2.2.4 oganic acid in culture solutioin of shake flask

P source	strain	Oxalic	Tartaric	Malic	Malonate	Acetic	Maleic	Citric	Succinic	SUM
		mg/L								
TCP	B									
	F	112.5		145.1		45.4				303.0
	G			trace		596.4	trace			596.4
	H					882.8		0.8		883.6
$FePO_4$	B									
	H						trace			trace
$AlPO_4$	B	24.9								24.9
	H					trace			0.3	0.3
Phytin	A	136.9		233.5		611.1		0.4	21.0	100.3
	B			288.7		54.8		trace	21.7	365.1
	F	330.0		28.0	trace				10.2	368.2
	G	284.1				95.5			12.3	391.9
	H			52.2		633.7		1.4	13.7	701.1
Lecithin	A									
	B	17.6		trace						17.6
	F	8.5							trace	8.5
	G									
	H	7.7				trace				7.7

2.2.4.2 Strain B release P from phosphates in shake flask

For strain B, the release amount of TCP is the largest in 3 inorganic phosphates as 290 mg/L, and the pH drops to 3.06 (Table 2.2.4.2a). Strain

B released about 1.80 mg/L $FePO_4$, and the pH dropped to 3.15. The release P by strain B from $AlPO_4$ is higher than that from $FePO_4$, reaching over 10 mg/L, and the pH dropped to 3.34.

Table 2.2.4.2a Inorganic phosphate release in shake flask with strain B inoculation

	TCP	$FePO_4$	$AlPO_4$
solution P concentration (mg/L)	290	1.80	10.15
pH	3.06	3.15	3.34

When release inorganic phosphates, strain B secreted low variety and quantity of organic acid (Table 2.2.4). No standard organic acid was detected in TCP and $FePO_4$ source culture solution. The organic acid in $AlPO_4$ source culture solution is mainly oxalic acid.

Release of phytin was higher than the release of inorganic phosphate, reaching 459 mg/L, and the pH decreased to 5.33 (Table 2.2.4.2b). There was almost no release of lecithin, even when the pH of the culture medium decreased to 3.31.

Table 2.2.4.2b Organic phosphate release in shake flask with strain B inoculation

	phytin	lecithin
solution P concentration (mg/L)	459	0.00
pH	5.33	3.31

Strain B secretes different variety and quantities of organic acids when releasing two organic phosphates (Table 2.2.4). When phytin was

released, malic acid was the main component, acetic acid and succinic acid are also secreted, and several unknown acids were detected. When lecithin was released, oxalic acid was mainly secreted, and malic acid was also slightly secreted.

2.2.4.3 Strain F release P from phosphates in shake flask

Strain F can release the three inorganic phosphates provided (Table 2.2.4.3a). The release of TCP is higher than that of $FePO_4$ and $AlPO_4$, reaching 477 mg/L, and the pH drops to 4.65. The release of $FePO_4$ was the lowest among the three, only 5.49 mg/L, and the pH decreased to 3.71. The release of $AlPO_4$ is slightly higher than that of $FePO_4$, which is 15.22 mg/L, and the pH drops to 3.72.

Table 2.2.4.3a Inorganic phosphate release in shake flask with strain F inoculation

	TCP	$FePO_4$	$AlPO_4$
solution P concentration (mg/L)	477	5.49	15.22
pH	4.65	3.71	3.72

It can be seen that when strain F releases TCP, it first continues to increase the P concentration in the solution, and then decreases, while the pH first drops rapidly, and when the P concentration decreases, the pH raised. This indicates that strain F may consume the inorganic P in the solution for its own biosynthesis, thus causing the decrease of P concentration in the solution.

Strain F mainly secreted oxalic acid, malic acid, and acetic acid in the process of dissolving TCP. In addition, an unknown acid was detected (Table 2.2.4).

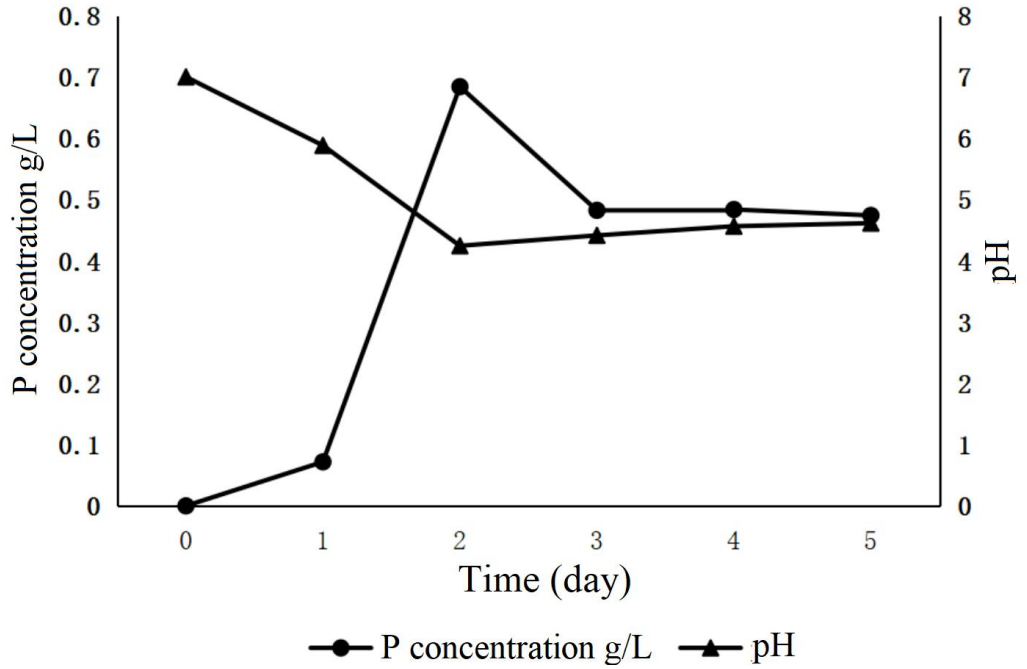


Fig. 2.2.4.3a Strain F release P from TCP in 5 days

The release amount of F to phytin was 371 mg/L, and the pH value reaches 3.83 (Table 2.2.4.3b). No release of lecithin from strain F was detected, and the pH decreased to 3.58.

Table 2.2.4.3b Organic phosphate release in shake flask with strain F inoculation

	phytin	Lecithin
solution P concentration (mg/L)	371	0.00
pH	3.83	3.31

From the 5-day release dynamics of F to phytin, it is consistent with

the release trend of F to TCP. The pH first drops sharply, then rises gently. The amount of P released corresponds to the pH, and continues to increase at the same time when the pH drops sharply, then decreases, and then tends to be flat. It seems like that when F releases P, it produced a climax of biosynthesis, thus consuming a large amount of inorganic P in the environment.

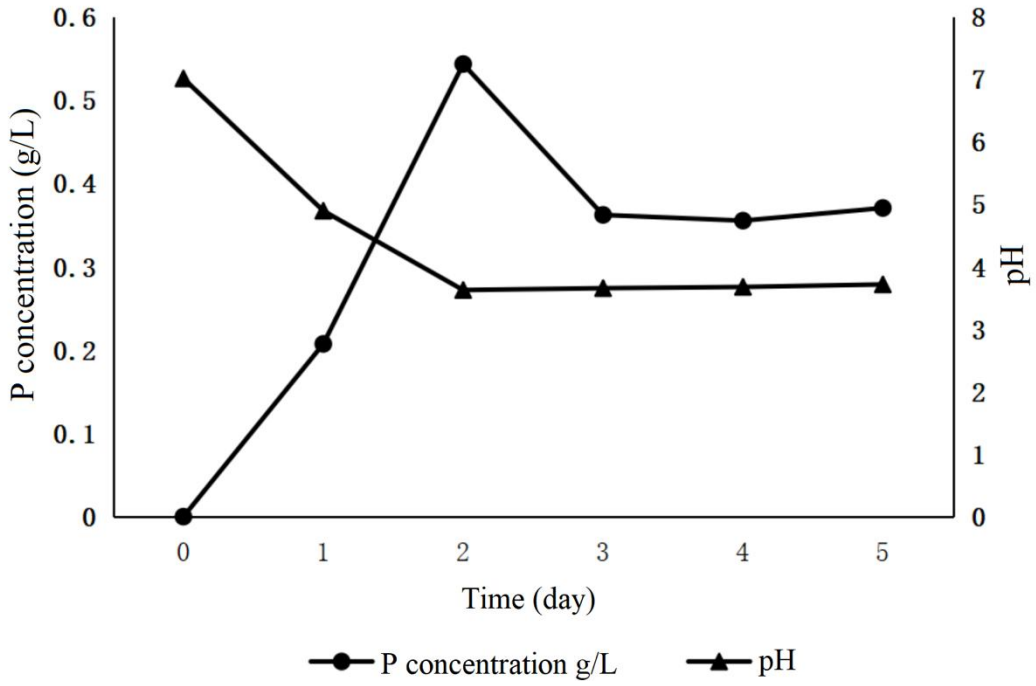


Fig. 2.2.4.3a Strain F release P from phytin in 5 days

Strain F mainly secretes oxalic acid when releasing two kinds of organic phosphates, but the amount secreted in phytin solution is much greater than that in lecithin solution (Table 2.2.4). In addition, strain F also secretes malic acid and a small amount of succinic acid in phytin-source culture solution, and trace succinic acid in lecithin-source culture solution.

2.2.4.4 Strain G release P from phosphates in shake flask

The release of G to TCP is 555 mg/L, and the pH drops to 4.56, higher than that of the other two (Table 2.2.4.4a). The release of G to $FePO_4$ is low, only about 4.01 mg/L, and the pH drops to 4.06. The release of $AlPO_4$ from G is about 14.26 mg/L, and the pH drops to 4.08.

Table 2.2.4.4a Inorganic phosphate release in shake flask with strain G inoculation

	TCP	$FePO_4$	$AlPO_4$
solution P concentration (mg/L)	555	4.01	14.26
pH	4.56	4.06	4.08

Strain G's release of P from TCP also has a rapid rise and then a decline process, and then a slow rise trend (Fig. 2.2.4.4a). The change of pH decreases rapidly with the rapid increase of the concentration of P in the solution. When the concentration of P decreases, the pH tends to be flat, and then when the concentration of P increases slowly, the pH also rises slowly. Although strain G consumed inorganic P in the environment, its release was still significantly higher than that of other bacteria.

Strain G secretes acetic acid and another unknown acid with high concentration in the process of releasing TCP (Table 2.2).

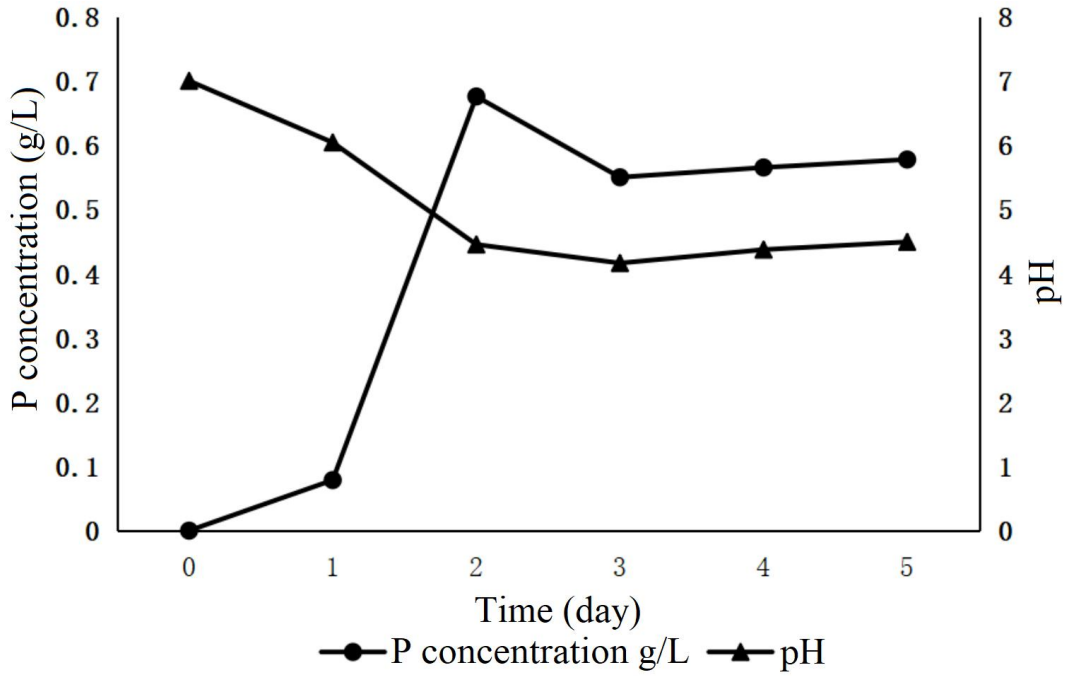


Fig. 2.2.4.4a Strain G release P from TCP in 5 days

The release amount of strain G to phytin was 363 mg/L, and the pH decreased to 3.96. The release of strain G to lecithin was also not detected, but the pH also decreased to 3.9 (Table 2.2.4.4b).

Table 2.2.4.4b Organic phosphate release in shake flask with strain G inoculation

	phytin	Lecithin
solution P concentration mg/L	363	0.00
pH	3.96	3.90

The release dynamics are consistent with the trend towards TCP (Fig.2.2.4.4b). After the release peak, the P concentration in the solution starts to decline, tends to be flat after one day, and then has an upward trend. The pH decreases rapidly at the same time of rapid release, then

tends to be flat, and basically remains unchanged after the next day. It is speculated that G will also have a rapid production period of biosynthesis, during which a large amount of inorganic P in the environment will be consumed.

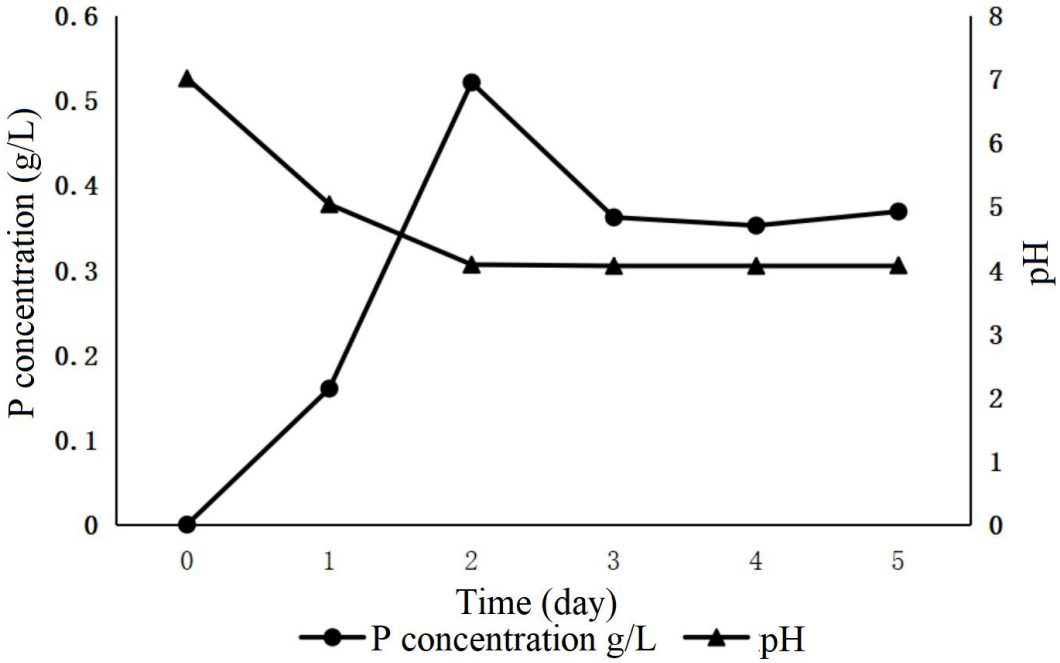


Fig. 2.2.4.4b Strain G release P from phytin in 5 days

Strain G mainly secreted oxalic acid, acetic acid and succinic acid when releasing phytin. Strain G was not detected to release, but traces of tartaric acid and acetic acid were still detected in lecithin-source culture solution.

2.2.4.5 Strain H release P from phosphates in shake flask

Strain H only released 174 mg/L of TCP, but the pH decreased to 4.30 (Table 2.2.4.5a). The release amount of H to $FePO_4$ and $AlPO_4$ is

close, 14.58 mg/L and 13.15 mg/L respectively, and the pH drops to 3.8 and 3.67.

Table 2.2.4.5a Inorganic phosphate release in shake flask with strain H inoculation

	TCP	$FePO_4$	$AlPO_4$
solution P concentration (mg/L)	174	14.58	13.15
pH	4.30	3.80	3.67

Strain H secretes a large amount of acetic acid when releasing TCP. When releasing $FePO_4$, no known organic acid was detected (Table 2.2.4). When releasing $AlPO_4$, trace amounts of acetic acid and succinic acid were detected.

The release of organic phosphate by strain H was 94 mg/L in phytin-source culture solution, and the pH value decreased to 4.59; The release of lecithin was 0.14 mg/L, and the pH decreased to 3.57 (Table 2.2.4.5b).

Table 2.2.4.5b Organic phosphate release in shake flask with strain H inoculation

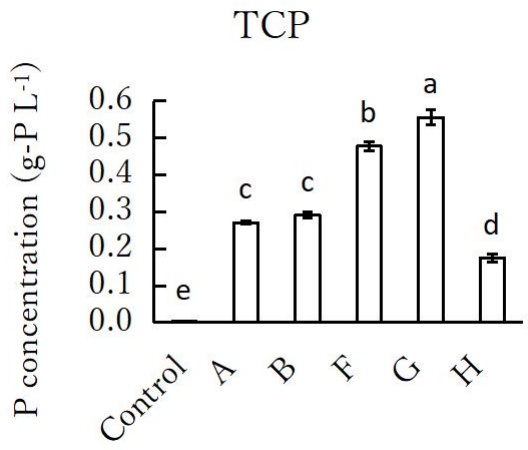
	phytin	Lecithin
solution P concentration (mg/L)	94	0.14
pH	4.59	3.57

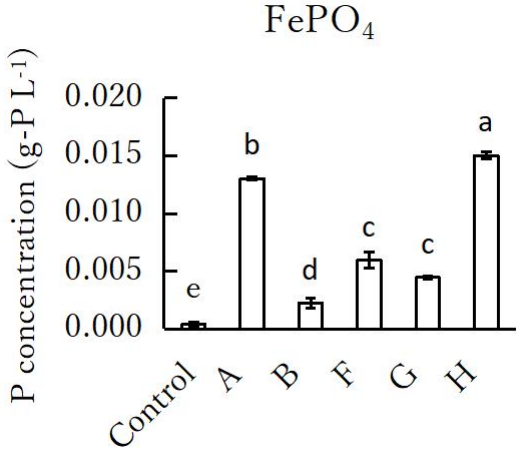
When strain H releases the two organic acids, it secretes acetic acid (Table 2.2.4). In addition, citric acid and succinic acid are also detected in phytin, and oxalic acid is mainly secreted in lecithin. But its secretion was lower than that in phytin.

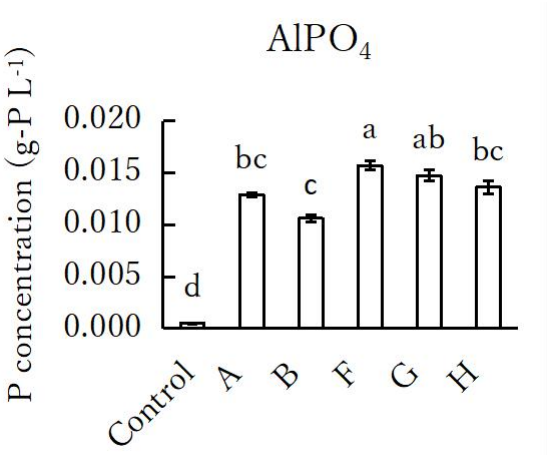
2.3 Discussion

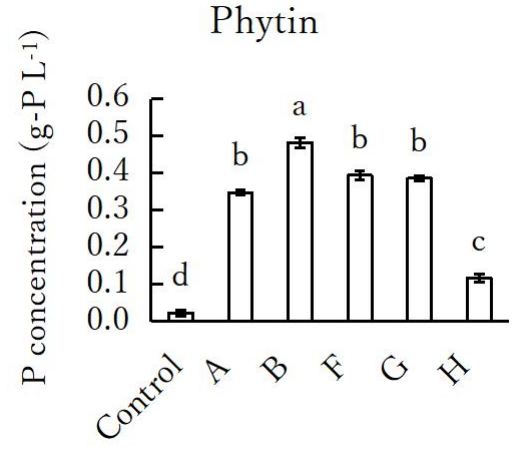
This chapter introduces the method of obtaining PSB used in this study. The difference in the release ability of the bacteria to three insoluble inorganic phosphates and two organic phosphates was detected through shake flask experiment. The survival ability of PSB in the soil was verified through the culture experiment inoculated to sterile soil. Finally, five PSB were selected as the strains used in this experiment. When the five strains were inoculated in the soil, they had the survival ability, and the release ability of the five strains to five kinds of phosphate in the shake flask was better than other strains.

As shown in Fig. 2.3.1, among the five strains, the largest TCP release was strain G, followed by strain F; The release amount of $FePO_4$ is the largest for strain H, followed by strain A, which is 2.6-7.5 times higher than strain B, F and G; strain F released the most $AlPO_4$, and there was only little difference among the five strains; The largest number of Phytin releases is B; The weak release of lecithin is strain A. It can be seen that strains with strong ability to release inorganic phosphates do not necessarily have a high release capacity for organic phosphates.


TCP
P concentration (g-P L-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
e
c
c
b
a
d
Control
A
B
F
G
H


FePO4
P concentration (g-P L-1)
0.020
0.015
0.010
0.005
0.000
e
b
d
c
c
a
Control
A
B
F
G
H


AlPO4
P concentration (g-P L-1)
0.020
0.015
0.010
0.005
0.000
d
bc
c
a
ab
bc
Control
A
B
F
G
H


Phytin
P concentration (g-P L-1)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
d
b
a
b
b
c
Control
A
B
F
G
H

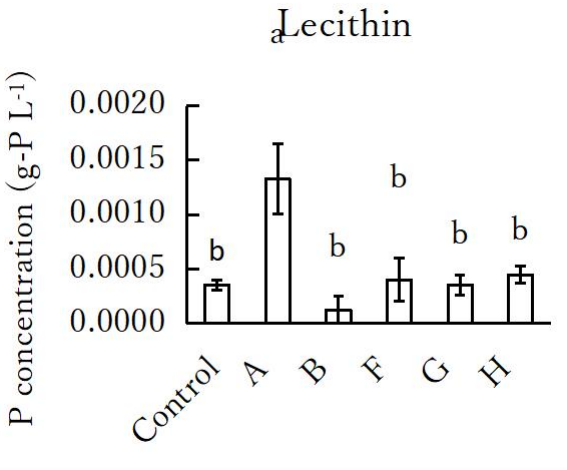


Figure 2.3.1 Soluble inorganic P concentration in culture solution containing different P sources after 5 days incubation with 5 PSB isolates (A-H) or no bacterial isolate (Control). Different letters indicate significant differences ($p < 0.05$; one-way ANOVA, Tukey, n = 3). Error bars = SE.

When five strains released five kinds of phosphates, strain B caused the maximum decrease of pH in inorganic phosphate TCP, $FePO_4$, $AlPO_4$ and organic phosphate lecithin, but the P release was lower than other strains; When strain B releases phytin, the decrease of pH is the smallest, but the release of P is the largest.

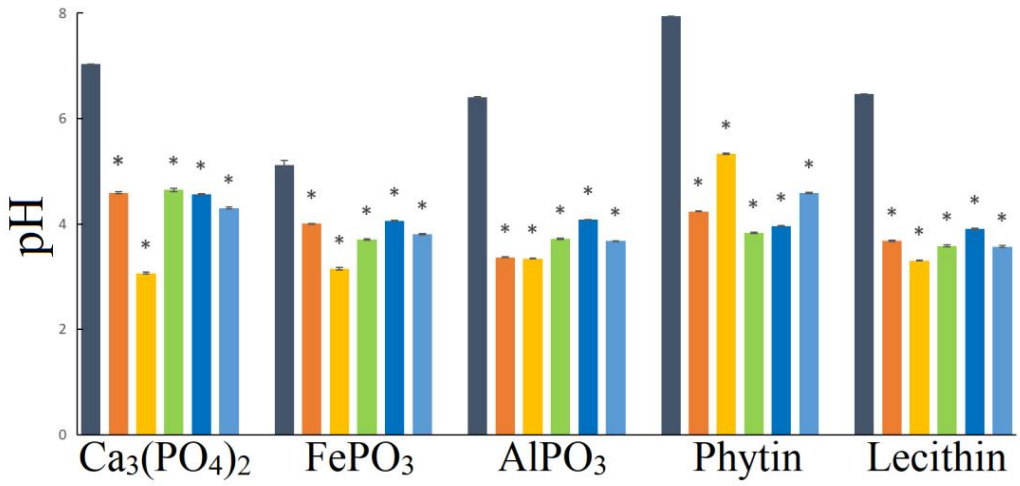


Figure 2.3.2 pH of culture solution after 5 days shake flask culture. The "*" symbol indicates significant differences ($p < 0.05$; one-way ANOVA, Tukey, n = 3). Error bars = SE.

Taking pH as a covariate, univariate analysis was conducted on strain, phosphate type, and solution P concentration (P release amount). It was found that the strain had a significant impact on the type of P release ($P < 0.05$) when PSB released inorganic phosphate. However, inorganic P sources did not have a significant impact on P release, and there was no interactive impact between strain type and P source type on P release. The influence of pH on the concentration of P in the solution was found to be statistically significant (Table 2.3.1).

Table 2.3.1 Tests of Between-Subjects Effects

Between-Subjects Factors		Dependent Variable:	P concentration
strains	phosphate	Source	*P*
A	TCP	pH	0.002
B	$FePO_4$	PSB	0.042
F	$AlPO_4$	phosphate	0.074
G		PSB * phosphate	0.198

H

When releasing organic phosphoric acid, the strain has a significant effect on the limited amount of P released by species, while the type of P source still has no significant effect on P release, but the strain and type of P source have a significant interaction on P release. pH cannot effectively reflect the concentration of P in solution (Table 2.3.2).

Table 2.3.2 Tests of Between-Subjects Effects

Between-Subjects Factors		Dependent Variable:	P concentration
strains	phosphate	Source	*p*
A	phytin	pH	0.647
B	lecithin	PSB	0.011
F		phosphate	0.739
G		PSB * phosphate	0
H			

Therefore, the decrease of pH has statistical significance for the release of inorganic phosphate, but not for the release of organic phosphate.

It can be seen from Table 2.2.4, when TCP was released, acetic acid was secreted by strain F, G and H, and strain H secreted 882.8 mg/L; oxalic acid and malic acid were found in F shake flask.

Limited organic acids were found in $FePO_4$ and $AlPO_4$ shake flask.

When phytin was released, oxalic acid was found in strain A, F and G culture solution, while malic acid was found in all except for strain G. Acetic acid was found in all except for strain F, and succinic acid was

found in all strains culture solutions.

Also limited organic acids were found in lecithin shake flask.

Based on the types and total amount of organic acids detected, it appeared that PSB secreted more types of organic acids when releasing TCP and phytin compared to the other three phosphates, with a higher total amount. This might be one of the reasons why PSB had a higher release amount of TCP and phytin.

Chapter III

PSB release P from sterilized soils (inoculation experiment)

In this chapter, inoculation experiment was done. Five PSB strains (A, B, F, G and H) which screened from chapter 2 were inoculated into La and Ci that with great different P fractions to observe the effect of them. The changes of soil P fractions, organic acid and phosphatase activity in the soils were detected to evaluate the ability of PSB release P of La and Ci.

3.1 Materials and methods

3.1.1 Materials and treatments

Two types of soils were used in this step (Table 3.1.1.1). Lateritic red earths (La), from Nanning, Guangxi, China (22°50'28.6"N 108°11'25.7"E), and Cinnamon soils (Ci) from Fenyang, Shanxi, China (37°17'10.0"N 111°43'11.8"E). Both soils are collected from non-cultivated lands.

Table 3.1.1.1 Soils used in this experiment

Soil group	Abbr.	Location	pH
Lateritic red earths	La	Nanning, Guangxi, China (22°50'28.6"N 108°11'25.7"E)	5.5
Cinnamon soils	Ci	Fenyang, Shanxi, China (37°17'10.0"N 111°43'11.8"E)	8.0

La is acidic with pH 5.4, and the content of available P is low as 0.1 mg/kg soil; Ci is alkaline with pH 8.0, and available P content is higher than La as 6.0 mg/kg soil. The total P content of the two is close, 0.6 and 0.7 g/kg soil respectively.

Soils were air dried after removal of non-soil components such as stones and plant roots. The dried soils were crushed and sieved by 1 mm sieve and then autoclaved as that in 2.1.2.3 (PSB soil viability test).

Strain A, B, F, G and H were used for the reason that strain F and G released more P from TCP, strain A and H released more P from $AlPO_4$ and $FePO_4$, and B from phytin. And all of these 5 strains survived in the sterilized soil.

PSB suspension was prepared as that in 2.1.3.1 (Shake flask culture).

Inoculation experiments included three treatments (PSB treatment, TCP treatment and combination treatment) and one control (Table 3.1.1.2). In the combination treatment, there were 5 experimental groups, which were strain A, B, F, G and H. Each treatment (or each experimental group) has three replicates.

Table 3.1.1.2 Treatments and conditions

Treatments	Soils and P supplement	Inoculation	Moisture support
Control	La / Ci 50g	2.5 mL P free NBRIP	12.5 mL sterile distilled water
PSB Treatment	La / Ci 50g	2.5mL PSB suspensions	12.5 mL sterile distilled water
TCP Treatment	La / Ci 50g + 0.5g TCP	2.5 mL P free NBRIP	12.5 mL sterile distilled water
Combination Treatment	La / Ci 50g + 0.5g TCP	2.5mL PSB suspensions	12.5 mL sterile distilled water

P free NBRIP: NBRIP exclude TCP
PSB suspensions: A/B/F/G/H cells suspended with P free NBRIP to OD_{600} = 0.1
incubate: 28℃ 5days
Control: La-Ctrl, Ci-Ctrl
PSB treatment: LaA~LaH; CiA~CiH
TCP treatment: LaP, CiP
Combination treatment: LaAP~LaHP; CiAP~CiHP

For PSB treatment, 2.5 mL PSB suspensions were inoculated into 50 g sterilized soil respectively. Then 12.5 mL sterilized distilled water per container was used to keep the moisture. For TCP treatment, 1% (w/w) TCP was added to 50 g soil and mixed well, followed by autoclaving twice at 121°C for 60 min. After cooling, 2.5 mL of P free NBRIP medium and 12.5 mL sterilized distilled water were added. For combination treatment, soils were prepared as that of TCP treatment. After cooling, 2.5 mL PSB suspensions and 12.5 mL sterilized distilled water were added into soils respectively. For use as a control, 2.5 mL of P free NBRIP and 12.5 mL sterilized distilled water were added to 50 g sterilized soil. The three treatments and the control were cultured at 28°C

for 7 days. After incubation, about 0.1 g soil was suspended in 99 mL normal saline and spread on NBRIP agar plate to confirm the survival of the strain. One gram soil was air dried for measuring soil phosphatase activity, and remaining soils were lyophilized for measuring P fractions and organic acid content.

3.1.2 Soil P fractions measuring

Using the Hedley method, P fractions were determined with the following procedure. First, 0.5 g dry soil was put into a 50 mL centrifuge tube for extraction. Extraction was done using (1) about 5 cm^2 anion-exchange resin (Selemion™ ion exchangeable resin; AGC Engineering Co. Ltd., Chiba, Japan) and 30 mL distilled water; (2) 30 mL 0.5 M $NaHCO_3$; (3) 30 mL 0.1 M NaOH, and (4) 20 mL 1 M HCl, in sequence. Each was conducted for 16 hr. Then, the resin was shaken with 20 mL 0.5 M HCl for 2 hr to extract resin P. Other extracts were $NaHCO_3$-Pi, NaOH-Pi and HCl-P. Then 5 mL $NaHCO_3$ extract mixed with 10 mL 0.9 M H_2SO_4, and 0.5 g $(NH_4)_2S_2O_8$ were autoclaved at 120°C for 60 min to obtain $NaHCO_3$-P (both Pi and Po). Also, 5 mL NaOH extract, 10 mL 0.9 M H_2SO_4, and 0.6 g $(NH_4)_2S_2O_8$ were autoclaved at 120°C for 90 min to obtain NaOH-PT. The difference between PT and the corresponding Pi is Po. The P concentrations of all extracts and the digested solution were

quantified using Molybdenum blue method as that in 2.1.2.2 (P content of culture solution measure).

3.1.3 Soil pH measuring

The pH is measured in soil-water (1:2.5) by pH meter after reciprocating vibration 30 min. The specific procedures are as follows: weigh 10 g of dry soil and put into a 50 ml centrifuge tube, add 25 ml of distilled water into the tube, cover the tube and shake with a reciprocating shaker at 120 g for 30 minutes. Leave the centrifuge tube standing for 1hr then measure the pH of the upper solution with a pH meter.

3.1.4 Soil organic acid detection

Add 10 g dry soil to a centrifuge tube containing 10 mL of HPLC mobile phase (here used 0.02 M KH_2PO_4 at pH 2.3). Reciprocating shake for 2 h, keep still for 24 h, centrifuge at 8,000 x *g* for 5min, filter the supernatant with a 4.5 μm filter.

HPLC analysis as same as 2.1.3.4 (Organic acid of culture solution detection).

3.1.5 Soil phosphatase measuring

Soil phosphatase is a kind of enzyme that catalyzes the mineralization of soil organic P compounds. Its activity directly affects

the decomposition, transformation and bioavailability of organic P in soil, and is an indicator to evaluate the direction and intensity of soil P biotransformation. Soil phosphatase was significantly affected by soil carbon, nitrogen content, available P content and pH. Generally, there are three types of phosphatase, alkaline, neutral and acidic, according to their optimum pH range.

This step used the Soil Phosphatase Activity Test Kit made by Beijing Solarbio Science and Technology Co., Ltd. There were S-NP (soil-neutral phosphatase) with Art. No. BC0460, S-AKP/ALP (soil-acid / alkaline phosphatase) with Art. No. BC0280 and S-ACP with Art. No. BC0140.

The principle of these kits is that, in the corresponding pH environment, phosphatase catalyzes the hydrolysis of disodium phenyl phosphate to produce phenol and disodium hydrogen phosphate (Fig.3.1.5). The enzyme activity can be calculated by measuring the amount of phenol generated.

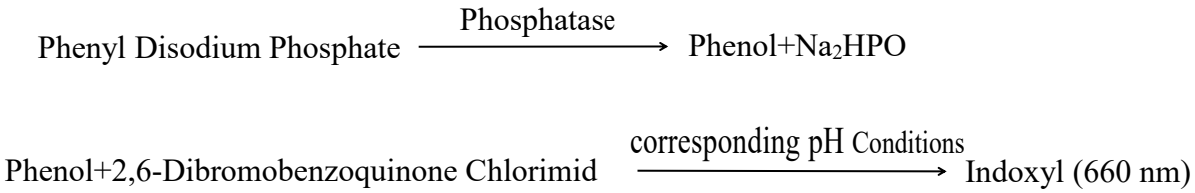


Fig.3.1.5 Principle of enzyme assay

1) Fresh soil samples shall be dried in an oven at 37 ℃, and pass through a 50 mesh sieve;

2) Weigh 0.1 g of air dried mixed soil, add 0.05 mL toluene, and shake gently for 15 min; Add 0.4 mL of reagent I and shake it well, place it in 37 ℃ for constant temperature culture, and catalyze the reaction for 24h. After that time, quickly add 1mL Reagent II and fully mix it to stop the enzyme catalyzed reaction. Centrifuge at 10,000 x *g* at room temperature for 10 min, take the supernatant and place it on ice for measuring.

3) Adjust the wavelength of the spectrophotometer to 660 nm, and adjust the distilled water to zero.

4) Add liquid into 1 mL glass cuvette according to the dosage in the Table 3.1.5.

Table 3.1.5 Composition of solution for measurement of enzyme activities

Reagent/μL	Blank	Standard	Sample
I	50		
III	100	100	100
IV	20	20	20
Distilled water	830	830	830
Standard		50	
Sample			50

Evenly mix and let stand at room temperature for 30 min. Measure the absorbance at 660 nm (expressed as strain A).

5) Calculation

Enzyme activity： At 37 ℃, 1 nmol phenol is released per gram of soil per day as one enzyme activity unit.

Enzyme activity（U/g soil）

=[C standard × (A sample - A blank) ÷ (A standard - A blank)] × VT ÷ W ÷ T × 1000

=725 × (A sample - A blank) ÷ (A standard - A blank) ÷ W

C standard: 0.5 μmol/mL; VT: Total volume of catalytic system, 1.45 mL; W: Soil sample weight, g; T: Catalytic reaction time, 24 h=1 d; 1,000: unit conversion factor, 1 μmol=1,000 nmol.

3.2 Results

3.2.1 Strain A release P from soils

After inoculation of strain A to La (LaA), four inorganic fractions were significantly changed, while the organic fractions were not affected. Inoculation to La with TCP (LaAP) caused significant changes in all fractions relative to LaP (Table 3.2.1a).

After inoculation to Ci (CiA), all fractions except NaOH-Pi were significantly changed; After inoculation to Ci added with TCP (CiAP), the fraction except NaOH-Pi and HCl-P changes significantly compared with CiP (Table 3.2.1b).

Inoculation of strain A in both soils resulted in a decrease in pH (Fig. 3.2.1a). When the content of calcium phosphate in the soil is high (LaAP,

CiA, CiAP), the oxalic acid in the soil is largely consumed (Fig. 3.2.1b). In all treatments, LaAP had the largest pH decline rate and the largest oxalic acid consumption rate.

Table 3.2.1a Soil fractions (g-P kg^{-1} soil) of strain A inoculation experiment in La

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaA	0.075±0.003**	0.029±0.004**	0.210±0.005	15.55±0.36**	9.84±0.28	0.0124±0.0032**
LaP	0.468±0.016**	0.263±0.008**	0.212±0.006	34.00±0.38**	9.43±0.23	452±6**
LaAP	1.051±0.022**	0.605±0.009**	0.288±0.006**	43.00±0.32**	10.78±0.19**	432±4**

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the LaA and LaP's fractions is calculated relative to La-Ctrl, while the significance of the LaAP's fractions is calculated relative to LaP.

**: $p < 0.01$

Table 3.2.1b Soil fractions (g-P kg^{-1} soil) of strain A inoculation experiment in Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci-Ctrl	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiA	3.05±0.01**	4.49±0.04**	1.44±0.02**	2.39±0.01	3.41±0.01**	300±3**
CiP	2.02±0.02**	5.62±0.02**	0.94±0.03	2.38±0.02	2.76±0.03	1800±9**
CiAP	4.10±0.01**	6.17±0.02**	1.43±0.02**	2.44±0.05	3.69±0.01**	1787±7**

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the CiA and CiP's fractions is calculated relative to La-Ctrl, while the significance of the CiAP's fractions is calculated relative to CiP.

**: $p < 0.01$

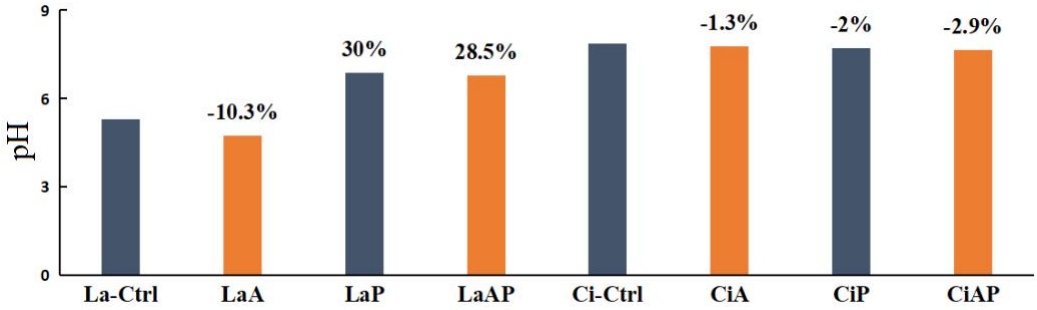


Fig. 3.2.1a Soil pH of strain A inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl in each treatment.

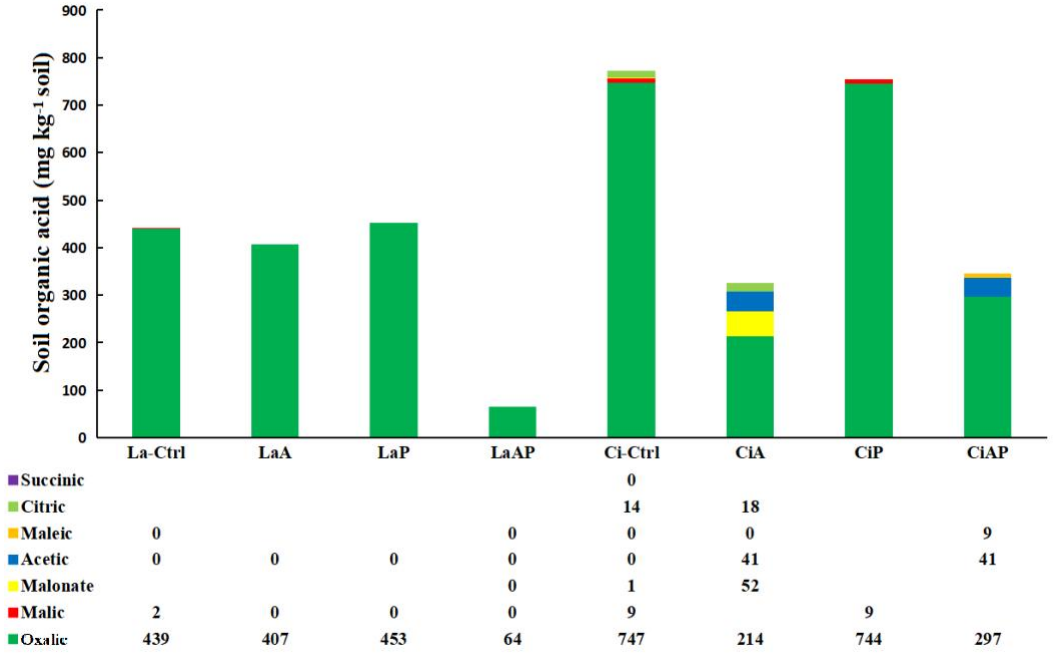


Fig. 3.2.1b Soil organic acid (mg kg^{-1} soil) of strain A inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

The activity of acid phosphatase (ACPase) in both soils were higher than that of neutral phosphatase (NPase) and alkaline phosphatase

(AKPase). The 3 phosphatase activities of all treatments of La were higher than those of all treatments of Ci (Fig.3.2.1c). Inoculation of A increased the activity of 3 phosphatase; Among them, the increase rate of ACPase and NPase in La is greater than that in Ci (Table.3.2.1c).

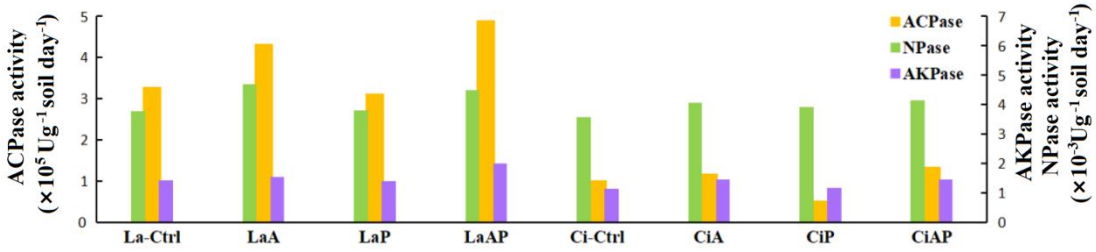


Fig. 3.2.1c Phosphatase activity of strain A inoculation experiment

Table.3.2.1c Change rate (%) of phosphatase activity of strain A inoculation experiment

	ACPase	NPase	AKPase		ACPase	NPase	AKPase
LaA	32	25	9	CiA	15	14	27
LaP	-5	1	-1	CiP	-48	10	2
LaAP	49	19	41	CiAP	31	16	27

Change rate is relative to control (La -Ctrl and Ci-Ctrl).
＞ 0: increased, ＜ 0: decreased.

Pearson correlation analysis was conducted on the fractions in two soils that had changed significantly in each treatment, and the factors that caused the change were found out from the five factors of soil pH, oxalic acid consumption, and three phosphatase activities.

It was found that NPase had a significant positive effect on resin P in La; pH had a significant negative effect on $NaHCO_3$-Pi, while AKPase activity had a positive effect. The significant change of other fractions

had no significant relationship with these five factors.

In La added with TCP, compared with LaP, all fractions had changed significantly. Five factors had significant effects on resin P, $NaHCO_3$-P and NaOH-Pi, among which pH was negatively correlated and the other four were positively correlated. NaOH-Po was not affected by pH, and HCl-P change was not caused by these five factors (Table.3.2.1d).

Among Ci and Ci added with TCP, five factors had significant effects on resin, $NaHCO_3$-Po and NaOH-Po, among which pH was negatively correlated and the rests were positively correlated. Furthermore, in Ci added with TCP, the reason for the significant increase of $NaHCO_3$-Pi was significantly related to the increase of oxalic acid consumption, ACPase and AKPase activity (Table.3.2.1d).

Strain A showed different phosphatase activity release and oxalic acid consumption in four treatments of two soils, and caused a decrease in pH. The changes of P fraction caused by inoculation had different correlations with pH, oxalic acid consumption and phosphatase activity in the four treatments, but generally showed negative correlation with pH and positive correlation with other factors.

Table.3.2.1d Pearson correlation between soil indicators and soil P fractions in strain A inoculation experiment

	soil pH	Oxalic consume	ACPase	NPase	AKPase	soil pH	Oxalic consume	ACPase	NPase	AKPase
	La					Ci				
Resin P	-	-	-	0.862*	-	-0.882*	1.000**	0.904*	0.986**	0.998**
$NaHCO_3$-Pi	-0.860*	-	-	-	0.889*	-	-	-	-	-
$NaHCO_3$-Po	-	-	-	-	-	-0.893*	1.000**	0.911*	0.989**	0.997**
NaOH-Pi	-	-	-	-	-	-	-	-	-	-
NaOH-Po	-0.811*	-	-	-	-	-0.930**	0.990**	0.958**	0.971**	0.984**
HCl-P	-	-	-	-	-	-	-	-	-	-
	La+TCP					Ci+TCP				
Resin P	-0.936**	0.998**	0.998**	0.994**	0.995**	-0.908*	0.999**	0.998**	0.866*	0.988**
$NaHCO_3$-Pi	-0.938**	0.978**	0.972**	0.964**	0.985**	-	0.963**	0.965**	-	0.953**
$NaHCO_3$-Po	-0.876*	0.920**	0.911*	0.889*	0.938**	-0.934**	0.987**	0.985**	0.817*	0.992**
NaOH-Pi	-0.940**	0.934**	0.924**	0.919**	0.944**	-	-	-	-	-
NaOH-Po	-	0.942**	0.945**	0.923**	0.941**	-0.863*	0.963**	0.965**	0.906*	0.928**
HCl-P	-	-	-	-	-	-	-	-	-	-

**: $p < 0.01$, *: $p < 0.05$, -: $p \geq 0.05$

-*: Significant negative correlation, *:Significant positive correlation

3.2.2 Strain B release P from soils

Inoculation of strain B to La caused significant changes in four inorganic fractions and $NaHCO_3$-Po. After inoculation to La added with TCP (LaBP), compared with LaP, $NaHCO_3$-P and NaOH-P changed significantly (Table 3.2.2a).

After being inoculated with Ci (CiB), strain B caused significant changes in fractions other than NaOH-P; After inoculation to Ci added with TCP (CiBP), compared with CiP, B caused a significant change in the removal of three fractions, and $NaHCO_3$-Po decreased significantly (Table 3.2.2b).

Inoculation of B caused a slight decrease in pH in the soil without TCP, but a slight increase in the soil with TCP (Fig. 3.2.2a). At the same time, when the content of calcium phosphate in the soil is high (LaBP, CiB, CiBP), the oxalic acid in the soil is largely consumed. Among all treatments, LaBP has the highest oxalic acid consumption rate (Fig. 3.2.2b).

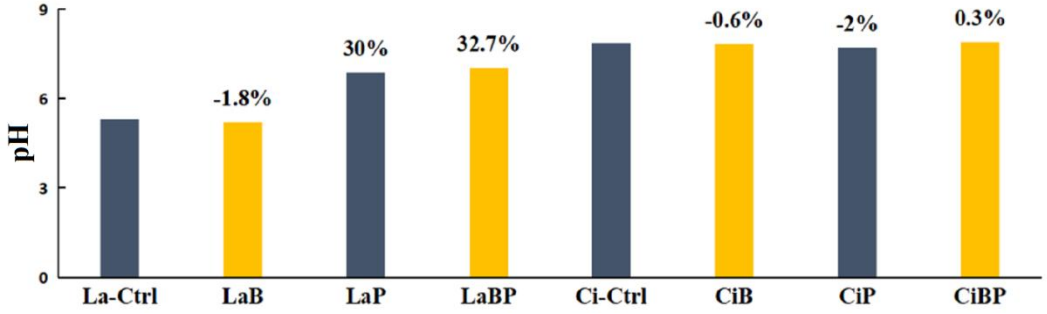


Fig. 3.2.2a Soil pH of strain B inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl

in each treatment.

Table 3.2.2a Soil fractions (g-P kg^{-1} soil) of strain B inoculation experiment in La

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaB	0.084±0.005**	0.070±0.008**	0.333±0.009**	19.96±0.39**	9.09±0.31	0.0007±0.0005**
LaP	0.468±0.016**	0.263±0.008**	0.212±0.006	34.00±0.38**	9.43±0.23	452±6**
LaBP	0.457±0.012	0.490±0.019**	0.280±0.005**	40.65±0.22**	11.39±0.23**	437±9

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the LaA and LaP's fractions is calculated relative to La-Ctrl, while the significance of the LaAP's fractions is calculated relative to LaP.

**: $p < 0.01$，*: $p < 0.05$.

Table 3.2.2b Soil fractions (g-P kg^{-1} soil) of strain B inoculation experiment in Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci-Ctrl	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiB	3.98±0.01**	4.36±0.05**	1.54±0.01**	2.38±0.01	2.73±0.02	299±2**
CiP	2.02±0.02**	5.62±0.02**	0.94±0.03	2.38±0.02	2.76±0.03	1800±9**
CiBP	4.10±0.01**	5.82±0.06	0.72±0.02**	2.49±0.04*	2.80±0.02	1790±6

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the CiA and CiP's fractions is calculated relative to Ci-Ctrl, while the significance of the CiAP's fractions is calculated relative to CiP.

**: $p < 0.01$，*: $p < 0.05$.

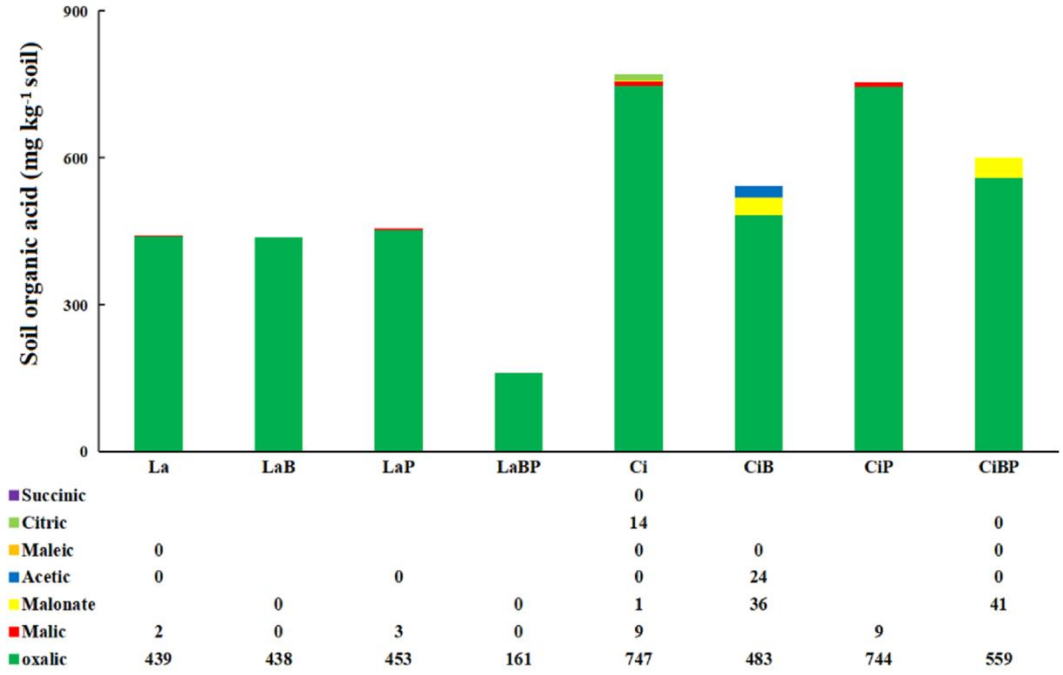


Fig. 3.2.2b Soil organic acid (mg kg^{-1} soil) of strain B inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

The activity of ACPase in both soils was higher than that of NPase and AKPase. The 3 phosphatase activities of all treatments in La were higher than those of all treatments in Ci. Inoculation of B increased the activity of 3 phosphatase; Among them, the increase rate of NPase and AKPase in La is greater than that in Ci, but the increase rate of ACPase activity in Ci is greater than that in La (Fig. 3.2.2c).

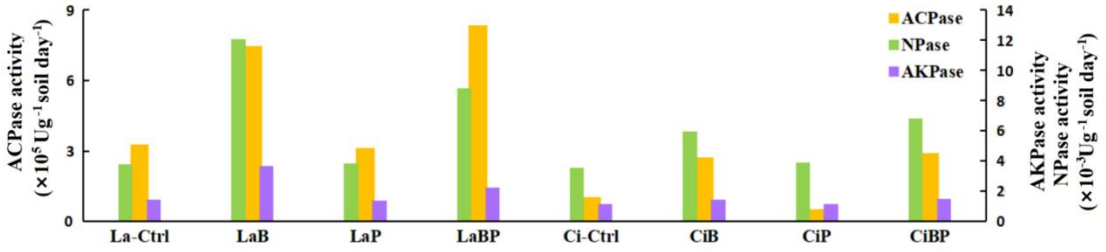


Fig. 3.2.2c Phosphatase activity of strain B inoculation experiment

Table 3.2.1c Change rate (%) of phosphatase activity of strain B inoculation experiment

	ACPase	NPase	AKPase		ACPase	NPase	AKPase
LaB	127	219	156	CiB	166	67	24
LaP	-5	1	-1	CiP	-48	10	2
LaBP	153	134	58	CiBP	183	90	29

Change rate is relative to control (La or Ci).
＞ 0: increased, ＜ 0: decreased.

Pearson correlation analysis was conducted on the fractions in two soils that had changed significantly in each treatment, and the factors that caused the change were found out from the five factors of soil pH, oxalic acid consumption, and three phosphatase activities.

It was found that the increases of resin P, $NaHCO_3$-Po and NaOH-Pi in La were related to the increase of oxalic acid consumption, ACPase and NPase activity; The factors affecting the increase of $NaHCO_3$-Pi and the decrease of HCl-P were not among the five factors.

In La added with TCP, the four fractions of $NaHCO_3$-P and NaOH-P have changed significantly compared with LaP. The increase of oxalic acid consumption and the activity of 3 phosphatase had significant positive effects on the four fractions; PH is positively correlated with

$NaHCO_3$-Po and NaOH-P.

In Ci and Ci with TCP added, five factors have significant effects on resin P and $NaHCO_3$-Po. Among them, pH is negatively correlated in Ci and positively correlated with resin P in Ci with TCP added; The change of $NaHCO_3$-Po was significantly decreased, and its change was negatively correlated with five factors.

Table 3.2.2d Pearson correlation between soil indicators and soil P fractions in strain B inoculation experiment

	soil pH	Oxalic consume	ACPase	NPase	AKPase	soil pH	Oxalic consume	ACPase	NPase	AKPase
			La					Ci		
Resin P	-	0.850*	0.853*	0.848*	-	-0.941**	0.999**	0.999**	1.000**	0.997**
$NaHCO_3$-Pi	-	-	-	-	-	-	-	-	-	-
$NaHCO_3$-Po	-	0.928**	0.929**	0.931**	-	-0.931**	0.998**	0.997**	0.999**	0.996**
NaOH-Pi	-	0.929**	0.928**	0.932**	-	-	-	-	-	-
NaOH-Po	0.889*	-0.878*	-0.880*	-0.882*	-	-	-	-	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-
			La+TCP					Ci+TCP		
Resin P	-	-	-	-	-	0.989**	0.999**	0.999**	1.000**	0.989**
$NaHCO_3$-Pi	-	0.821*	0.813*	0.811*	0.831*	-	-	-	-	-
$NaHCO_3$-Po	0.850*	0.935**	0.933**	0.933**	0.940**	-0.941**	-0.948**	-0.949**	-0.957**	-0.920**
NaOH-Pi	0.879*	0.928**	0.928**	0.928**	0.935**	-	-	-	-	-
NaOH-Po	0.900*	0.950**	0.948**	0.946**	0.948**	-	-	-	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-

**: $p < 0.01$, *: $p < 0.05$, -: $p \geq 0.05$

-*: Significant negative correlation, *: Significant positive correlation

Strain B also showed different phosphatase activity release and oxalic acid consumption in four treatments of two soils, and caused a decrease in soil pH in the soil environment without TCP, while it caused a rise in pH in the environment with TCP. The changes of P fraction caused by inoculation were correlated with the five factors, and there were significant differences among the four treatments.

3.2.3 Strain F release P of soils

After inoculation of strain F to La (LaF), four inorganic fractions were significantly changed, while the organic fractions were not affected. After inoculation to La with TCP (LaFP), only NaOH-Pi changed significantly compared with LaP (Table 3.2.3a).

After inoculation to Ci (CiF), all fractions except HCl-P were significantly changed, in which NaOH-Pi was significantly decreased. After inoculation to Ci added with TCP (CiFP), the fraction except NaOH-Pi and HCl-P increased significantly compared with CiP (Table 3.2.3b).

After inoculation to Ci (CiF), all fractions except HCl-P were significantly changed, in which NaOH-Pi was significantly decreased. After inoculation to Ci added with TCP (CiFP), the fraction except NaOH-Pi and HCl-P increased significantly compared with CiP (Table.3.2.3b).

Table 3.2.3a Soil fractions (g-P kg^{-1} soil) of strain F inoculation experiment in La

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaF	0.079±0.003**	0.043±0.004**	0.210±0.005	15.44±0.25**	9.56±0.26	0.0121±0.0033**
LaP	0.468±0.016**	0.263±0.008**	0.212±0.006	34.00±0.38**	9.43±0.23	452±6**
LaFP	0.506±0.016	0.266±0.007	0.216±0.009	35.36±0.29**	9.56±0.27	448±8

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the LaA and LaP's fractions is calculated relative to La-Ctrl, while the significance of the LaAP's fractions is calculated relative to LaP.

** $p < 0.01$，* $p < 0.05$

Table 3.2.3b Soil fractions (g-P kg^{-1} soil) of strain F inoculation experiment in Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci-Ctrl	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiF	2.39±0.01**	5.84±0.11**	1.78±0.05**	2.28±0.02**	2.83±0.02**	300±4
CiP	2.02±0.02**	5.62±0.02**	0.94±0.03	2.38±0.02	2.76±0.03	1800±9**
CiFP	2.82±0.07**	6.17±0.06**	1.78±0.06**	2.36±0.03	3.01±0.02**	1792±9

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the CiA and CiP's fractions is calculated relative to Ci-Ctrl, while the significance of the CiAP's fractions is calculated relative to CiP.

**: $p < 0.01$，*: $p < 0.05$.

F inoculation only caused a slight decrease in pH in La, but caused a slight increase in the soil added with TCP (Fig.3.2.3a). When the content of calcium phosphate in the soil is high (LaFP, CiF, CiFP), the oxalic acid in the soil is largely consumed. Among all treatments, CiF has the highest oxalic acid consumption rate (Fig.3.2.3b).

The activity of ACPase in both soils was higher than that of NPase and AKPase. The ACPase and AKPase activities of all treatments of La were higher than those of all treatments of Ci (Fig.3.2.3c). The inoculation of F increased the activity of AKPase in La, but decreased the activity of AKPase in Ci. The increase rate of AKPase in La is greater than that in Ci (Table 3.2.3c).

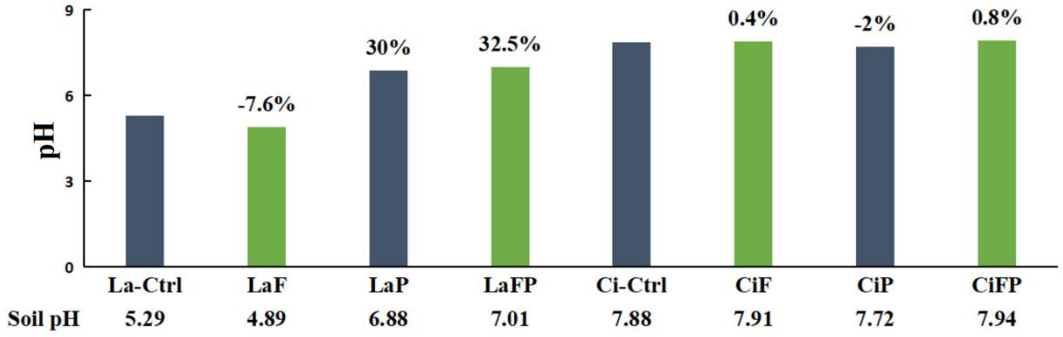


Fig. 3.2.3a Soil pH of strain F inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl in each treatment.

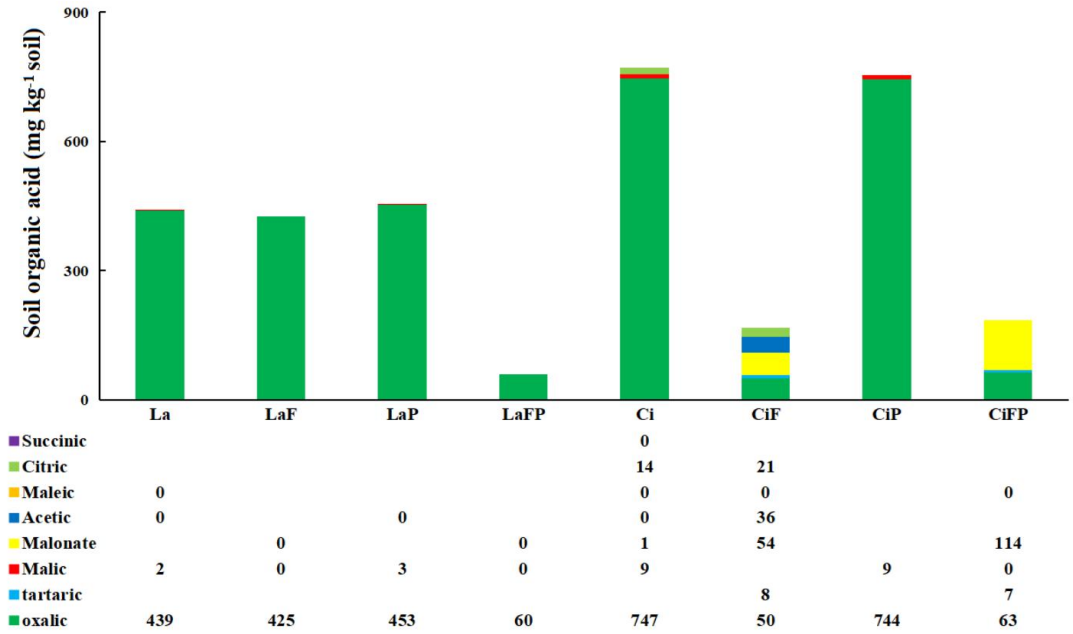


Fig. 3.2.3b Soil organic acid (mg kg^{-1} soil) of strain F inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

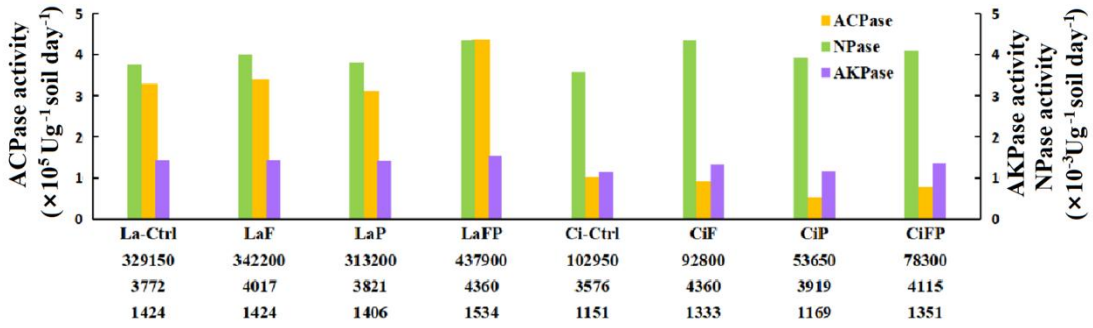


Fig. 3.2.3c Phosphatase activity of strain F inoculation experiment

Table 3.2.3c Change rate (%) of phosphatase activity of strain F inoculation experiment

	ACPase	NPase	AKPase		ACPase	NPase	AKPase
LaF	4	6	0	CiF	-10	22	16
LaP	-5	1	-1	CiP	-48	10	2
LaFP	33	16	8	CiFP	-24	15	17

Change rate is relative to control (La or Ci).

＞ 0: increased, ＜ 0: decreased.

Pearson correlation analysis was conducted on the fractions in two soils that had changed significantly in each treatment, and the factors that caused the change were found out from the five factors of soil pH, oxalic acid consumption, and three phosphatase activities.

It was found that in La, resin P increased due to the decrease of pH and the increase of oxalic acid consumption, and $NaHCO_3$-Pi increased due to the decrease of pH and the increase of oxalic acid consumption, as well as the increase of acid and NPase activities; NaOH-Pi is positively affected by NPase activities.

In La added with TCP, no significant factors related to the change of NaOH-Pi were found.

In Ci and Ci added with TCP, pH shows a positive correlation, while oxalic acid consumption still shows a positive correlation. ACPase activity shows a positive correlation in Ci with TCP, and a negative correlation in Ci without TCP.

Strain F also showed different phosphatase activity release and oxalic acid consumption under four treatments of two soils. Inoculation caused pH rise and oxalic acid consumption of other three treatments except LaF. The changes of P fraction caused by inoculation were correlated with the five factors, and there were significant differences among the four treatments.

Table 3.2.3d Pearson correlation between soil indicators and soil P fractions in strain F inoculation experiment

	soil pH	Oxalic consume	ACPase	NPase	AKPase	soil pH	Oxalic consume	ACPase	Npase	AKPase
			La					Ci		
Resin P	-0.867*	0.867*	-	-	-	-	0.999**	-0.927**	0.993**	0.978**
$NaHCO_3$-Pi	-0.896*	0.887*	0.865*	0.909*	-	0.837*	0.883*	-	0.858*	0.860*
$NaHCO_3$-Po	-	-	-	-	-	0.944**	0.938**	-0.906*	0.937**	0.959**
NaOH-Pi	-	-	-	0.826*	-	-	-	-	-	-
NaOH-Po	-	-	-	-	-0.849*	-	-	-	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-
			La+TCP					Ci+TCP		
Resin P	-	-	-	-	-	0.991**	0.982**	0.983**	-	0.956**
$NaHCO_3$-Pi	-	-	-	-	-	0.856*	-	0.816*	-	-
$NaHCO_3$-Po	-	-	-	-	-	0.933**	0.937**	0.916*	-	0.893*
NaOH-Pi	-	-	-	-	-	-	-	-	-	-
NaOH-Po	-	-	-	-	-	-	-	-	0.831*	-
HCl-P	-	-	-	-	-	-	-	-	-	-

**: $p < 0.01$, *: $p < 0.05$, -: $p \geq 0.05$

-*: Significant negative correlation, *:Significant positive correlation

3.2.4 Strain G release P of soils

After strain G was inoculated to La (LaG), significant changes in $NaHCO_3$-Pi and NaOH-Pi were caused, and the organic fractions were not affected. After inoculation to La with TCP (LaGP), the fraction except NaOH-Pi and HCl-P increased significantly compared with LaP (Table 3.2.4a).

After inoculation with Ci (CiG), the fraction increased significantly, except NaOH-Po and HCl-P. After inoculation to Ci with TCP (CiGP), the fraction increased significantly compared with CiP, except HCl-P (Table 3.2.4b).

Inoculation of strain G caused a decrease in pH of La and Ci without TCP, but caused a slight increase in pH of soil with TCP (Fig. 3.2.4a). The consumption of soil oxalic acid was greater in the combination treatment of the two soils (Fig. 3.2.4b).

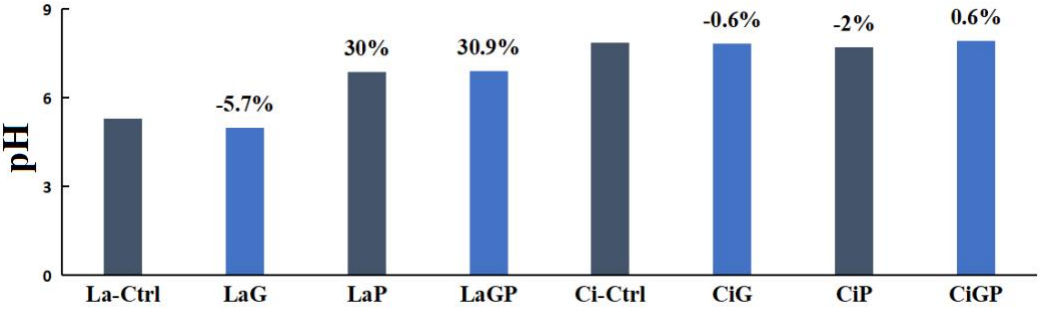


Fig. 3.2.4a Soil pH of strain G inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl in each treatment.

Table 3.2.4a Soil fractions (g-P kg^{-1} soil) of strain G inoculation experiment in La

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaG	0.062±0.007	0.056±0.005**	0.210±0.005	16.77±0.35**	9.66±0.35	0.0017±0.0008**
LaP	0.468±0.016**	0.263±0.008**	0.212±0.006	34.00±0.38**	9.43±0.23	452±6**
LaGP	0.542±0.009**	0.335±0.013**	0.284±0.006**	34.86±0.30	12.68±0.22**	451±6

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the LaA and LaP's fractions is calculated relative to La-Ctrl, while the significance of the LaAP's fractions is calculated relative to LaP.

**: $p < 0.01$，*: $p < 0.05$.

Table 3.2.4b Soil fractions (g-P kg^{-1} soil) of strain G inoculation experiment in Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci-Ctrl	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiG	1.92±0.01**	4.97±0.07**	1.34±0.11**	2.44±0.02*	2.74±0.01	304±3
CiP	2.02±0.02**	5.62±0.02**	0.94±0.03	2.38±0.02	2.76±0.03	1800±9**
CiGP	2.84±0.02**	5.03±0.03**	1.36±0.09**	2.56±0.03**	3.38±0.04**	1793±9

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the CiA and CiP's fractions is calculated relative to Ci-Ctrl, while the significance of the CiAP's fractions is calculated relative to CiP.

**: $p < 0.01$，*: $p < 0.05$.

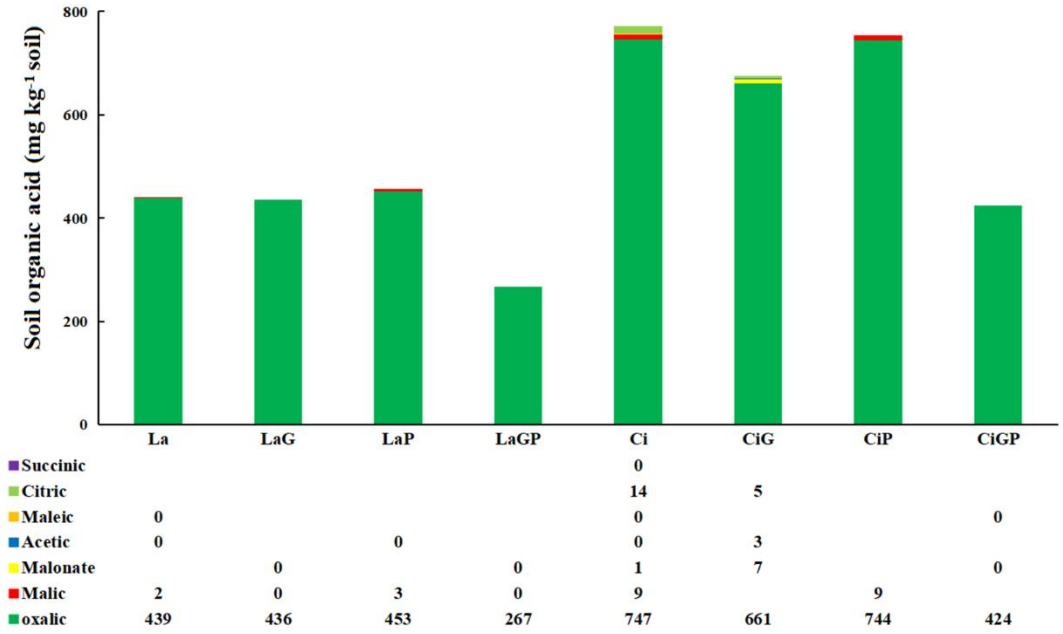


Fig. 3.2.4b Soil organic acid (mg kg^{-1} soil) of strain G inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

The activities of ACPase in both soils were higher than that of NPase and AKPase (Fig. 3.2.4c). The ACPase activity of LaGP was much higher than that of the other three treatments, with an increase rate at 370% (Table 3.2.4c).

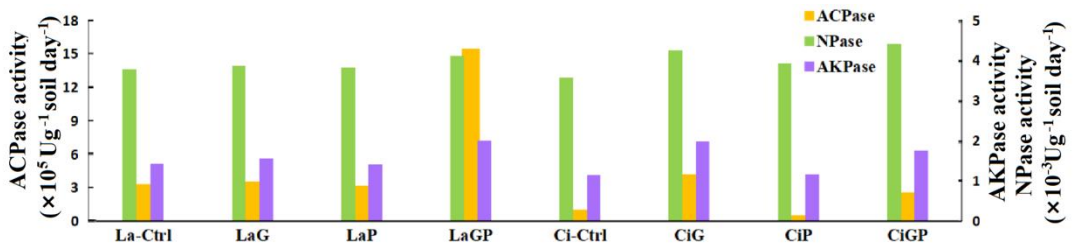


Fig. 3.2.4c Phosphatase activity of strain G inoculation experiment

Table 3.2.4c Change rate (%) of phosphatase activity of strain G inoculation experiment

	ACPase	NPase	AKPase		ACPase	NPase	AKPase
LaG	8	3	9	CiG	310	19	73
LaP	-5	1	-1	CiP	-48	10	2
LaGP	370	9	41	CiGP	152	23	52

Change rate is relative to control (La or Ci).
＞ 0: increased, ＜ 0: decreased.

As Table 3.2.4d showed, it was found that in La, $NaHCO_3$-Pi and NaOH-Pi were negatively correlated with pH, and positively correlated with oxalic acid consumption and phosphatase activity. None of the five factors was significant reasons causing changes in HCl-P.

In La added with TCP, the correlation of these five factors with the change of each fraction is positive.

In Ci, $NaHCO_3$-Pi and NaOH-Pi were negatively correlated with pH, and positively correlated with oxalic acid consumption and phosphatase activity.

In Ci with TCP added, the five factors all had positive effects on resin, while they had negative effects on $NaHCO_3$-Pi ($NaHCO_3$-Pi of CiGP is significantly reduced compared with CiP).

Strain G also showed different phosphatase activity release and oxalic acid consumption under four treatments of two soils, and caused a decrease in soil pH in the soil environment without TCP, while caused a rise in pH in the environment with TCP. The changes of P fraction caused by inoculation were related to all the five factors, but there were different among the four treatments.

Table.3.2.4d Pearson correlation between soil indicators and soil P fractions in strain G inoculation experiment

	soil pH	Oxalic consume	ACPase	NPase	AKPase	soil pH	Oxalic consume	ACPase	NPase	AKPase
			La					Ci		
Resin P	-	-	-	-	-	-0.924**	0.953**	0.949**	0.901*	0.928**
$NaHCO_3$-Pi	-0.945**	0.917**	0.870*	-	0.865*	-0.931**	0.841*	0.848*	0.895*	0.836*
$NaHCO_3$-Po	-	-	-	-	-	-	-	-	-	-
NaOH-Pi	-0.882*	0.892*	0.951**	0.826*	0.898*	-	-	-	-	-
NaOH-Po	-	-	-	-	-	-	-	-	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-
			La+TCP					Ci+TCP		
Resin P	0.842*	0.935**	0.936**	0.837*	0.915*	0.997**	0.994**	0.994**	0.968**	0.989**
$NaHCO_3$-Pi	-	-	-	-	-	-0.861*	-0.922**	-0.918**	-0.841*	-0.909*
$NaHCO_3$-Po	-	0.945**	0.944**	0.864*	0.937**	-	-	-	-	-
NaOH-Pi	-	-	-	-	-	-	-	-	-	-
NaOH-Po	-	0.991**	0.991**	0.932**	0.979**	0.820*	0.828*	0.824*	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-

**: $p < 0.01$, *: $p < 0.05$, -: $p \geq 0.05$

-*: Significant negative correlation, *:Significant positive correlation

3.2.5 Strain H release P of soils

Table 3.2.5a Soil fractions (g-P kg^{-1} soil) of strain H inoculation experiment

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaH	0.069±0.023	0.011±0.002	0.224±0.006	16.62±0.27**	9.57±0.38	0.0210±0.0049**
LaP	0.468±0.016**	0.263±0.008**	0.212±0.006	34.00±0.38**	9.43±0.23	452±6**
LaHP	0.445±0.009	0.456±0.015**	0.350±0.008**	34.03±0.33	12.71±0.32**	443±9

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the LaA and LaP's fractions is calculated relative to La-Ctrl, while the significance of the LaAP's fractions is calculated relative to LaP.

**: $p < 0.01$，*: $p < 0.05$.

Table 3.2.5b Soil fractions (g-P kg^{-1} soil) of strain H inoculation experiment in Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiH	2.43±0.01**	4.89±0.05**	1.12±0.02**	2.52±0.01**	2.87±0.01**	302±3
CiP	2.02±0.02**	5.62±0.02**	0.94±0.03	2.38±0.02	2.76±0.03	1800±9**
CiHP	2.84±0.01**	6.05±0.14a**	1.12±0.07*	2.53±0.04**	2.99±0.01**	1793±10

Values represent the mean ± SE (standard errors).

The significance was analyzed using an Independent-Samples T test. The significance of the CiA and CiP's fractions is calculated relative to Ci-Ctrl, while the significance of the CiAP's fractions is calculated relative to CiP.

**: $p < 0.01$，*: $p < 0.05$.

Inoculation of strain H to La (LaH) caused significant changes in NaOH-Pi and HCl-P fractions. Inoculation to La added with TCP (LaHP) caused significant changes in $NaHCO_3$-P and NaOH-Po relative to LaP (Table 3.2.5a).

After inoculation to Ci and C with TCP (CiH, CiHP), all fractions except HCl-P were significantly changed (Table 3.2.5b).

The pH of four treatments in both soils decreased due to H inoculation (Fig.3.2.5a). At the same time, when the content of calcium phosphate in the soil is high (LaHP, CiH, CiHP), the oxalic acid in the soil is largely consumed (Fig.3.2.5b).

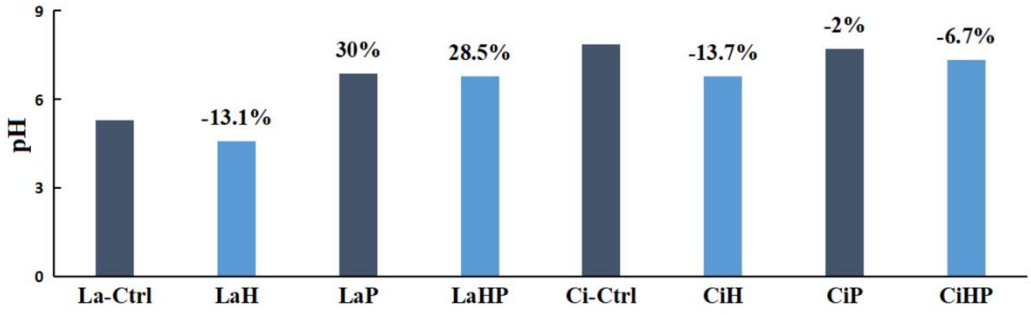


Fig. 3.2.5a Soil pH of strain H inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl in each treatment.

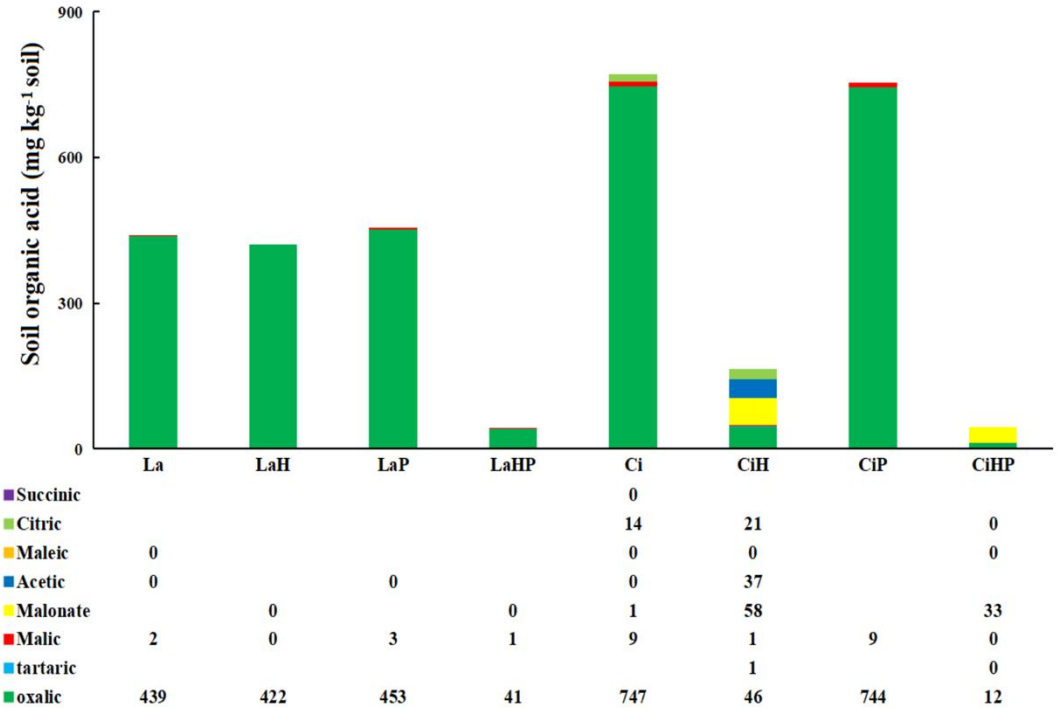


Fig. 3.2.5b Soil organic acid (mg kg^{-1} soil) of strain H inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

The activity of ACPase in both soils was higher than that of NPase and AKPase. The ACPase of La was higher than that of Ci. Compared with the control (La, Ci), the content and increase of three phosphatases of LaHP are higher than that of CiHP.

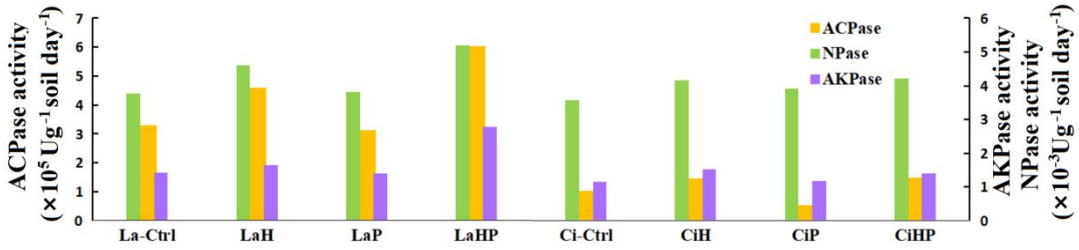


Fig. 3.2.5c Phosphatase activity of strain H inoculation experiment

Table 3.2.5c Change rate (%) of phosphatase activity of strain H inoculation experiment

	ACPase	NPase	AKPase		ACPase	NPase	AKPase
LaH	40	22	15	CiH	41	16	33
LaP	-5	1	-1	CiP	-48	10	2
LaHP	84	38	95	CiHP	44	18	22

Change rate is relative to control (La or Ci).

＞ 0: increased, ＜ 0: decreased.

From the Table 3.2.5d, it can be found that pH was negatively correlated with fractions in the four inoculation treatments.

In La, oxalic acid consumption and acid phosphatase activity are positively correlated with NaOH-Pi.

In La added with TCP, oxalic acid consumption and phosphatase activity are positively correlated with $NaHCO_3$-P and NaOH-Po.

In Ci, resin, $NaHCO_3$-P and NaOH-Po were significantly correlated with the five factors; In Ci with TCP added, only resinP is related to the five factors.

Strain H showed different phosphatase activity release and oxalic acid consumption in four treatments of two soils, but both caused the decrease of pH value. The changes of P fractionation caused by inoculation had different correlations with pH, oxalic acid consumption and phosphatase activity in the four treatments, but generally showed negative correlation with pH and positive correlation with other factors.

Table 3.2.5d Pearson correlation between soil indicators and soil P fractions in strain H inoculation experiment

	soil pH	Oxalic consume	ACPase	NPase	AKPase	soil pH	Oxalic consume	ACPase	NPase	AKPase
	La					Ci				
Resin P	-	-	-	-	-	-0.993**	0.993**	0.990**	0.987**	0.954**
$NaHCO_3$-Pi	-	-	-	-	-	-0.872*	0.889*	0.922**	0.861*	0.889*
$NaHCO_3$-Po	-	-	-	-	-	-0.905*	0.952**	0.939**	0.921**	0.992**
NaOH-Pi	-0.851*	0.863*	0.878*	-	-	-	-	-	-	-
NaOH-Po	-	-	-	-	-	-0.906*	0.910*	0.941**	0.857*	0.853*
HCl-P	-	-	-	-	-	-	-	-	-	-
	La+TCP					Ci+TCP				
Resin P	-	-	-	-	-	-0.992**	0.999**	0.997**	0.904*	0.974**
$NaHCO_3$-Pi	-0.889*	0.910*	0.907*	0.908*	0.911*	-	-	-	-	-
$NaHCO_3$-Po	-	0.972**	0.969**	0.970**	0.972**	-	-	-	-	-
NaOH-Pi	-	-	-	-	-	-	-	-	-	-
NaOH-Po	-	0.991**	0.992**	0.991**	0.990**	-	-	-	-	-
HCl-P	-	-	-	-	-	-	-	-	-	-

**: $p < 0.01$, *: $p < 0.05$, -: $p \geq 0.05$

-*: Significant negative correlation, *:Significant positive correlation

3.3 Discussion

This chapter introduces the experiment of PSB on the change of soil P fractions. Five PSB strains with higher ability to release 3 kinds of insoluble inorganic phosphate and 2 kinds of organic phosphate in shake flask culture were inoculated into two soils. The two soils are La and Ci, which have very different P fractions. For P fractionation of two soils, excluding residual P, P in La is mainly concentrated in NaOH extractable fraction, while P content distributed in other levels is very low. Due to the high content of iron and aluminum, La has a great potential to retain P; on the other hand, La provide little calcium phosphate for PSB to release, so whether PSB can change the P fractionation of La is studied in this experiment. Another kind of soil is Ci from Shanxi Province, China. This soil is weakly alkaline, with a high content of HCl extractable P, low iron and aluminum content. The content of NaOH extractable P is much lower than that of La. Compared with La, it is easier for PSB to release HCl extractable P.

According to the pH change data presented in Figure 3.3.1, the five strains tested caused a greater range of pH changes in La (in both PSB and combined treatments) compared to Ci. In all treatments, strains A and H caused a decrease in pH in La. Notably, H resulted in the lowest pH values across all four La series (with strain A being the same as strain H in the LaP series). On the other hand, strain B had the highest pH values

in two La's treatments, while strain F had the highest pH values in two Ci's treatments. Furthermore, the addition of TCP led to an increase in pH levels in LaP as well as in LaBP, LaFP, CiBP, CiFP, and CiGP, when compared to their respective controls.

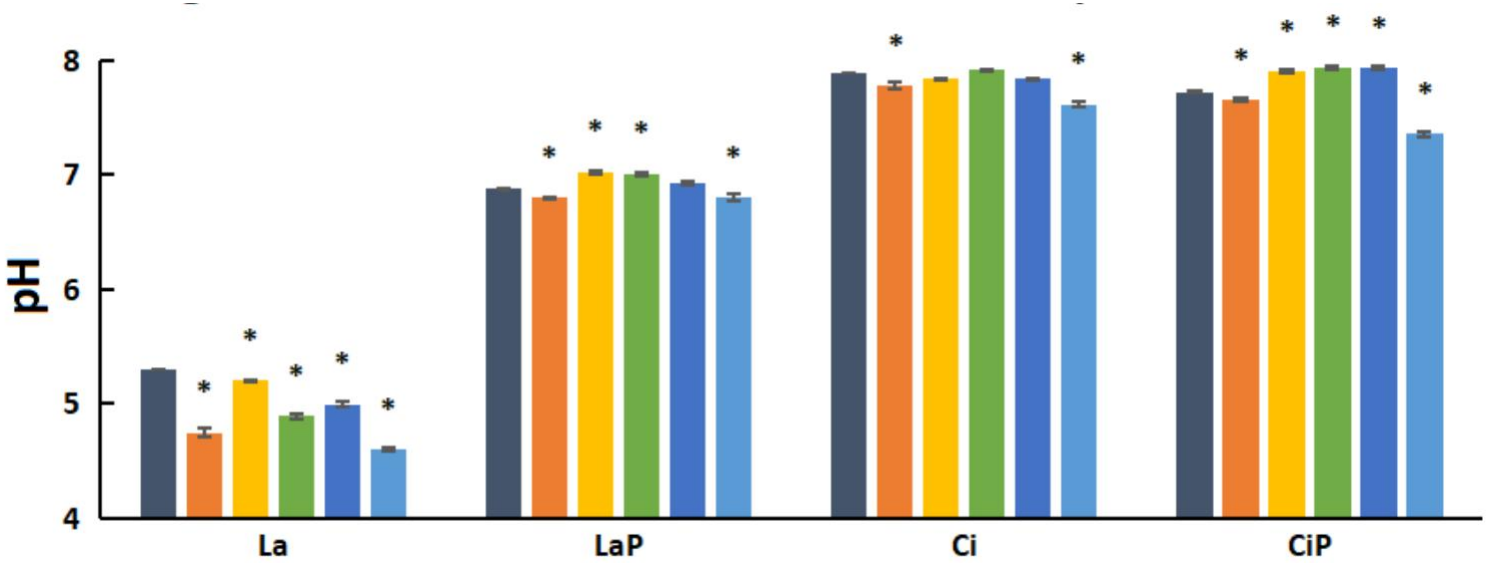


Fig. 3.3.1 Treatments' pH of inoculation experiment

The pH changes caused by PSB in soil inoculation experiments were found to be inconsistent with the pH changes

observed in shake flask experiments. For example, strain B caused lower pH levels in TCP, $FePO_4$, and $AlPO_4$ in shake

flask experiments than other strains, but it led to higher pH levels in soil inoculation experiments than some other strains. These results suggest that the pH responses of PSB vary depending on the environment.

From the perspective of soil organic acid detection (Table 3.3.1), oxalic acid was detected in all soil samples. The amount of oxalic acid detected in non-inoculated soil samples was higher than in samples inoculated with PSB. The content of oxalic acid was nearly the same in treatments without TCP supply and with TCP supply. TCP supply does not appear to alter the composition and content of organic acids in La soil without inoculation, but it slightly affects the composition of some Ci soil.

In addition to oxalic acid, malic acid was detected in uninoculated La soil with a content of 1.9-3.3 mg/kg soil, and in uninoculated Ci soil with a content of 9.3-9.4 mg/kg soil. Furthermore, malic acid, malonic acid, acetic acid, and citric acid were detected in the control group of Ci soil.

In the PSB treatment of La soil, the inoculation of the strain slightly reduced the content of oxalic acid in the soil, resulting in a reduction of 7.2-24.2 mg/kg soil. Malic acid was detected in all experimental groups, and malonic acid was detected in all experimental groups except group A. Group A also exhibited trace amounts of acetic acid.

In the combined treatment of La soil, the reduction in oxalic acid

caused by inoculation ranged from 186 to 411 mg/kg soil, with the largest reduction caused by strain H. Strain H secreted 0.6 mg/kg soil of malic acid. Malic acid and malonic acid were detected in all experimental groups, while trace amounts of maleic acid were detected in group A.

In the PSB treatment of Ci soil, the reduction in oxalic acid caused by inoculation ranged from 264 to 701 mg/kg soil, with the largest reduction caused by strain H. The content of both malonic acid and acetic acid secreted by all experimental groups was higher than in the control group, with malonic acid being 5.8-56.6 mg/kg higher and acetic acid being 2.5-41 mg/kg higher than in the control soil. The citric acid secreted by strain A, F, and H was 3.9-6.7 mg/kg higher than in the control soil. Additionally, strains F and H secreted 7.5 mg/kg and 0.8 mg/kg of tartaric acid, respectively.

In the combined treatment of Ci soil, the reduction in oxalic acid caused by inoculation ranged from 186 to 733 mg/kg soil, with the largest reduction caused by strain H. In addition to oxalic acid, strain A secreted 40.6 mg/kg soil of acetic acid and 8.7 mg/kg soil of malic acid, strain B secreted 41.0 mg/kg soil of malonic acid and 11.4 mg/kg soil of citric acid, strain F secreted 114.2 mg/kg soil of maleic acid and 7.0 mg/kg soil of tartaric acid, and strain H secreted 32.9 mg/kg soil of malonic acid and 2.5 mg/kg soil of citric acid. Trace amounts of maleic acid were detected in all experimental groups except group A.

Overall, the type and content of organic acids secreted by the same strain in Ci soil are generally higher than in La soil. For soils with high calcium phosphate content such as LaP, Ci, and CiP, the degree of change in oxalic acid is much greater than in La soil with low calcium phosphate content. It was also observed that strain H secreted malic acid in LaP, resulting in the largest reduction of oxalic acid. In Ci soil, the total amount of other organic acids secreted by strains H and F was 118.2 and 118.3, respectively, with their oxalic acid being reduced to 45.7 and 49.8, much higher than the reduction observed in group A, B and G.

Table 3.3.1 Soil organic acid (mg kg^{-1} soil) of inoculation experiment

group	Oxalic	Tartaric	Malic	Malonate	Acetic	Maleic	Citric	Succinic	SUM
La-Ctrl	439.0		1.9		trace	trace			440.9
LaA	406.7		trace		trace				406.7
LaB	438.2		trace	trace					438.2
LaF	425.4		trace	trace					425.4
LaG	436.0		trace	trace					436.0
LaH	421.7		trace	trace					421.7
LaP	452.6		3.3		trace				455.9
LaAP	64.4		trace	trace	trace	trace			64.4
LaBP	161.3		trace	trace					161.3
LaFP	59.9		trace	trace					59.9
LaGP	266.9		trace	trace					266.9
LaHP	41.2		0.6	trace					41.8
Ci-Ctrl	746.8		9.3	0.9	0.2	0.4	14.4	trace	772.0
CiA	213.8			52.2	41.2	trace	183		325.5
CiB	482.5			35.8	24.2	trace			542.5
CiF	49.7	7.5		53.7	36.0	0.1	21.1		168.0
CiG	661.5			6.7	2.7		4.8		675.7
CiH	45.7	0.8	1.4	57.5	37.4	trace	21.1		163.9
CiP	744.3		9.4						753.7
CiAP	296.6				40.6	8.7			345.9
CiBP	558.6			41.0	trace	trace	11.4		611.0
CiFP	63.1	7.0	trace	114.2		trace			184.3
CiGP	424.4			trace		trace			424.4
CiHP	11.7	trace	trace	32.9		trace	2.5		47.1

Based on an analysis of soil phosphatase activity, La soil generally exhibits higher levels of phosphatase activity compared to Ci soil. ACPase activity was found to be the highest among the three types of phosphatase. Additionally, all strains except F caused the increase of phosphatase activity in all treatments of both soils.

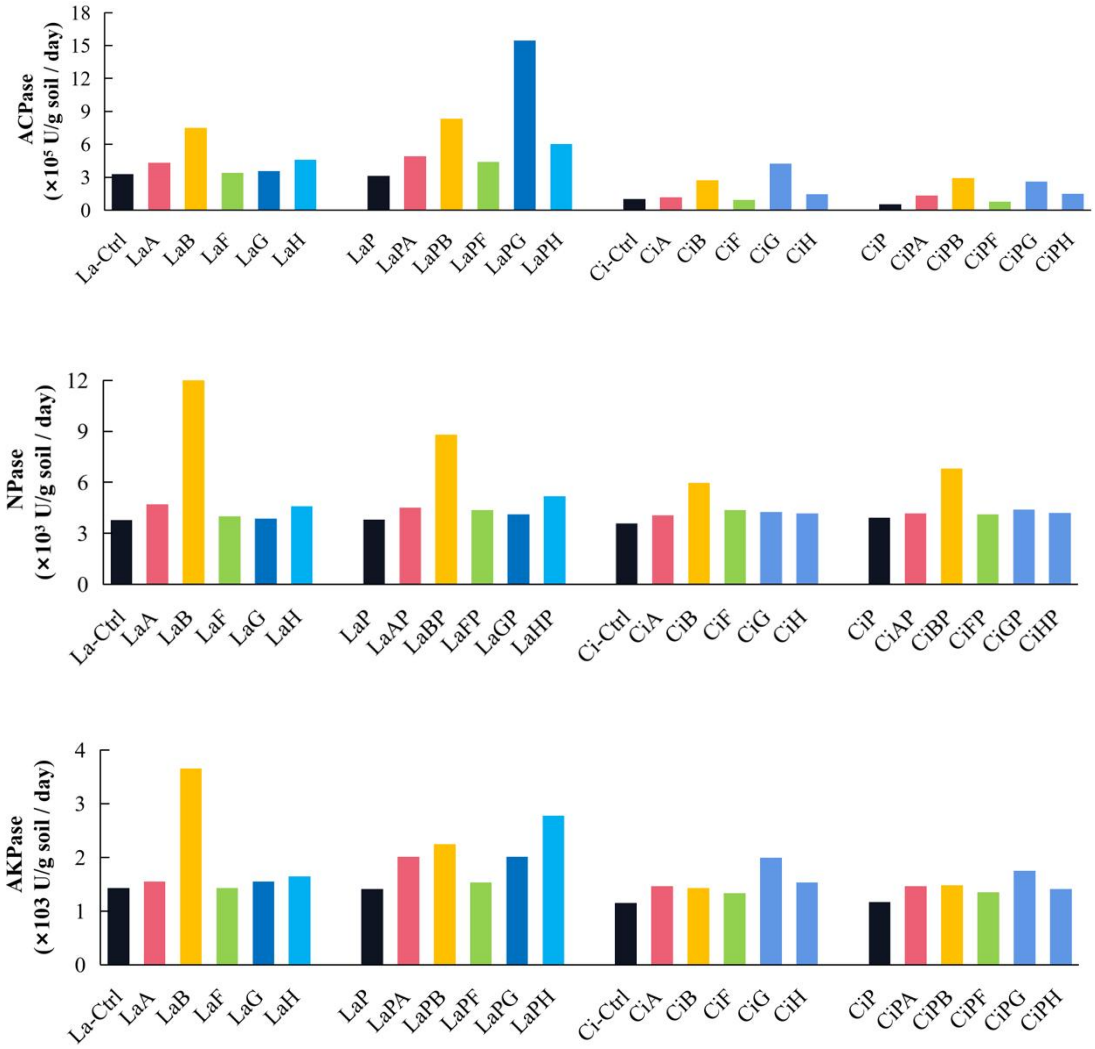


Fig.3.3.2 Soil phosphatase activity (U/g soil/ day) of inoculation experiment

While the application of PSB resulted in increased soil phosphatase activities, the magnitude of the changes varied depending on the strain

species, soil type, and treatment. Specifically, strains A, B, F, and H consistently displayed increased ACPase activity across all four treatments in both soil types. However, the enzyme activity caused by strain G differed significantly across the four treatments. NPase activity remained relatively stable in both La and Ci soils, while AKPase activity showed irregular fluctuations.

In La inoculation experiment (Table 3.3.2a), compared with the control (La-Ctrl), all groups of the PSB treatment were found to have significantly increased NaOH-Pi and decreased HCl-P. The TCP fertilization changed the size of the inorganic P fractions (i.e. resin P, $NaHCO_3$-Pi, NaOH-Pi, and HCl-P), but organic P fractions ($NaHCO_3$-Po, NaOH-Po) remained similar. Compared with the TCP treatment (LaP), all groups of the combination treatment, except LaFP, increased significantly in $NaHCO_3$-Pi, $NaHCO_3$-Po and NaOH-Po; LaAP, LaBP and LaFP increased significantly in NaOH-Pi. The interaction between PSB inoculation and TCP supply was found to be significant for all P fractions except HCl-P. Table 3.3.2b showed the changes of labile P, moderately labile P and HCl-P in La inoculation experiment. La-Ctrl's moderately labile P (23.71 mg-P kg^{-1} soil) is much higher than that of labile P (0.27 mg-P kg^{-1} soil). When TCP was added, the increase of moderately labile P (19.72 mg-P kg^{-1} soil) was greater than that of labile P (0.67 mg-P kg^{-1} soil). When PSB were inoculated, the increases of moderately labile P

were also greater than those of labile P in both PSB and combination treatments.

For Ci inoculation experiment (Table 3.3.3a), compared with Ci-Ctrl, all groups of the PSB treatment were found to have significantly increased resin P and $NaHCO_3$-Pi. TCP supply was found to have significantly different resin P, $NaHCO_3$-Pi, and HCl-P, but organic P fractions were unaffected, which is a similar finding to that for LaP. Compared with the CiP, all groups of the combination treatment were found to have significantly increased resin P. The interaction between PSB inoculation and TCP supply was found to be significant for resin P, $NaHCO_3$-Pi, $NaHCO_3$-Po, and NaOH-Po, whereas NaOH-Pi and HCl-P were unaffected by it. As Table 3.3.3b shows, Ci-Ctrl's labile P and moderately labile P were similar in their pool size: 6.84 and 5.13 mg-P kg^{-1} soil. TCP supply increased labile P by 1.74 mg kg^{-1} soil but no increase in moderately labile P was detected. When PSB were inoculated, the increases of labile P were greater or slightly greater than those of moderately labile P in both PSB and combination treatments, except CiGP.

Pearson correlation analysis of the two soils with TCP supply (TCP and combined treatment) and without TCP supply (control and PSB treatment) showed no significant correlation between labile P and HCl-P (data not shown).

Table 3.3.2a Soil P fractions (mg-P kg^{-1} soil) in La inoculation experiment

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
La-Ctrl	0.057±0.003	0.005±0.002	0.205±0.007	14.29±0.24	9.41±0.24	0.0729±0.0108
LaA	0.075±0.003	0.029±0.004*	0.210±0.005	15.55±0.36*	9.84±0.28	0.0124±0.0032*
LaB	0.084±0.005	0.070±0.008*	0.333±0.009*	19.96±0.39*	9.09±0.31	0.0007±0.0005*
LaF	0.079±0.003	0.043±0.004*	0.210±0.005	15.44±0.25*	9.56±0.26	0.0121±0.0033*
LaG	0.062±0.007	0.056±0.005*	0.210±0.005	16.77±0.35*	9.66±0.35	0.0017±0.0008*
LaH	0.069±0.023	0.011±0.002	0.224±0.006	16.62±0.27*	9.57±0.38	0.0210±0.0049*
LaP	0.468±0.016*	0.263±0.008*	0.212±0.006	34.00±0.38*	9.43±0.23	452±6*
LaAP	1.051±0.022*	0.605±0.009*	0.288±0.006*	43.00±0.32*	10.78±0.19*	432±4
LaBP	0.457±0.012	0.490±0.019*	0.280±0.005*	40.65±0.22*	11.39±0.23*	437±9
LaFP	0.506±0.016	0.266±0.007	0.216±0.009	35.36±0.29*	9.56±0.27	448±8
LaGP	0.542±0.009*	0.335±0.013*	0.284±0.006*	34.86±0.30	12.68±0.22*	451±6
LaHP	0.445±0.009	0.456±0.015*	0.350±0.008*	34.03±0.33	12.71±0.32*	443±9
PSB	***	***	***	***	***	-
TCP	***	***	***	***	***	***
PSB * TCP	***	***	***	***	***	-

Values represent the mean of three replicates ± SE (standard errors).

Significant differences among means were tested with one-way ANOVA (Dunnett. Use La-Ctrl as control category for LaA ~LaH and LaP; use LaP for LaAP ~ LaHP).

La-Ctrl: control, La with P-free NBRIP adding; LaA ~ LaH: PSB treatment, La with A ~ H suspensions inoculation; LaP: TCP treatment, La with TCP supply and P-free NBRIP adding; LaAP~LaHP: Combination treatment, La with A~H suspensions inoculation and TCP supply.

PSB, TCP, and PSB*TCP: Two-way ANOVA for the factors PSB inoculation, TCP supply, and the interaction of PSB inoculation ×TCP supply.

***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, −: $p \geqq 0.05$.

Table 3.3.2b Changes of labile P, moderately labile P and HCl-P in La inoculation experiment (mg-P kg-1 soil)

group	labile P	relative to La		moderately labile P	relative to La
		labile P Increase	HCl-P decrease		moderately labile P Increase
La-Ctrl	0.27			23.71	
LaA	0.31	0.05	0.06	25.39	1.69
LaB	0.49	0.22	0.07	29.05	5.34
LaF	0.33	0.06	0.06	25.00	1.29
LaG	0.33	0.06	0.07	26.43	2.73
LaH	0.30	0.03	0.05	26.20	2.49
LaP	0.94	0.67		43.43	19.72
		relative to LaP			
		labile P Increase	HCl-P decrease		
LaAP	1.94	1.68	1.00	20.00	53.78	30.07
LaBP	1.23	0.96	0.29	14.63	52.04	28.33
LaFP	0.99	0.72	0.05	4.26	44.92	21.21
LaGP	1.16	0.89	0.22	1.11	47.54	23.83
LaHP	1.25	0.98	0.31	9.26	46.75	23.04

La-Ctrl control; *LaA ~ LaH* PSB treatment, La with A ~ H strains inoculating；*LaP* TCP treatment, La with TCP supply；*LaAP ~ LaHP* Combination treatment, La with A~H strains inoculating and TCP supply；

Table 3.3.3a Soil P fractions (mg-P kg^{-1} soil) in Ci inoculation experiment

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P
Ci-Ctrl	1.76±0.01	4.13±0.01	0.95±0.01	2.38±0.01	2.76±0.01	307±2
CiA	3.05±0.01*	4.49±0.04*	1.44±0.02*	2.39±0.01	3.41±0.01*	300±3
CiB	3.98±0.01*	4.36±0.05*	1.54±0.01*	2.38±0.01	2.73±0.02	299±2
CiF	2.39±0.01*	5.84±0.11*	1.78±0.05*	2.28±0.02*	2.83±0.02*	300±4
CiG	1.92±0.01*	4.97±0.07*	1.34±0.11*	2.44±0.02*	2.74±0.01	304±3
CiH	2.43±0.01*	4.89±0.05*	1.12±0.02	2.52±0.01*	2.87±0.01*	302±3
CiP	2.02±0.02*	5.62±0.02*	0.94±0.03	2.38±0.02	2.76±0.03	1800±9*
CiAP	4.10±0.01*	6.17±0.02*	1.43±0.02*	2.44±0.05	3.69±0.01*	1787±7
CiBP	4.10±0.01*	5.82±0.06	0.72±0.02*	2.49±0.04	2.80±0.02	1790±6
CiFP	2.82±0.07*	6.17±0.06*	1.78±0.06*	2.36±0.03	3.01±0.02*	1792±9
CiGP	2.84±0.02*	5.03±0.03*	1.36±0.09*	2.56±0.03*	3.38±0.04*	1793±9
CiHP	2.84±0.01*	6.05±0.14*	1.12±0.07	2.53±0.04*	2.99±0.01*	1793±10
PSB	***	***	***	***	***	-
TCP	***	***	***	***	***	***
PSB * TCP	***	***	***	-	***	-

Values represent the mean of three replicates ± SE (standard errors).

Significant differences were assessed using One-way ANOVA (Dunnett. Use Ci-Ctrl as control category for CiA ~CiH and CiP; use CiP for CiAP ~ CiHP).

Ci-Ctrl: control, Ci with P-free NBRIP adding; CiA ~ CiH: PSB treatment, Ci with A ~ H suspensions inoculation; CiP: TCP treatment, Ci with TCP supply and P-free NBRIP adding; CiAP~CiHP: Combination treatment, Ci with A~H suspensions inoculation and TCP supply.

PSB, TCP, and PSB*TCP: Two-way ANOVA for the factors PSB inoculation, TCP supply, the interaction of PSB inoculation ×TCP supply.

***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, −: $p \geqq 0.05$.

Table 3.3.3b Changes of labile P, moderately labile P and HCl-P in Ci inoculation experiment (mg-P kg^{-1} soil)

group	labile P	relative to Ci			moderately labile P	relative to Ci
		labile P Increase	HCl-P decrease			moderately labile P Increase
Ci-Ctrl	6.84				5.13	
CiA	8.98	2.14	7.04		5.80	0.66
CiB	9.88	3.04	7.96		5.11	-0.02
CiF	10.01	3.17	7.41		5.11	-0.02
CiG	8.23	1.39	3.33		5.18	0.04
CiH	8.44	1.60	5.37		5.38	0.25
CiP	8.58	1.74	relative to CiP		5.14	0.00
			labile P Increase	HCl-P decrease		
CiAP	11.70	4.86	3.12	13.15	6.14	1.01
CiBP	10.63	3.79	2.05	10.37	5.29	0.16
CiFP	10.77	3.93	2.19	8.15	5.37	0.24
CiGP	9.22	2.38	0.64	6.85	5.94	0.81
CiHP	10.01	3.17	1.43	7.22	5.52	0.39

Ci-Ctrl control; *CiA ~ CiH* PSB treatment, Ci with A ~ H strains inoculating; *CiP* TCP treatment, Ci with TCP supply; *CiAP ~ CiHP* Combination treatment, Ci with A~H strains inoculating and TCP supply;

To further clarify how PSB inoculation affected the soil labile and M labile P, Pearson correlation were calculated.

Table 3.3.4 Pearson correlation between PSB inoculation and soil physicochemical indicators

	Labile P	M labile P	pH	Oxalic	ACP	Labile P	M labile P	pH	Oxalic	ACP
	in La without TCP					in Ci without TCP				
PSB	—	.471*	-.624**	—	—	.735**	—	—	-.607**	—
Labile P		.805**	—	—	.831**		—	—	-.559*	—
M labile P			—	—	.683**			-.476*	—	—
pH				.797**	—				—	—
Oxalic					—					.504*
	in La with TCP					in Ci with TCP				
PSB	—	.536*	—	-.848**	—	.621**	—	—	-.679**	.551*
Labile P		.803**	-.527*	-.471*	—		—	—	—	—
M labile P			—	—	—			—	—	—
pH				—	—				—	—
Oxalic					—					—

**: $p < 0.01$; *: $p < 0.05$; -: $p \geq 0.05$

-*: Significant negative correlation; *: Significant positive correlation

As Table 3.3.4 showed that in both La with and without TCP, PSB inoculation showed a positive correlation with soil M labile P, but no correlation showed with labile P. In in both Ci with and without TCP, PSB inoculation showed a

positive correlation with soil labile P, while no correlation showed with soil M labile P.

Considering the information above, in the PSB treatment of La, strain B with a higher release capability for phytin caused the greatest increase in soil labile P. It can be seen that in La, the three phosphatase activities of the group inoculated with B were higher than those of the group inoculated with other strains, which may help B release certain organic phosphates in the soil. At the same time, it can be seen that the decrease in soil pH of the group inoculated with B was smaller than that of the group inoculated with other strains, which is consistent with B's pH changes in the phytin bottle. Overall, in La with low HCl-P content, phosphatase may mainly be relied upon to change the soil P structure. This can be seen in Table 3.3.4, where soil ACPase activity was positively correlated with soil labile P content and M labile P content.

In the combination treatment of La, the supply of TCP has changed the original P fractionation in the soil, thereby affecting the performance of various strains in releasing P from the treated soil. Significant decreases in soil pH were observed in the groups inoculated with strains A and H, while the group inoculated with strain G exhibited higher acid phosphatase activity than the other groups. According to Table 3.3.4, soil pH was negatively correlated with soil labile P content, and higher labile P content was detected in the soil of group A with lower pH than in other

groups. This may indicate that the pH decrease is one of the reasons for the increase in labile P in the soil with high HCl-P. It is worth noting that, although the soil pH in the group inoculated with strain H was the same as that in group A, there was no significant increase in labile P compared to other strains. Therefore, the pH decrease is not the absolute cause of the increase in labile P in the soil with high HCl-P.

In PSB treatment of Ci, which was Ca-P rich soil, the largest pH decrease was observed in the group inoculated with strain H, while the maximum acidic phosphatase activity was found in the group inoculated with G. However, the greater decrease in soil HCl-P and the larger increase in labile P were observed in groups B and F. The correlation analysis in Table 3.3.4 also indicates that soil pH and acidic phosphatase are not related to labile P content in Ci with TCP.

In the combination treatments of Ci, the maximum pH decrease occurred in the group inoculated with strain H, while the highest activity of acidic phosphatase was found in the group inoculated with strain B. The maximum decrease in HCl-P and the maximum increase in labile P were observed in the group inoculated with strain A. As shown in Table 3.3.4, similar to the Ci without TCP, the analysis results of Ci with TCP also indicated that there was no correlation between soil pH and acidic phosphatase with soil labile P.

It could be seen that PSB exhibited different P release efficiencies in

different environments. This might be related to the release strategies of PSB itself (secretion of phosphatase or lowering of pH), as well as the influence of the environment. In this experiment, regardless of Ci or La, strain B caused the maximum decrease in soil HCl-P when TCP was not supplied. After TCP supply, strain A caused the maximum decrease in HCl-P. Therefore, the same environmental changes might have different effects on the phosphorus release efficiency of different PSB.

Chapter IV

PSB affect maize seedling P accumulation in sterilized soils (sterilized co-culture experiment)

To investigate whether PSB affects maize seedling P accumulation in soils, a sterilized co-culture experiment was carried out. In this experiment, PSB strain A was inoculated into the roots of maize seedlings growing in sterile La and Ci soils, with or without TCP added. Strain A was selected because it caused an increase in labile P of 1.68 mg-P kg^{-1} soil in LaAP and 4.86 mg-P kg^{-1} soil in CiAP, which were higher than other strains. One week later, strain A was found to have survived, and there was no bacterial growth in the TCP treatment or the control. The maize shoots and soils were then collected for measurement.

4.1 Materials and methods

4.1.1 Materials and treatments

Soils used in sterilized co-culture experiment are the same as 3.2.1, and were set the treatments as follows (Table 4.1.1):

Table 4.1.1 Treatments and conditions

Treatments	Soil and P supplements	Inoculation	Moisture support
Control	La / Ci 50g	2.5 mL P free NBRIP	12.5 mL sterile distilled water
PSB treatment	La / Ci 50g	2.5mL A suspension	12.5 mL sterile distilled water
TCP treatment	La / Ci 50g + 0.5g TCP	2.5 mL P free NBRIP	12.5 mL sterile distilled water
Combination treatment	La / Ci 50g + 0.5g TCP	2.5mL A suspension	12.5 mL sterile distilled water

Add 5 mL P free Hoagland nutrient solution was added to support plant growth every other day.
Add sterile distilled water by weight.

Strain A suspension was prepared as that in 2.1.2.1 (Shake flask culture).

Maize seed: *Zea mays* L. cv. Guidan 162 Guangxi Zhaohe Seed Industry, China (Fig.4.1.1).

Fig.4.1.1 Package of used maize seeds

4.1.2 Seedling culture

The flow of cultivation treatment was shown in Fig.4.1.2. Maize seeds were well washed with tap water. After draining off the water, seeds were washed once with 75% (v/v) ethanol. They were then rinsed off with sterile distilled water. The seeds were immersed in 1% (w/v) mercuric chloride for 2 min and were rinsed with sterile distilled water. Sterilized seeds were covered with moistened sterile paper towels and were placed in sterile petri dishes. Germination was conducted for 24 hr at 28°C.

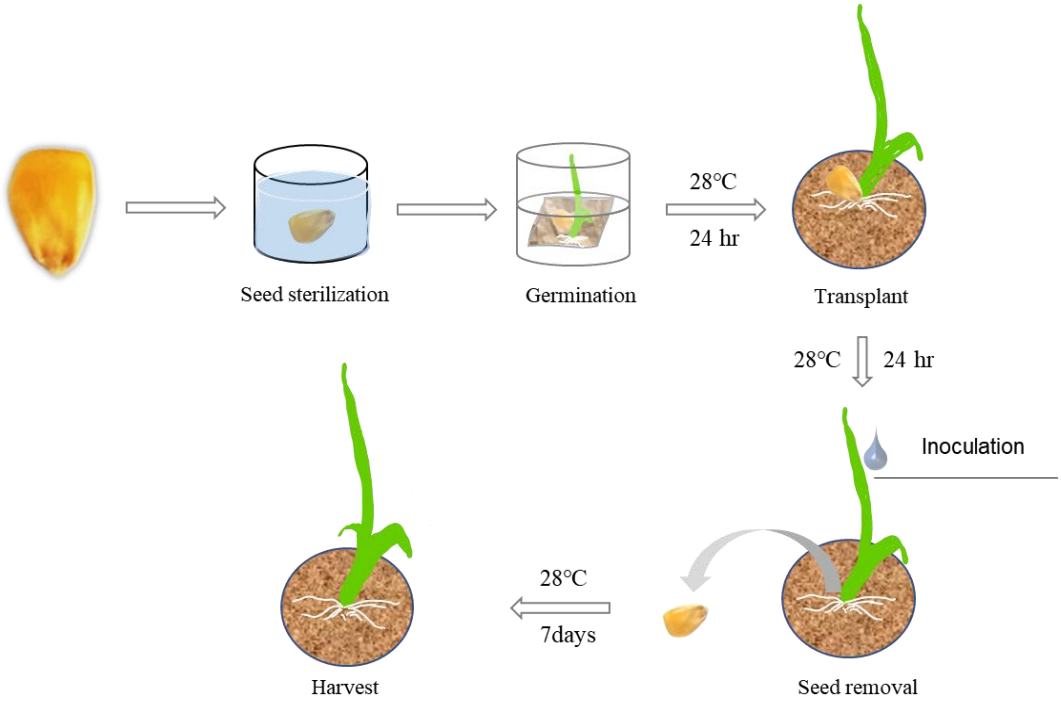


Fig.4.1.2 Flow of the inoculation experiment

The germinated maize seeds were transplanted into culture containers containing the control and treatment soils. To each container, 5 milliliters of P-free Hoagland nutrient solution (Hoagland and Arnon, 1950) and 12.5 milliliters of sterile distilled water were added. After a 24-hour incubation at 28°C, the maize seeds were carefully removed from the seedlings. Two point five milliliters of PSB suspension were added to the PSB treatment and combination treatment, while 2.5 milliliters of P-free NBRIP were added to the control and TCP treatment. Then, 5 milliliters of P-free Hoagland nutrient solution were added to each container every other day to support plant growth. Additionally, sterile distilled water was added to maintain moisture, which was controlled by

weight. Seven days later, the above-ground plant parts were harvested, dried at 70°C, weighed, and measured to determine total P content. Zero point one gram of soil was used to confirm strain survival, while 1 gram of soil was air dried to measure soil phosphatase activity. The remaining soils were lyophilized to measure P fractions and organic acid content.

4.1.3 Seedling P content measuring

The content of total P in plant shoot was determined by H_2SO_4 decomposition method:

Weigh 0.05g of each sample into a 25 mL glass test tube, and then add 1 mL concentrated H_2SO_4 into the tube. Place the test tube at 200 ℃ for heat preservation. After 5 minutes, add 200 μL 30% hydrogen peroxide to the tube, and then add it every 7 minutes until the solution becomes clear and transparent. After the solution becomes clear and transparent, keep it at 200 ℃ for 2 hours to drive away the remaining hydrogen peroxide.

After cooling the solution, add distilled water to a constant volume of 20 mL, and measure the P content in the solution with molybdenum blue method. Then calculate the P content of the plant sample according to the digested weight.

4.1.4 Soil P fraction, pH, organic acid and phosphatase measuring

Soil P fractions was measured by Hedley method which is the same as 3.1.2. Soil pH was measured by pH meter, the content of organic acid in soil is detected by HPLC, and the enzyme activity is measured by kit, which is the same as 3.1.3-3.1.5.

4.2 Results

4.2.1 Maize seedling P accumulation of two soils

Maize seedling shoot dry weight in La was generally heavier than that in Ci (Fig. 4.2.1a). PSB inoculation or TCP supply increased shoot dry weight. Combination treatment showed heavier than single treatment as PSB or TCP treatment. Relative to control, combination treatment increased 74% in La and 51% in Ci.

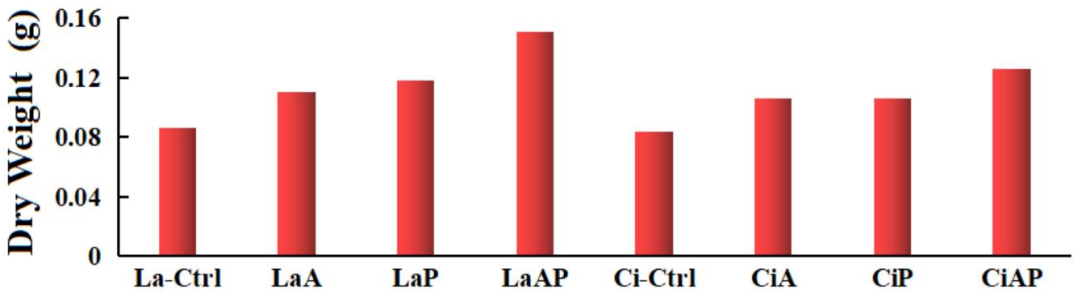


Fig. 4.2.1a Dry weight of maize seedling shoot

Maize seedling shoot P content in La is almost half of that in Ci, and almost same within La treatments (Fig. 4.2.1b).

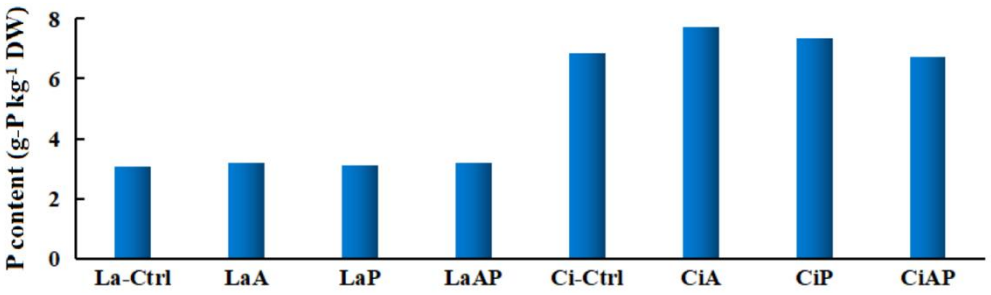


Fig. 4.2.1b P content of maize seedling shoot

In terms of P accumulation of maize seedling (Fig. 4.2.1c), LaA and CiA were higher than La-Ctrl and Ci-Ctrl, respectively. LaP and CiP were also higher than La-Ctrl and Ci-Ctrl, respectively, LaAP was higher than La-Ctrl, LaA and LaP, and CiAP was higher than Ci-Ctrl and CiP. There were no significant differences between LaA and LaP and between CiA and CiP.

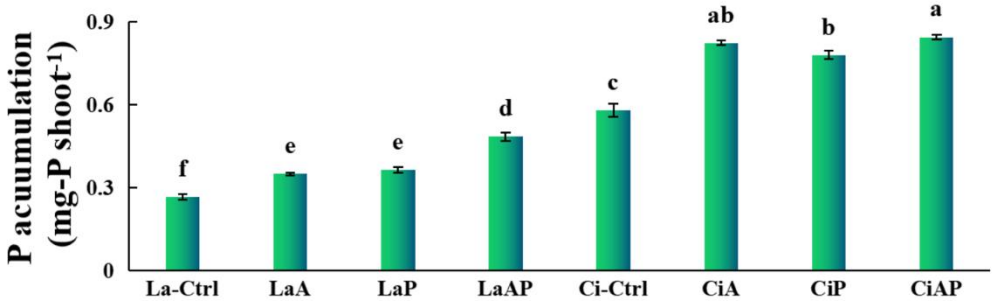


Fig. 4.2.1c P accumulation of maize seedling shoot. Different letters indicate significant differences ($p < 0.05$; one-way ANOVA, Tukey, n = 3). Error bars = SE.

The results in Table 4.2.1 indicated that In La, PSB inoculation increased the P accumulation by 0.082 mg, increase rate was around 31%; TCP supply increased the P accumulation by 0.097 mg, increase rate was

around 36%; and the combination treatment increased the P accumulation by 0.217 mg, increase rate was around 81%. The absolute increase and increase rate of LaAP was higher than that of LaA. In Ci, PSB inoculation increased the P accumulation by 0.244 mg, increase rate was around 42%; TCP supply increased the P accumulation by 0.200 mg, increase rate was around 35%; and the combination treatment increased the P accumulation by 0.265 mg, increase rate was around 46%. The absolute increase and increase rate of CiAP was similar to CiA.

In both soils, all treatments increased maize seedling P accumulation higher than the control, and there was no significant difference between the PSB treatment and the TCP treatment.

In terms of the increase in quantity, the combined treatment of LaAP showed a greater increase compared to the combined increase of the PSB-treated LaA and TCP-treated LaP, while it was lower in Ci soils.

In terms of the increase rate, PSB inoculation was more efficient in Ci soil compared to La soil, while TCP supply showed a similar increase in both soils. However, the combined utilization of PSB and TCP in La soil showed an increase nearly twice as much as in Ci soil.

In general, for La soil, although the overall P accumulation is low, the combined utilization of PSB and TCP significantly improved the planting effects. In Ci soil, PSB inoculation alone showed promising results.

Table 4.2.1 Maize seeding P accumulation and variation

group	P accumulation (mg-P/ shoot)	relative to La-Ctrl	
La-Ctrl	0.268±0.011c	Increase (mg)	Increase rate %
LaA	0.350±0.005b	0.082	30.60
LaP	0.365±0.009b	0.097	36.19
LaAP	0.485±0.016a	0.217	80.97
		relative to Ci-Ctrl	
Ci-Ctrl	0.580±0.024c	Increase (mg)	Increase rate %
CiA	0.824±0.009ab	0.244	42.07
CiP	0.780±0.016b	0.200	34.48
CiAP	0.845±0.007a	0.265	45.69

P accumulation represent the mean of three replicates ± SE (standard errors).
Values in a column not followed by a common letter indicate significant differences ($p < 0.05$; one -way ANOVA, Tukey).
La, Ci control; LaA, CiA PSB treatment； LaP, CiP TCP treatment；
LaPA, CiPA Combination treatment.

4.2.2 Soils pH

In sterilized co-cultivation experiments, soil pH measurements were conducted on different treatments, and it was found that inoculation with PSB strain A did not significantly alter the soil pH values (Fig.4.2.2). In La, the addition of TCP increased soil pH, while in Ci, the addition of TCP had no significant effect on soil pH. When compared with the soil inoculation experiments, it was found that after strain A was inoculated into La, it caused a 10.3% decrease in soil pH, while in La planted with

corn, strain A caused a 3.5% decrease in soil pH. Using LaP as a control, strain A's inoculation caused an 11.6% decrease in LaP soil pH, while in LaP planted with maize seedling, there was a 1.4% increase in pH caused by strain A inoculation. In Ci, regardless of whether TCP was added or not, strain A's inoculation caused a slight decrease in soil pH, while in Ci planted with maize seedling, strain A's inoculation did not cause a decrease in soil pH. Thus, the alteration of soil pH by PSB is influenced by the roots of plants to a certain extent.

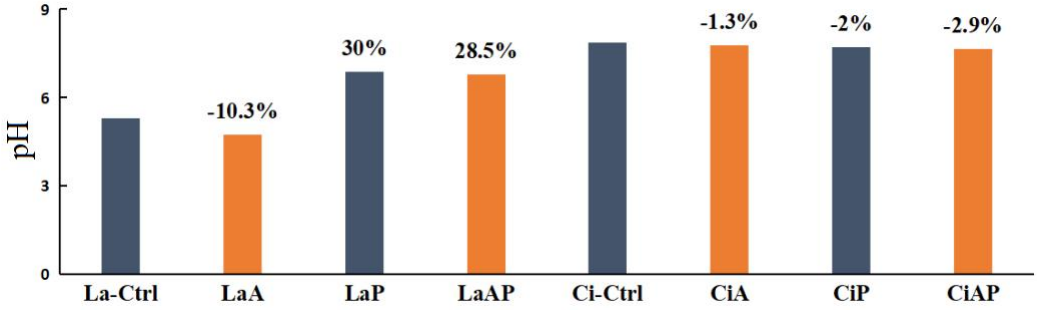


Fig. 4.2.2 Soil pH of strain A inoculation experiment. The percentages indicated on LaA, LaP, and LaAP represent the percentage of pH change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of pH change relative to Ci-Ctrl in each treatment.

4.2.3 Soils organic acid

Soil organic acids, mainly oxalic acid, were detected in all treatments of sterilized co-culture of both soils (Fig.4.2.3ab). In La, the inoculation of strain A caused a 2.1% decrease in soil oxalic acid content, while in TCP-added La, the inoculation of strain A also caused a 15.6% decrease in soil oxalic acid content. In Ci, the inoculation of strain A

caused a 2.2% decrease in soil oxalic acid content, but in TCP-added La, the inoculation of strain A did not cause a decrease in soil oxalic acid content.

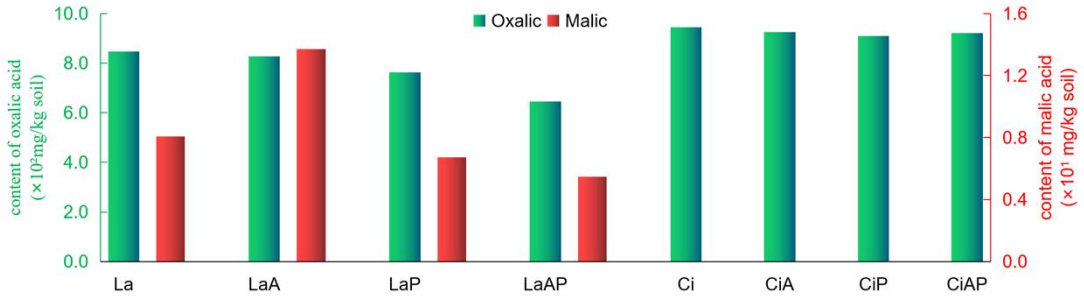


Fig. 4.2.3a Content of organic acid in the sterilized co-culture soil

Comparing these results with the organic acid detection results of the soil inoculation experiment with strain A, it was found that the soil oxalic acid content in La and Ci, where no maize seedlings were planted, was lower than that in the soil where maize seedlings were planted. In terms of inoculation experiment, the inoculation of strain A caused 7.3% and 85.9% decrease in La and TCP-added La's oxalic acid content, which were both greater than the decreases in soil oxalic acid content in the same treatment where maize seedlings were planted. The inoculation of strain A also caused 71.4% and 60.0% decrease in Ci and TCP-added Ci's oxalic acid content, which were both greater than the decrease in soil oxalic acid content in the same treatment where maize seedlings were planted.

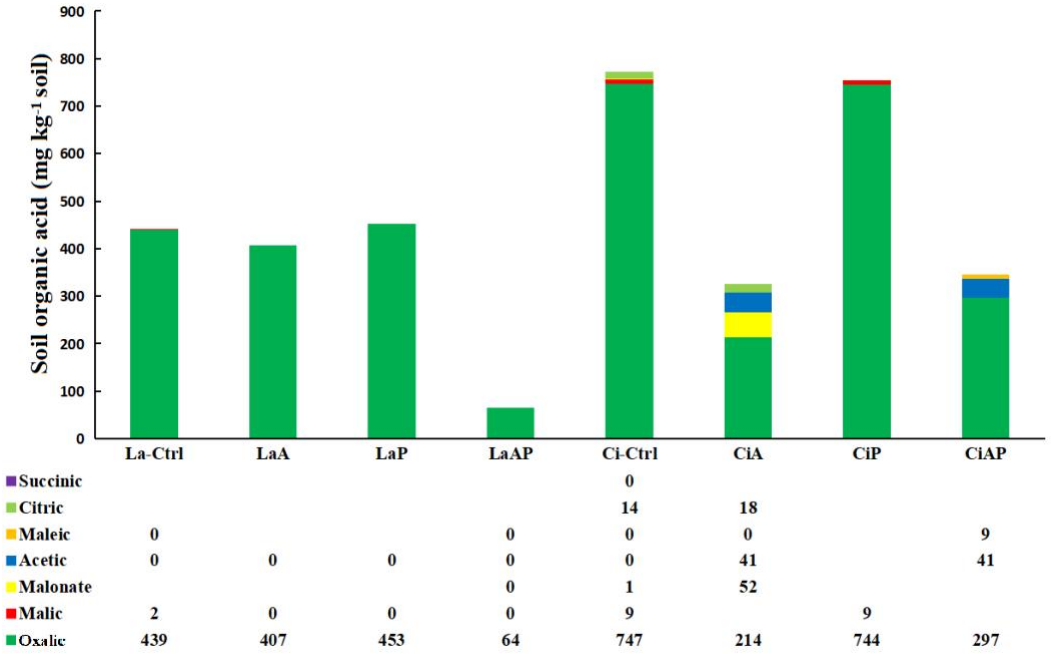


Fig. 4.2.3b Soil organic acid (mg kg^{-1} soil) of strain A inoculation experiment. The value "0" represents trace amounts. The terms used in the context are as follows: "Succinic" refers to succinic acid, "Citric" refers to citric acid, "Maleic" refers to maleic acid, "Acetic" refers to acetic acid, "Malonate" refers to malonic acid, "Malic" refers to malic acid, and "Oxalic" refers to oxalic acid.

It is worth noting that in the soil inoculation experiment with strain A, a certain amount of malic acid was present in Ci and TCP-added Ci where strain A was not inoculated, but it was not found after inoculation, but it was not detected after inoculation. However, in the sterilized co-culture experiment, malic acid was not detected in any of the treatments of Ci, but a certain amount of malic acid was detected in all treatments of La, except for the high content of oxalic acid.

The author considered that the decrease in soil oxalic acid content in both experiments might be due to the consumption by the inoculated PSB. However, in the soil where maize seedlings were planted, the root system

might provide oxalic acid, which resulted in a smaller decrease in oxalic acid content than in the soil where no maize seedlings were planted. As for malic acid, it might also be consumed by PSB, but in the sterilized co-culture experiment, maize roots in La might secrete more malic acid than in Ci, resulting in a higher detection of malic acid in La where maize seedlings were planted than in Ci where maize seedlings were planted (no malic acid was detected in any treatments of Ci where maize seedlings were planted).

4.2.4 Soils phosphatase

As shown in Figure 4.2.4a, the activities of soil acid phosphatase (ACPase) in the La treatments were lower than those in the Ci treatments. In both soils, whether with or without added TCP, inoculation with PSB strain A increased the activity of soil ACPase. Comparing the soil inoculation experiment with strain A (Figure 4.2.4a'), it can be observed that the presence of maize seedling roots reduced the soil ACPase activities in the La treatments, but increased them in the Ci treatments.

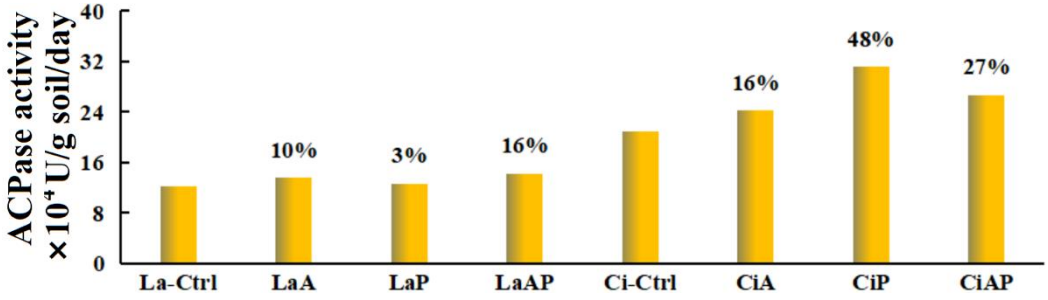


Fig. 4.2.4a ACPase activity of sterilized co-culture soil

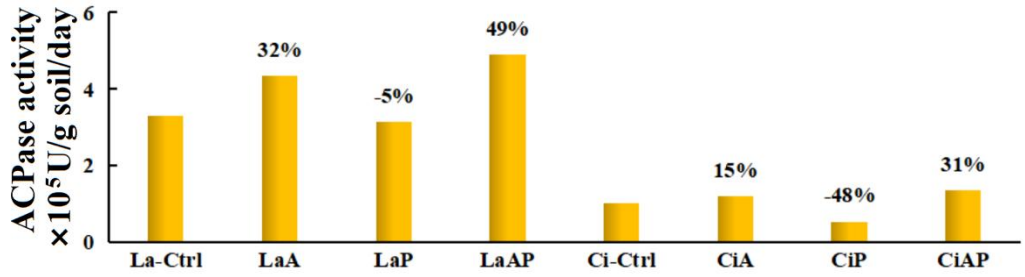


Fig 4.2.4a' ACPase activity of strain A inoculation experimental soil.
The percentages indicated on LaA, LaP, and LaAP represent the percentage of activity change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of activity change relative to Ci-Ctrl in each treatment.

The activity of neutral phosphatase (NPase) detected in the soil in this study was approximately only 1% of the activity of acid phosphatase (ACPase) (Figure 4.2.4b). Similar to ACPase activity, the NPase activities in the La treatments were lower than those in the Ci treatments, but the differences were smaller compared to the differences in ACPase activities between the two soils. Inoculation with the PSB strain A also led to increases in soil NPase activities. Compared to the unplanted soil (Figure 4.2.4b'), the presence of maize seedling roots had little influence on the soil NPase activity in the La treatments. However, it increased NPase activity in the Ci treatments by approximately 30%, which was lower than the increases in ACPase activities (approximately 50%-80%).

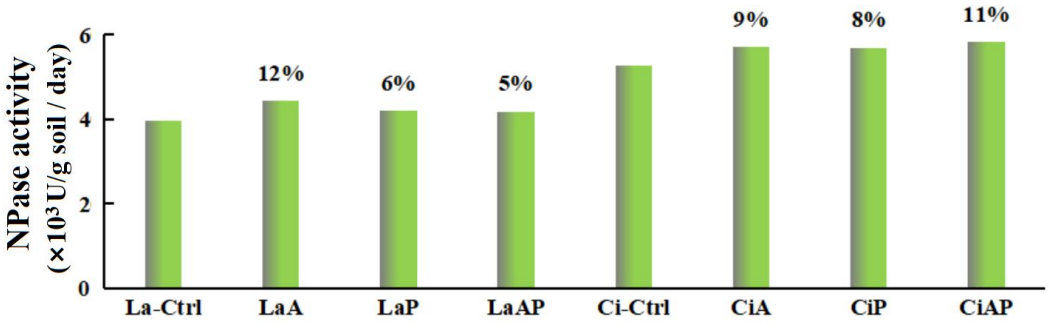


Fig. 4.2.4b NPase activity of sterilized co-culture soil

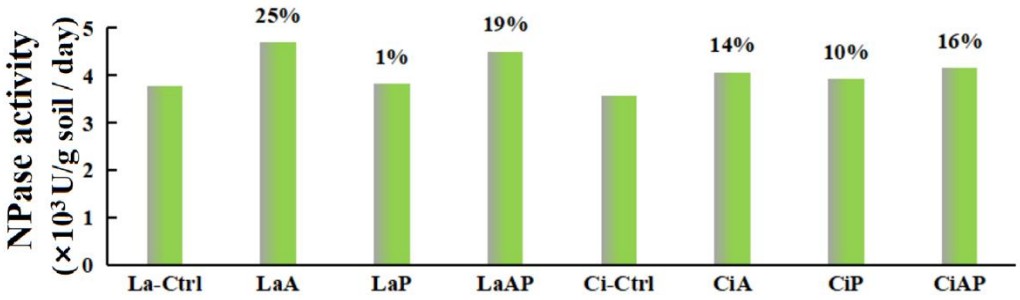


Fig 4.2.4b' NPase activity of strain A inoculation experimental soil.
The percentages indicated on LaA, LaP, and LaAP represent the percentage of activity change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of activity change relative to Ci-Ctrl in each treatment.

Although the activity of alkaline phosphatase (AKPase) in the soil was very low (less than 1% of ACPase), a positive effect of PSB on the AKPase activity in various treatments can still be observed from Figure 4.2.4c: regardless of the addition of TCP, the inoculation of strain A increased the AKPase activity in the corresponding soil treatments. The AKPase activity in the Ci treatments remained higher than that in the La treatments. Compared to the unplanted soil (Figure 4.2.4c'), the presence of maize seedling roots had little influence on the soil AKPase activity in the La treatments, but it led to an increase in AKPase activity in the Ci

treatments by approximately 40-60%.

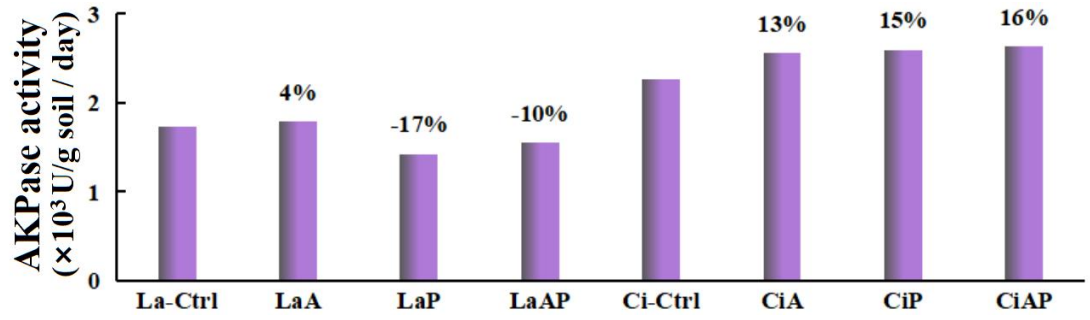


Fig. 4.2.4c AKPase activity of sterilized co-culture soil

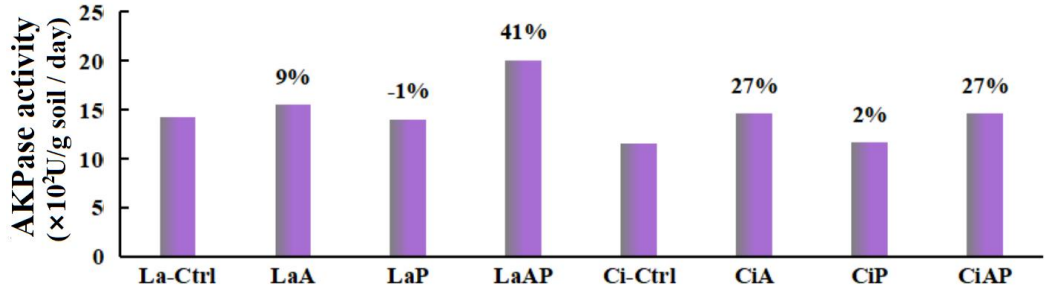


Fig. 4.2.4c' AKPase activity of strain A inoculation experimental soil.
The percentages indicated on LaA, LaP, and LaAP represent the percentage of activity change relative to La-Ctrl in each treatment; The percentages indicated on CiA, CiP, and CiAP represent the percentage of activity change relative to Ci-Ctrl in each treatment.

In summary, in both the La and Ci treatments with maize seedlings, inoculation with PSB strain A still increased the soil phosphatase activity. Unlike in unplanted soil, the extent of increase is influenced not only by soil type and the addition of TCP but also by the presence of plant root systems.

4.2.5 Soils fractions

As Table 4.2.5.1 showed, in sterilized co-culture experiment, La

treated with PSB isolate A (LaA) showed differences in all fractions except $NaHCO_3$-Po relative to the uninoculated control soil (La-Ctrl), while LaAP (relative to LaP) significantly changed all fractions except HCl-P; CiA (relative to Ci-Ctrl) significantly changed all fractions except NaOH-Po, while CiAP (relative to CiP) significantly changed all fractions except NaOH-Po and HCl-P. TCP supply significantly increased all except NaOH-Po fraction in LaP whereas it increased all except NaOH-Pi and NaOH-Po in CiP. The interaction between PSB inoculation and TCP supply was found to be significant for resin P, $NaHCO_3$-Pi, and NaOH-Pi in La, whereas for labile P (resin P, $NaHCO_3$-Pi and $NaHCO_3$-Po) in Ci.

Observing the changes in labile P and M labile P in the soil, as shown in Table 4.2.5.2, it was found that in La, all three treatments (PSB treatment, TCP treatment, and combination treatment) showed an increase in M labile P higher than labile P. However, in Ci, all three treatments showed an increase in labile P higher than M labile P. Comparing with the inoculation experiment of strain A (Table 4.2.5.2'), it can be seen that regardless of whether maize seedlings were planted in the two soils or not, the trend of changes in labile P and M labile P under the three treatments was consistent. The difference is that in La with maize seedlings planted, the soil M labile P content is slightly higher than that in unplanted La, while in Ci with maize seedlings planted, both the soil labile P and M

labile P contents are slightly higher than those in unplanted Ci. Additionally, the presence of roots also slightly increased the increment of labile P and M labile P in each treatment of La and increased the increment of labile P in each treatment of Ci. Therefore, the authors considered that changes in soil P fractionation due to the presence of roots might also have some degree of influence

Table 4.2.5.1 Soil P fractions (mg-P kg^{-1} soil) in sterilized co-cultured La and Ci

Group	Resin P	$NaHCO_3$-Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	HCl-P	Total
La-Ctrl	0.027±0.003a	0.023±0.002a	0.212±0.004a	14.795±0.265a	9.431±0.230a	0.065±0.006a	585±16a
LaA	0.061±0.003b	0.068±0.004b	0.226±0.004a	18.004±0.231b	10.991±0.203b	0.007±0.002b	584±10a
LaP	0.564±0.006c	0.361±0.006c	0.256±0.006b	40.991±0.341c	10.113±0.234a	436±3c	2113±9b
LaAP	1.174±0.015d	0.649±0.021d	0.292±0.011c	46.998±0.243d	12.103±0.305c	427±2c	2112±12b
PSB	***	***	***	***	***	*	-
TCP	***	***	***	***	***	***	***
TCP * PSB	***	***	-	***	-	*	-
Ci-Ctrl	1.86±0.02a	3.82±0.08a	1.72±0.05a	2.05±0.01a	4.36±0.02a	306±4a	684±14a
CiA	2.47±0.03b	5.11±0.03b	2.19±0.05b	2.39±0.01b	4.39±0.02ab	291±2b	683±14a
CiP	3.89±0.09c	6.09±0.03c	2.10±0.05b	2.06±0.04a	4.30±0.09ab	1704±8c	2170±12b
CiAP	4.20±0.07d	6.92±0.08d	3.32±0.05c	2.40±0.02b	4.49±0.04b	1701±9c	2168±11b
PSB	***	***	***	***	*	-	-
TCP	***	***	***	-	-	***	***
TCP * PSB	*	***	***	-	-	-	-

Values represent the mean of three replicates ± SE (standard errors).

Significant differences in a column under each group are indicated by different letters (P≤0.05). Significant was analyzed with One-way ANOVA (Games-Howell).

La-Ctrl, Ci-Ctrl control; *LaA, CiA* PSB treatment； *LaP, CiP* TCP treatment； *LaAP, CiAP* Combination treatment；

PSB/TCP/PSB*TCP Two-way Anova for the factors PSB inoculation/TCP supply/ the interaction of PSB inoculation ×TCP supply.

***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$, −: $p \geqq 0.05$.

Table 4.2.5.2 Changes of labile P, moderately labile P and HCl-P in sterilized co-culture experiment (mg-P kg^{-1} soil)

group	labile P	Increase	moderately labile P	Increase
La-Ctrl	0.26	relative to La-Ctrl	24.23	relative to La-Ctrl
LaA	0.36	0.10	29.00	4.77
LaP	1.18	0.92	51.10	26.87
LaAP	2.11	1.85	59.10	34.87
Ci-Ctrl	7.40	relative to Ci-Ctrl	6.41	relative to Ci-Ctrl
CiA	9.77	2.37	6.78	0.37
CiP	12.08	4.68	6.36	-0.05
CiAP	14.44	7.04	6.88	0.47

La-Ctrl, Ci-Ctrl control; *LaA, CiA* PSB treatment；*LaP, CiP* TCP treatment；*LaAP, CiAP* combination treatment；

Table 4.2.5.2’ Changes of labile P, moderately labile P and HCl-P in inoculation experiment (mg-P kg^{-1} soil)

group	labile P	Increase	moderately labile P	Increase
La-Ctrl	0.27	relative to La-Ctrl	23.71	relative to La-Ctrl
LaA	0.31	0.05	25.39	1.69
LaP	0.94	0.67	43.43	19.72
LaAP	1.94	1.68	53.78	30.07
Ci-Ctrl	6.84	relative to Ci-Ctrl	5.13	relative to Ci-Ctrl
CiA	8.98	2.14	5.80	0.66
CiP	8.58	1.74	5.14	0.00
CiAP	11.70	4.86	6.14	1.01

La-Ctrl, Ci-Ctrl control; *LaA, CiA* PSB treatment；*LaP, CiP* TCP treatment；*LaAP, CiAP* combination treatment；

4.3 Discussion

4.3.1 PSB inoculation affect maize seedling P accumulation

Based on the Pearson correlation analysis investigating the relationship between maize seedling P accumulation and PSB inoculation, incorporating the measured soil physicochemical properties mentioned above, the following conclusions can be drawn. In Ci condition,

Table 4.3.1 Pearson correlation between PSB inoculation, maize P accumulation and soil indicators

	P accum.	labile P	M labile P	pH	Oxalic	ACPase	P accum.	labile P	M labile P	pH	Oxalic	ACPase
			in La (La-Ctrl & PSB treat.)						in Ci (Ci-Ctrl & PSB treat.)			
PSB	-	.857*	.878*	-	-	-	.842*	.866*	.947**	-	-	.958**
P accum.		-	-	-.919**	-	-		.956**	-	-	-	-
labile P			.859*	-	-	-			-	-	-	.812*
M labile P				-.818*	-	-				-	-	.924**
pH					-	-					-	-
Oxalic						-						-
			in La with TCP (TCP treat. & PSB treat.)						in Ci with TCP (TCP treat. & PSB treat.)			
PSB	-	.935**	.942**	-	-	.828*	-	-	-	-	-	-.996**
P accum.		-	-	-	-.939**	-		-	.984**	-	-	-
labile P			.938**	-	-	-			-	-	-	-
M labile P				-	-	-				.828*	-	-
pH					-	-					-	-
Oxalic						-.866*						-

**: $p < 0.01$; *: $p < 0.05$; -: $p \geq 0.05$

-*: Significant negative correlation; *: Significant positive correlation

maize seedling P accumulation is positively correlated with PSB inoculation. In the La condition, regardless of the addition of TCP, no direct relationship between PSB inoculation and maize seedling P accumulation was found. However, observations revealed that in the La condition, maize seedling P accumulation is negatively correlated with soil pH, soil pH is negatively correlated with soil M labile P, and soil M labile P is positively correlated with PSB inoculation. These relationships can be expressed using the following proportional relationships (Note: "$\propto$" indicates a positive correlation, not proportionality):

$$\text{P accum.} \propto -\text{pH} \propto -\text{M labile P} \propto \text{PSB}$$

In the La with TCP, maize seedling P accumulation is negatively correlated with soil oxalate content, soil oxalate content is negatively correlated with soil acid phosphatase activity, and soil acid phosphatase activity is positively correlated with PSB inoculation. These relationships can be expressed using the following proportional relationships:

$$\text{P accum.} \propto -\text{Oxalic} \propto -\text{ACPase} \propto \text{PSB}$$

Therefore, although maize seedling P accumulation is not directly related to PSB inoculation in the La condition, an indirect positive correlation can still be established between the two through soil physicochemical properties.

Additionally, in the Ci condition with TCP, no direct or indirect relationship was found between maize seedling P accumulation and PSB

inoculation. From Table 4.2.1, it can be seen that there is no significant difference in maize seedling P accumulation between PSB inoculation alone and combined inoculation. However, TCP supply has a significant impact on maize phosphorus accumulation. Therefore, in the combined treatment, it is difficult to determine whether it is the PSB inoculation or TCP supply that is responsible for the effect.

Considering the release of soil P by PSB inoculation, it can be observed that PSB inoculation positively affects soil labile P content in the La, La with TCP, and Ci conditions, effectively facilitating the release of soil P.

4.3.2 Exploration of Factors Influencing Maize Seedling P Accumulation

In order to investigate the impact of PSB inoculation on maize seedling P accumulation in the sterilized co-culture experiment, the author employed multiple linear regression analysis to analyze the relevant variables.

To establish a multiple linear regression model with [maize seedling P accumulation] as the dependent variable and [soil type], [TCP supply], and [PSB inoculation] as independent variables, we can set the continuous numerical variable of [maize seedling P accumulation] as the dependent variable and the categorical variables of [soil type], [TCP supply], and [PSB inoculation] as independent variables. We can assign

La = 0 and Ci = 1 for [soil type], no supply = 0 and supply = 1 for [TCP supply], and no inoculation = 0 and inoculation = 1 for [PSB inoculation].

Table 4.3.2a Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Durbin-Watson
1	.940[a]	.884	.867	.08433045	2.128

a. Predictors: (Constant), TCP supply, PSB inoculation, Soil type

Dependent Variable: maize seedling P accumulation

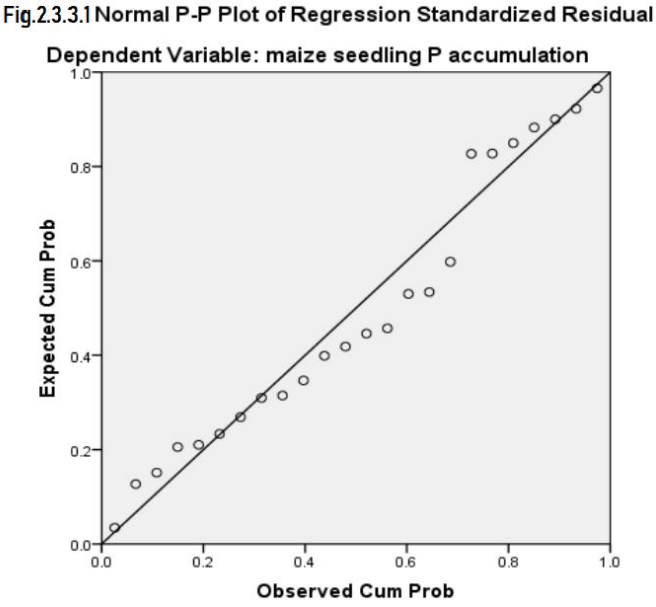


Fig.4.3.2 Normal P-P plot of regression standardized residual dependent variable: maize seedling P accumulation

Table 4.3.2b Coefficients

		Unstandardized Coefficients		Standardized Coefficients		Collinearity Statistics	
Model		B	Std. Error	Beta	*p*	Tolerance	VIF
1	(Constant)	.247	.034		.000		
	Soil type	.390	.034	.862	.000	1.000	1.000
	PSB inoculation	.128	.034	.282	.001	1.000	1.000
	TCP supply	.113	.034	.250	.004	1.000	1.000

Dependent Variable: maize seedling P accumulation

Based on the findings from Table 4.3.2a, the R Square statistic value of 0.884 indicates that the three independent variables in the present study, namely [soil type], [PSB inoculation], and [TCP supply], can explain approximately 88.4% of the variation in the dependent variable, maize seedling P accumulation. Additionally, as observed in Fig. 4.3.2, the regression standardized residuals exhibit a reasonably normal distribution. Moreover, referring to the results in Table 4.3.2b, the collinearity statistics indicate a lack of multicollinearity among the independent variables, as evidenced by the VIF values of 1. This implies that the examined variables do not substantially influence each other. Further analysis from Table 4.3.2b reveals that all three independent variables exhibit significance levels (p) below 0.05, indicating their statistically significant effects on the dependent variable. Therefore, the current model exhibits a good fit.

According to the regression results, the Unstandardized Coefficients (B values) for [soil type], [PSB inoculation], and [TCP supply] are estimated to be 0.39, 0.128, and 0.113, respectively. These values suggest that, compared to planting in La, cultivating maize in Ci soil type leads to an increase of 0.39 mg-P shoot^{-1} in P accumulation. Similarly, PSB inoculation enhances maize seedling P accumulation by 0.128 mg-P shoot^{-1} compared to non-inoculation, while TCP supply contributes to a rise of 0.113 mg-P shoot^{-1} compared to no supply. Based on these

regression findings, the following prediction equation can be derived:

1) Maize seedling P accumulation

$$= 0.247 + (0.39 \times \text{soil type}) + (0.128 \times \text{PSB inoculation}) + (0.113 \times \text{TCP supply})$$

Using the prediction equation, the maize seedling P accumulation in the co-cultivation experiment was as follows:

Table 4.3.3a Predicted maize seedling P accumulation (mg-P shoot^{-1}) based on the regression equation

	Control	PSB treatment	TCP treatment	Combination treatment
La	0.247	0.375	0.360	0.488
Ci	0.637	0.765	0.750	0.878

Table 4.3.3b Actual measured maize seedling P accumulation (mg-P shoot^{-1})

	Control	PSB treatment	TCP treatment	Combination treatment
La	0.268	0.350	0.365	0.485
Ci	0.580	0.824	0.780	0.845

As observed, the predicted values closely approximate the actual measured values, indicating that the equation provides a good fit.

Based on the co-culture experiments conducted separately with La and Ci soil types, an analysis of the factors influencing maize seedling P accumulation was performed. Keeping [maize seedling P accumulation] as the dependent variable, the independent variable [soil type] was removed, while the independent variables [PSB inoculation] and [TCP supply] were retained. Regression analysis was conducted. Model

diagnostics revealed the absence of multicollinearity among the independent variables, and the regression standardized residuals exhibited a roughly normal distribution. Based on the model, the prediction equation is written as follows:

2) Maize seedling P accumulation in La

$= 0.258 + (0.101 \times \text{PSB inoculation}) + (0.116 \times \text{TCP supply})$ $R^2=0.601$

3) Maize seedling P accumulation in Ci

$= 0.625 + (0.154 \times \text{PSB inoculation}) + (0.110 \times \text{TCP supply})$ $R^2=0.531$

From the three regression results, when conducting a regression analysis of factors influencing maize seedling P accumulation using the co-cultivation results of two soil types, all three independent variables have a significant impact on maize seedling P accumulation. Among them, [soil type] has the greatest influence, followed by [PSB inoculation], and [TCP supply] has the smallest influence. When conducting a regression analysis using the co-cultivation results of La, it was found that both [PSB inoculation] and [TCP supply] have a significant impact on maize seedling P accumulation, with the impact of [TCP supply] slightly higher than that of [PSB inoculation]. When conducting a regression analysis using the co-cultivation results of Ci, it was found that both [PSB inoculation] and [TCP supply] also have a significant impact on maize seedling P accumulation, with the impact of [PSB inoculation] slightly higher than that of [TCP supply]. In conclusion, the magnitude of PSB's

impact on maize seedling P accumulation varies depending on the soil environment and different treatments, but the impact of PSB is positive and certain.

Chapter V

PSB affect maize seedling P accumulation in natural soil (non-sterilized co-culture experiment)

In this chapter, a non-sterilized co-culture experiment was conducted to investigate the effect of PSB on the rhizosphere bacterial community, using fresh La as the cultivation substrate. Non-fresh soil (Shanxi Cinnamon soils) was not utilized in this step due to its incomplete microbial structure. Maize seedlings were transplanted on the first day after sprouting, and the soil was collected and potted on the same day.

For soil DNA extraction, rhizosphere soil samples were collected on the day of collection. Leveraging long-read sequencing technologies, these samples were subjected to single-molecule real-time (SMRT) sequencing on PacBio platforms, which generated circular consensus sequences (CCS). The CCS reads were then filtered, clustered, and de-noised to generate full-length amplicon tags for species annotation and abundance analysis. Further analyses included alpha diversity, beta diversity, differential analysis between groups, correlation analysis, function prediction, and more.

5.1 Materials and methods

5.1.1 Materials and treatments

Strains A, B, F, G, and H were utilized for non-sterilized co-culture. However, during the culture process, groups B and H experienced non-experimental fatalities. Consequently, the results only reflect the outcomes of groups A, F, G in the PSB treatment, control, and the P treatments.

The PSB suspension was prepared as described in section 2.1.3 (Shake flask culture).

The inoculation experiments consisted of two treatments (P treatment and PSB treatment) and one control (Table 5.1.1). All treatments and the control employed the same soil, supplemented with TCP.

For the PSB treatment, 25 mL of PSB suspension was inoculated. For the P treatment and control, 25 mL of P-free NBRIP solution was used. Subsequently, 125 mL of sterilized distilled water was added to each container to maintain moisture. In addition, 5 mL of P-free Hoagland nutrient solution was added to both the control and PSB treatment, while a complete nutrient solution was provided to the P treatment every other day to support plant growth. The treatments and control were cultured at 28°C for 30 days.

Table 5.1.1 Treatments and conditions of the experiment

Treatments	Soils and P supplements	Inoculation	Moisture support	Hoagland
Control	La 500g + 5g TCP	25 mL P free NBRIP	125 mL sterile distilled water	P free
P treatment	La 500g + 5g TCP	25 mL P free NBRIP	125 mL sterile distilled water	Complete nutrition
PSB treatment	La 500g + 5g TCP	25mL PSB suspensions	125 mL sterile distilled water	P free

Add 5 mL Hoagland nutrient solution was added to support plant growth every other day.

Add sterile distill water by weight.

28℃, 30 days.

The scheme of soil preparation was shown in Fig.5.1.1.1. The soil samples were collected from an outdoor field and subsequently cleared of stones and other non-soil materials to the greatest extent possible. The soil was then passed through a 2 mm sieve. To ensure uniformity, the soil was evenly spread on sterile plastic cloth. The soil samples were divided into 21 large grids, with each large grid further divided into 21 small grids. One small grid soil sample was taken from each large grid and combined to create a composite sample. In total, 21 composite soil samples were obtained.

For each composite sample, 500 g of soil was weighed and mixed with 5 g of TCP. The mixture was thoroughly blended and transferred to individual pots. All equipment used in the process was disinfected with 75% alcohol to prevent the introduction of non-soil bacteria.

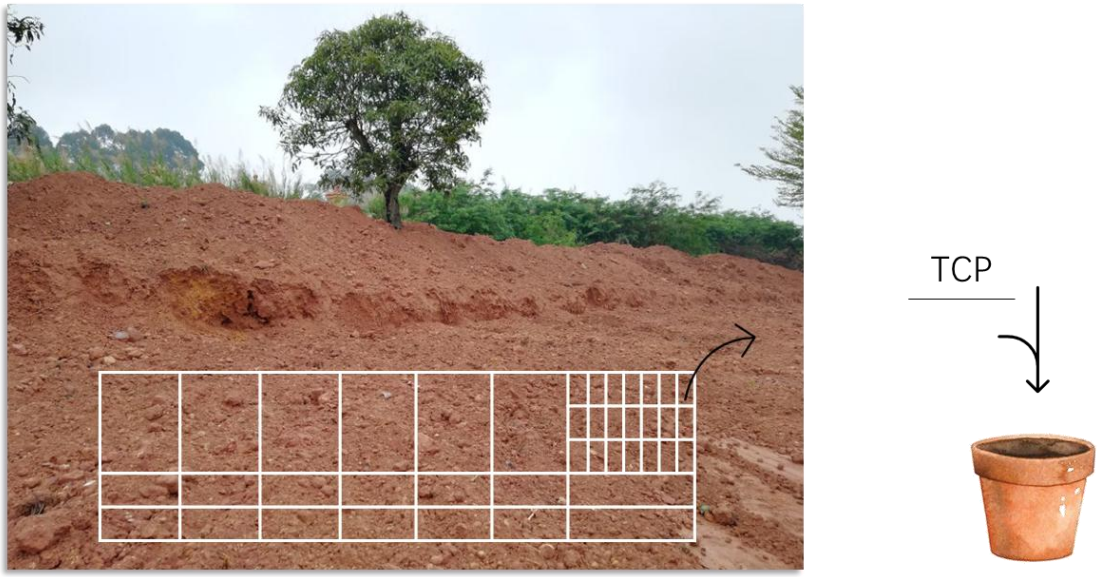


Fig.5.1.1.1 Scheme of soil preparation in this experiment

Flow of the cultivation experiment was shown in Fig.5.1.1.2. The seed sterilization, germination, transplant, and culture followed the procedure outlined in section 4.1.2. After one month, both the plants and the rhizosphere soil were sampled.

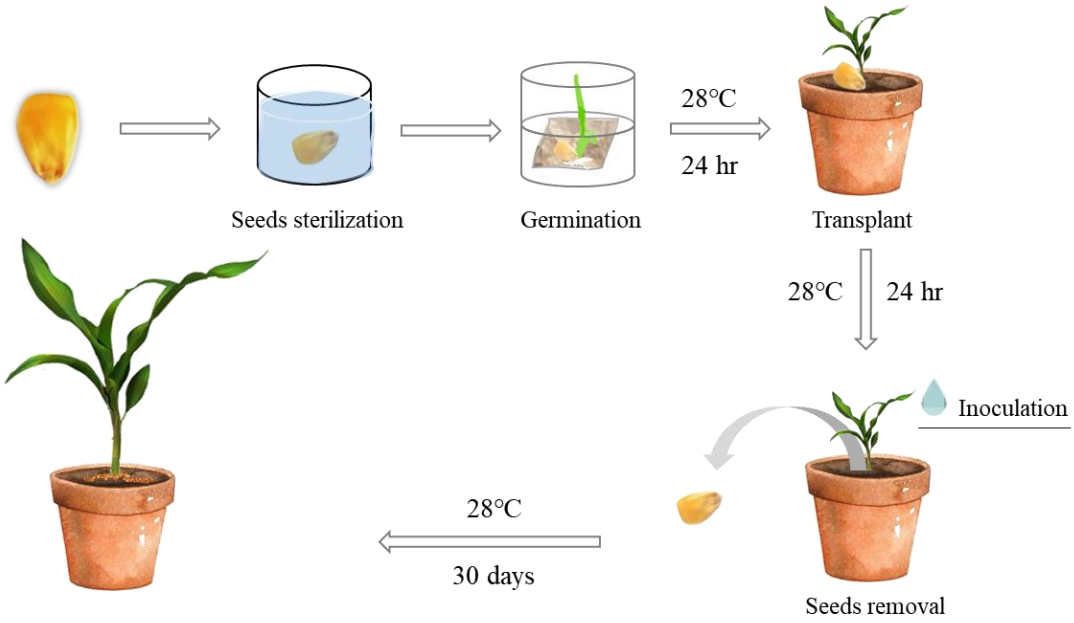


Fig.5.1.1.2. Flow of cultivation experiment

The aboveground portion of the plants was harvested and weighed, and various indicators such as height and leaf width were measured. Following the measurements, the plants were dried to a constant weight in a constant temperature oven set at 70 ℃. Subsequently, the dried plants were pulverized for the measurement of total P.

To collect rhizosphere soil, carefully extract the maize roots from the cultivation pot, gently shaking them to remove bulk soil. Place a sterile bag over the maize roots after removing the bulk soil, shake the roots, causing the soil tightly attached to the roots to fall into the sterile bag, thereby collecting rhizosphere soil (Fig.5.1.1.3).

Fig. 5.1.1.3 Collection of the rhizosphere soil in a bag

The collected rhizosphere soil was immediately weighed and used for soil DNA extraction. One gram of soil was air-dried to measure soil phosphatase activity, while the remaining soil samples were lyophilized to measure organic acid content.

5.1.2 Soil pH, organic acid and phosphatase measuring

Soil pH was measured by pH meter, the content of organic acid in soil is detected by HPLC, and the enzyme activity is measured by kit, which is the same as 3.1.3-3.1.5.

5.1.3 Seedling P content measuring

The content of total P in plant shoot was determined by H_2SO_4 decomposition method as same as 4.1.3.

5.1.4 Soil DNA extraction

TGuide S96 Magnetic Soil DNA Kit (Tianggen Biotech) was used to extract soil DNA.

5.1.5 Amplicon sequencing

Soil DNA was sequenced by Biomarker Technologies Co., Ltd., Beijing, China.

5.1.6 Bioinformatic Analysis

Bioinformatic Analysis was conducted through BMK Cloud (Biomarker Technologies).

5.2 Results

5.2.1 Soil physicochemical indicators

Regarding pH, none of the treatments significantly altered soil pH or, in other words, the changes were almost negligible (Table 5.2.1). In Chapter 4, the combination treatment of LaAP and the TCP treatment of LaP did not result in any significant changes in soil pH. The results were consistent between the two.

In terms of soil phosphatase activity, the PSB treatment slightly increased the activity, while the soluble P treatment reduced the activity

(Table 5.2.1). Similar trends were observed for neutral phosphatase.

The soil oxalic acid content showed a significant decrease in the PSB treatment, especially in groups A and F (Table 5.2.1). The soluble P supply only caused a minor decrease. A similar pattern was observed for tartaric acid, while malic acid showed a significant decrease in group A and P treatment but an increase in groups F and G. Tartaric acid was hardly detected in both Chapters 3 and 4, although it was present at considerable levels in the experimental soil. This could be due to the decomposition of tartaric acid after autoclaving of the soil.

Overall, in terms of physicochemical indicators, PSB inoculation had a significant impact on the content of organic acids in the soil, particularly oxalic acid content, and also had some influence on phosphatase activity.

Table 5.2.2 Soil physicochemical indicators

Treatment and Group	pH	ACPase	NPase	AKPase	Oxalic	Tartaric	Malic
		$\times 10^6$ U/g soil	——$\times 10^3$ U/g soil——		——————mg/kg soil——————		
Control	7.20	1.19	4.79	2.72	794.80	84.70	33.40
P	7.20	1.01	3.31	2.34	623.30	79.30	16.20
A	7.23	1.52	5.74	2.81	297.90	19.80	18.90
F	7.20	1.38	5.22	2.62	291.80	38.10	42.60
G	7.19	1.66	6.31	3.09	521.20	47.00	54.50

5.2.2 Maize seedling physiological indicators

From the physiological indicators of maize, P treatment increased the indicators significantly while PSB treatment did not (Table 5.2.2).

Table 5.2.2 Physiological indicators of maize seedlings

Treatment	Height cm	Leaf width cm	Fresh weight g	Dry weight g	Shoot P content mg-P/g DW	Shoot P accumulation mg
Control	62.67	2.93	18.64	1.88	4.96±0.05	9.34±0.78
A	66.00	3.43	23.3	2.28	5.2±0.07	11.63±0.80
F	65.00	3.37	22.71	2.17	4.92±0.18	10.54±0.72
G	62.33	3.6	22.24	2.16	5.22±0.15	11.08±0.54
P	89.00	4.33	40.01	3.74	7.37±0.20*	28.71±0.47*

Fig.5.2.2 Appearance of maize plants at harvest

It can be observed that inoculating PSB in a 7-day sterilized co-culture of 2.3 significantly increased P accumulation in maize seedlings. However, in non-sterilized co-culture, although the growth of

maize seedlings with PSB inoculation appeared better than that of the control group, the difference was not statistically significant (Fig.5.2.2). This can be attributed to two factors: Firstly, the duration of the culture in the two experiments differed. Secondly, PSB colonization and survival were influenced by other soil microorganisms in the non-sterilized co-culture. Hence, there are likely variations in the colonization or survival effects of PSB and the efficiency of P release into the soil between the two co-culture experiments.

5.2.3 Rhizosphere bacteria species annotation and taxonomic

The following number of species were annotated in this sequencing：

Table 5.2.3 Statistics of species annotation

Sample	Phylum	Class	Order	Family	Genus	Species
control	22	42	89	143	220	321
A	21	40	85	126	199	281
F	21	43	95	152	252	375
G	23	45	99	153	247	376
P	23	45	96	151	245	369

It can be seen from Table 5.2.3 that the species annotated in the treatment inoculated with A were less than other treatments, and other inoculation and soluble P supply slightly increased the species in the soil.

Mapping comparison of the top 30 genera among groups shows that A has relatively high abundance on *Lactiplanatibaillus* and *Staphylococcus*, and the highest relative abundance of control is

Candidatus Udaeobacter and *Paenibacillus*, the relative abundance of G on *Flavoisolibacter* is higher than that of other treatments. In the P treatment with sufficient P supply, the relative abundance of *Haliscomenobacter* is higher (Fig. 5.2.3.1).

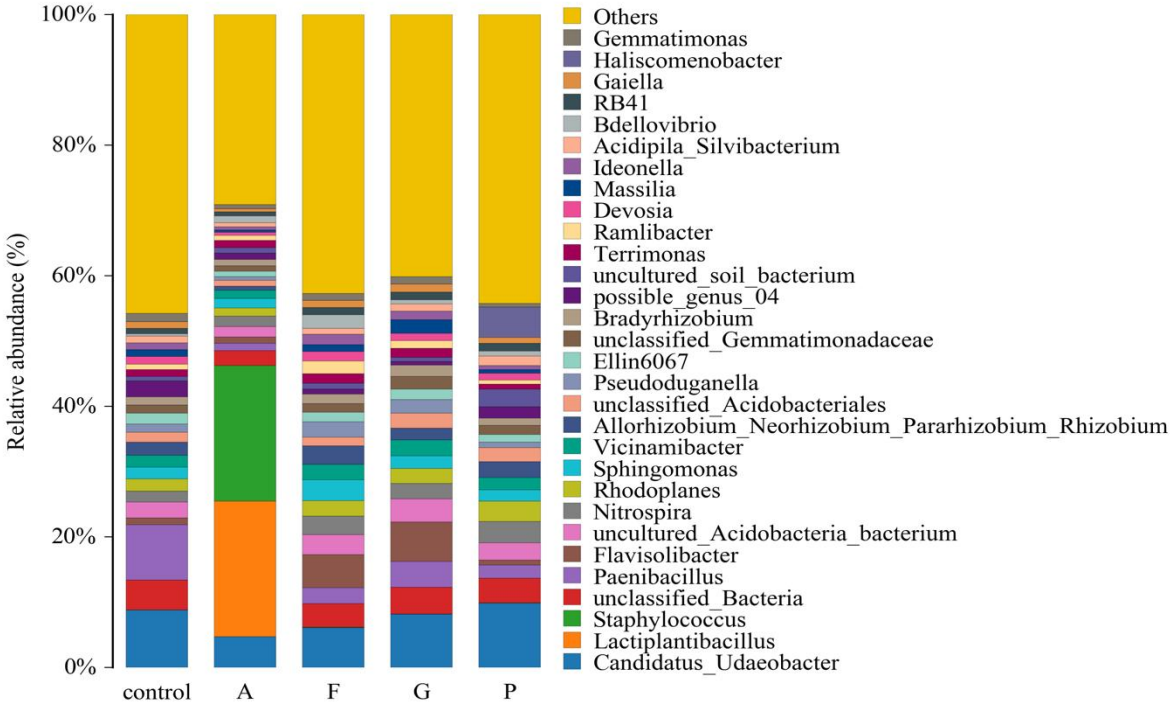


Fig. 5.2.3.1 Histogram of top 30 species distribution at genus level

It can be observed that in the P treatment and PSB treatment, the three replicates of groups F and G exhibit consistent high-abundance species, and the differences among the treatments or groups are also evident. The three replicates of the Control treatment also share some similar high-abundance species composition, but control2 shows significantly higher abundance in these species compared to the other two

replicates. In group A, apart from the three high-abundance genera, there are hardly any other genera present in A2. The distribution of high-abundance genera in A1 and A2 also differs significantly, indicating poor reproducibility (Fig. 5.2.3.2).

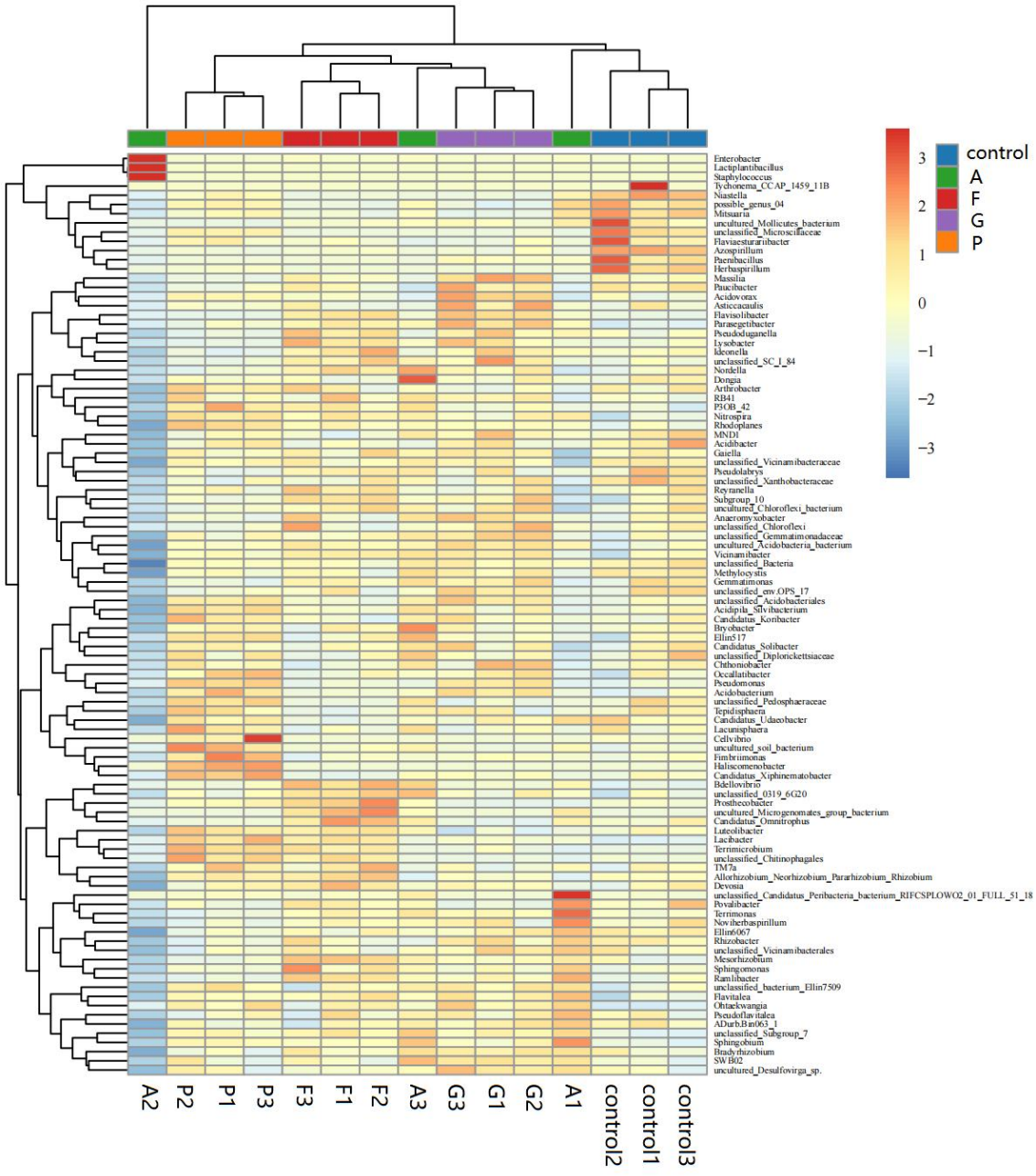


Fig. 5.2.3.2 Heat map of species richness clustering

5.2.4 Rhizosphere bacterial diversity

Simpson index shows that PSB and the supplied soluble P did not significantly alter species diversity; the Chao1 index shows that PSB and the supplied soluble P did not significantly alter species richness (Fig.5.2.4.1).

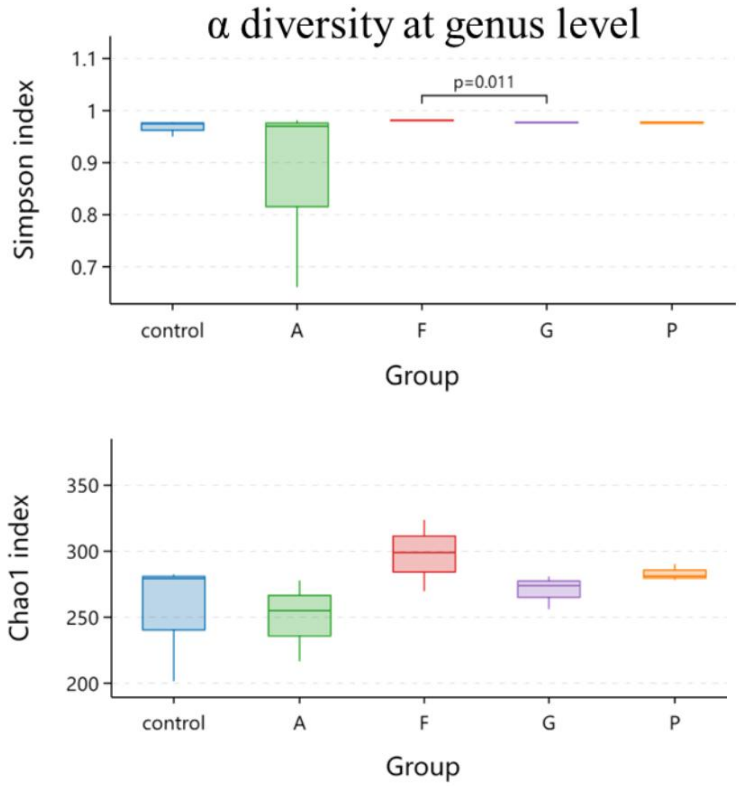


Fig. 5.2.4.1 α diversity at genus level

β diversity, as assessed through Principal Component Analysis (PCoA), revealed significant differences among the treatments in the PC2 direction (Fig.5.2.4.2). This indicates that the treatment had an impact on the composition of rhizobacterial communities.

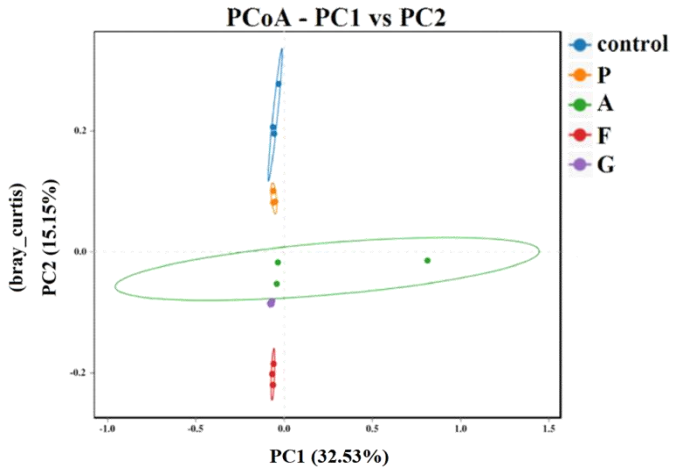


Fig. 5.2.4.2 Analysis of similarities (Anosim) at genus level

Furthermore, Anosim was performed to assess inter-group and intra-group differences among the samples (Fig.5.2.4.3). The calculated R value was 0.768, indicating that the inter-group difference in this sample grouping was greater than the intra-group difference (p = 0.001). This suggests high test reliability. The figure depicts β distances greater than 0.5, while intra-group β distances are less than 0.4.

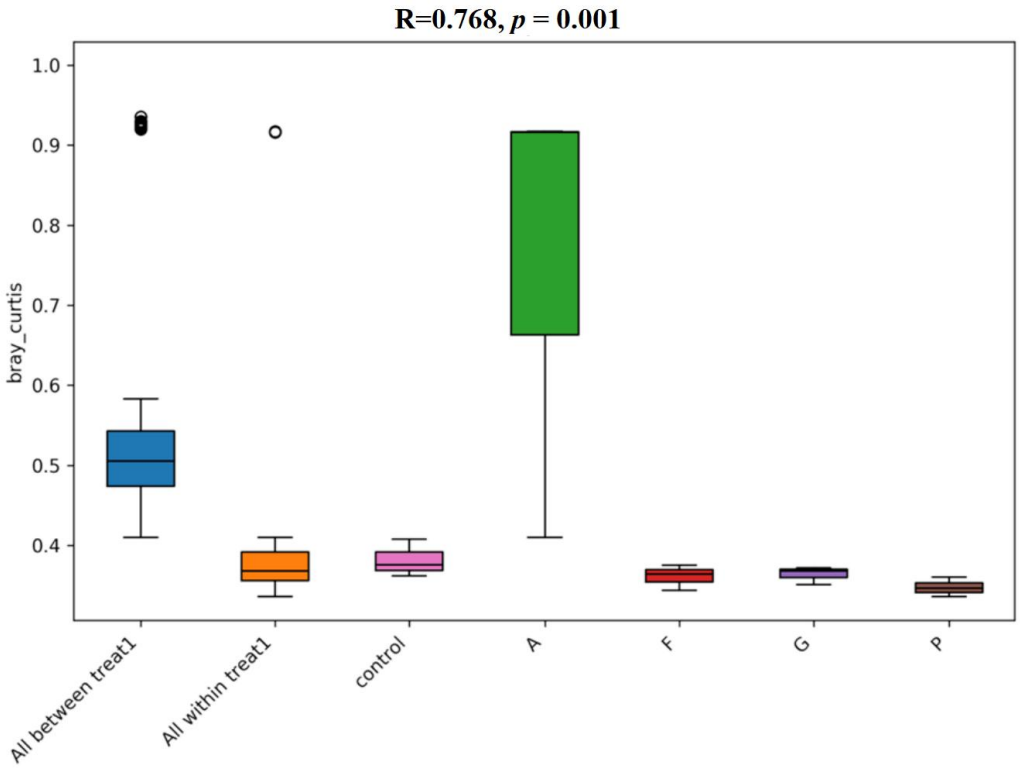


Fig. 5.2.4.3 Analysis of similarities (Anosim) at genus level

5.2.5 Difference of rhizosphere bacteria between groups

Two pictures were selected from the Marker intergroup ambiguity histogram, one of which showed that the marker species in treatment A, *Enterobacter*, had a high abundance in only one sample (Fig.5.2.5a). Strain A happened to belong to *Enterobacter*. The results of this investigation may be due to sampling errors, or it may be due to the high degree of colonization of A in this sample.

Fig. 5.2.5a Marker intergroup abundance histogram

In addition, it can be seen from the following figure that inoculation of PSB increases the abundance of uncultured bacteria (except A2) (Fig.5.2.5b).

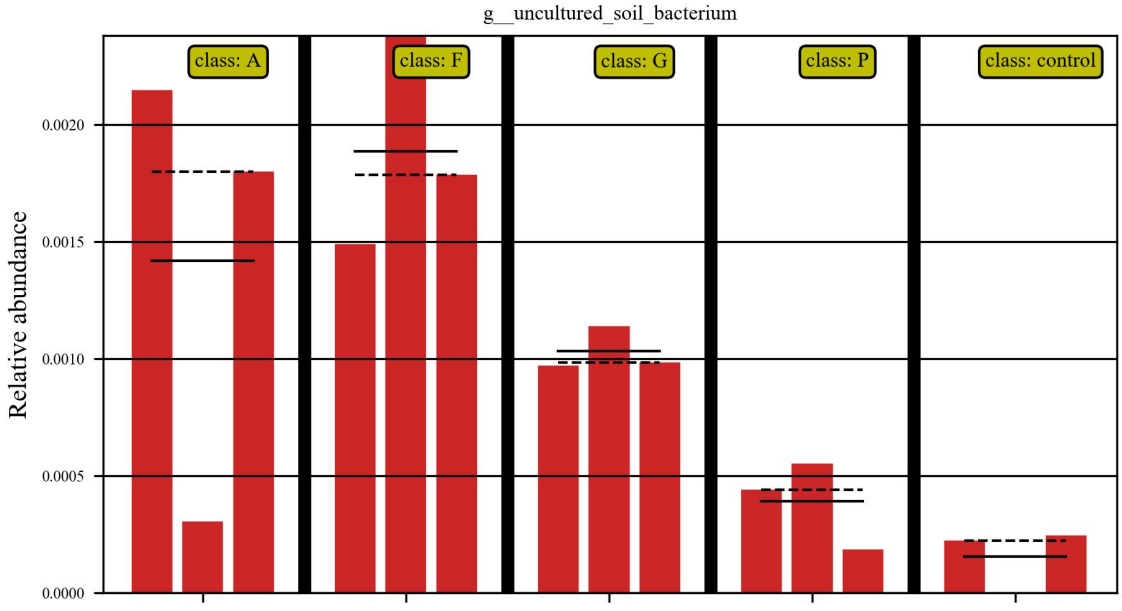


Fig. 5.2.5b Marker intergroup abundance histogram

5.2.6 Correlation network of rhizosphere bacteria

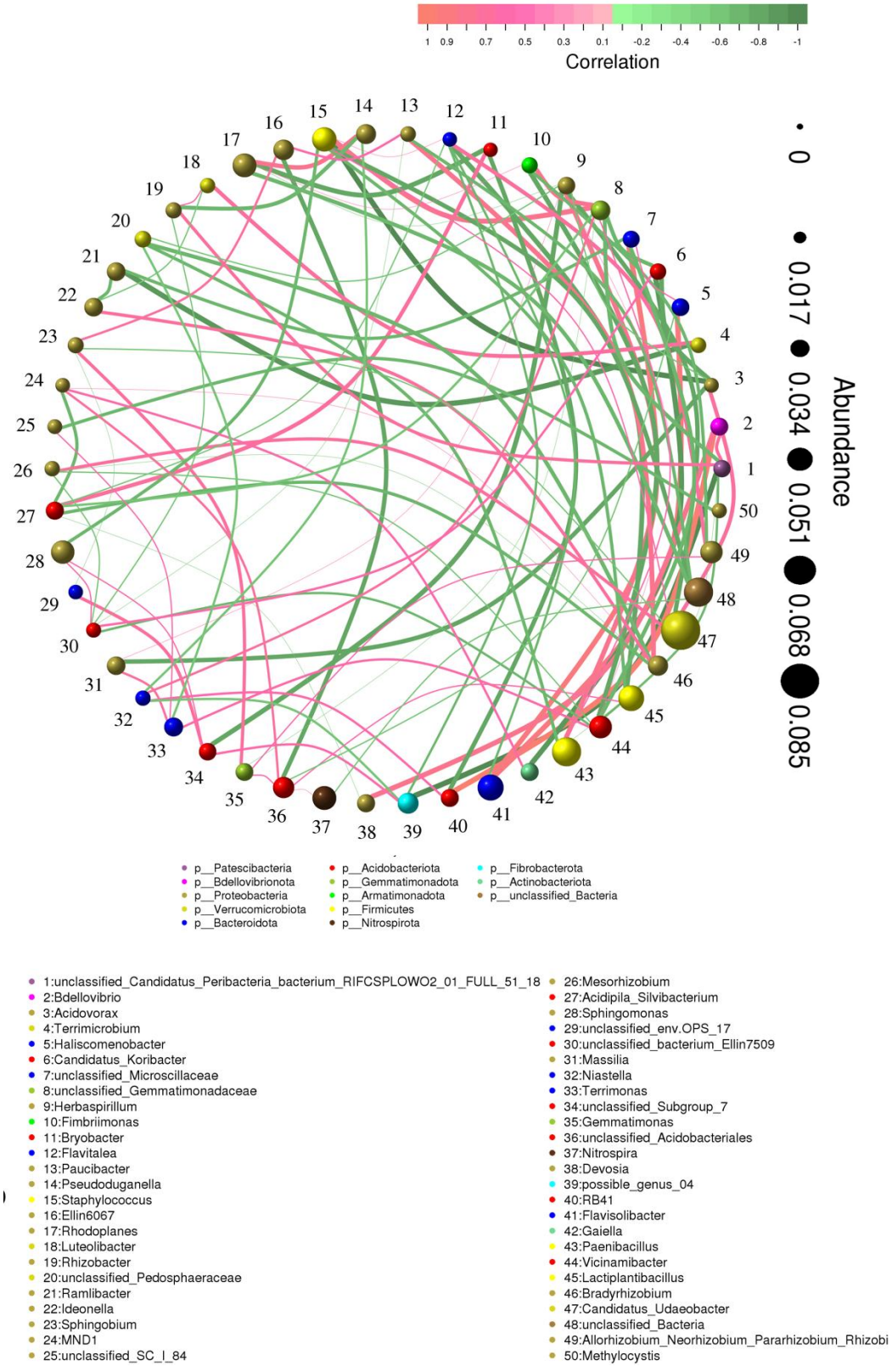


Fig. 5.2.6 Correlation network of top 50 correlated genus

Spearman rank correlation analysis was used to calculate the significant correlation among the top 50 genera with the highest abundance in each sample (Fig.5.2.6, Table 5.2.6). The results showed that *Lactiplanatibaillus* and Staphylococcus, which had the highest abundance in the sample of treatment A, showed negative correlation with other genera with significant correlation. These two genera have the highest abundance in treatment A, which is likely to result in that the annotation species found in treatment A are less than those in other treatments.

Table 5.2.6 Edge properties

Source	Target	weight
Lactiplantibacillus	*Vicinamibacter*	-0.6068
	unclassified_Acidobacteriales	-0.5779
	Acidipila_Silvibacterium	-0.5784
	RB41negative	-0.5644
	Gemmatimonas	-0.5779
	Pseudomonas	-0.6536
	SWB02	-0.5317
	Nordella	-0.7246
Staphylococcus	unclassified_*Acidobacteriales*	-0.6036
	unclassified_Gemmatimonadaceae	-0.5754
	MND1	-0.5149
	unclassified_env.OPS_17	-0.6052
	SWB02	-0.5564
	Pseudolabrys	-0.5260

5.2.7 Functional genes prediction

It can be observed that Group F shows a significant difference

compared to the control in terms of predatory exparasitic, with the mean

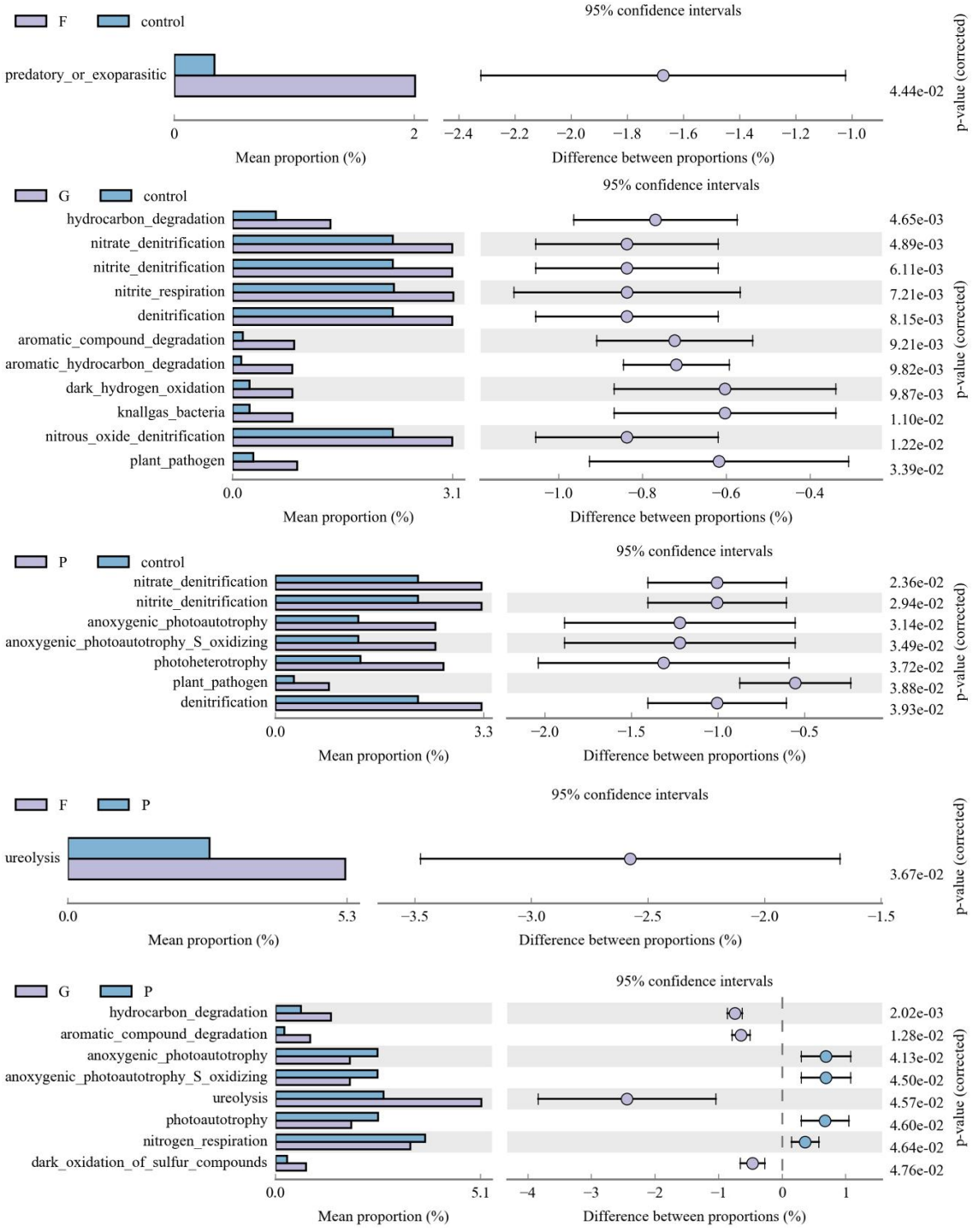


Fig.5.2.7 Functional genes prediction of the rhizosphere bacterial communities

proportion of Group F being higher than that of the control. Group G is

predicted to have significant differences compared to the control in multiple functional aspects of nitrogen cycling, particularly excelling in hydrocarbon degradation compared to both the control and P treatment. P treatment, compared to the control, exhibits advantages in certain genes associated with carbon and nitrogen cycling. Additionally, Group F and Group G are predicted to have higher proportions in ureolysis functionality compared to P treatment. Moreover, the mean proportion of Group G in anoxygenic_photoautotrophy is lower than that of P treatment. No significant differences in functional gene prediction were found between Group A and the control, as well as between P treatment and the control.

5.2.8 Canonical Correspondence Analysis of Environmental Factors and Maize Seedling P accumulation

The Canonical Correspondence Analysis (CCA) was conducted to assess the relationship between environmental factors, including PSB inoculation, soluble P supply, and maize seedling P accumulation, with the rhizobacterial community (Fig.5.2.8). As it revealed, *Pseudomonas*, which genus strain G belong to, was showed a positive correlation with maize seedling P accumulation. The genus *Flavisolibacter*, *Lactiplantibacillus* and *Staphylococcus* which exhibited the highest relative abundance in the groups F, G and A, were positively correlated

with PSB inoculation. However, none of these bacterial genera, including *Paenibacillus* (the dominant genus in the control), exhibited a significant positive correlation with maize seedling P accumulation.

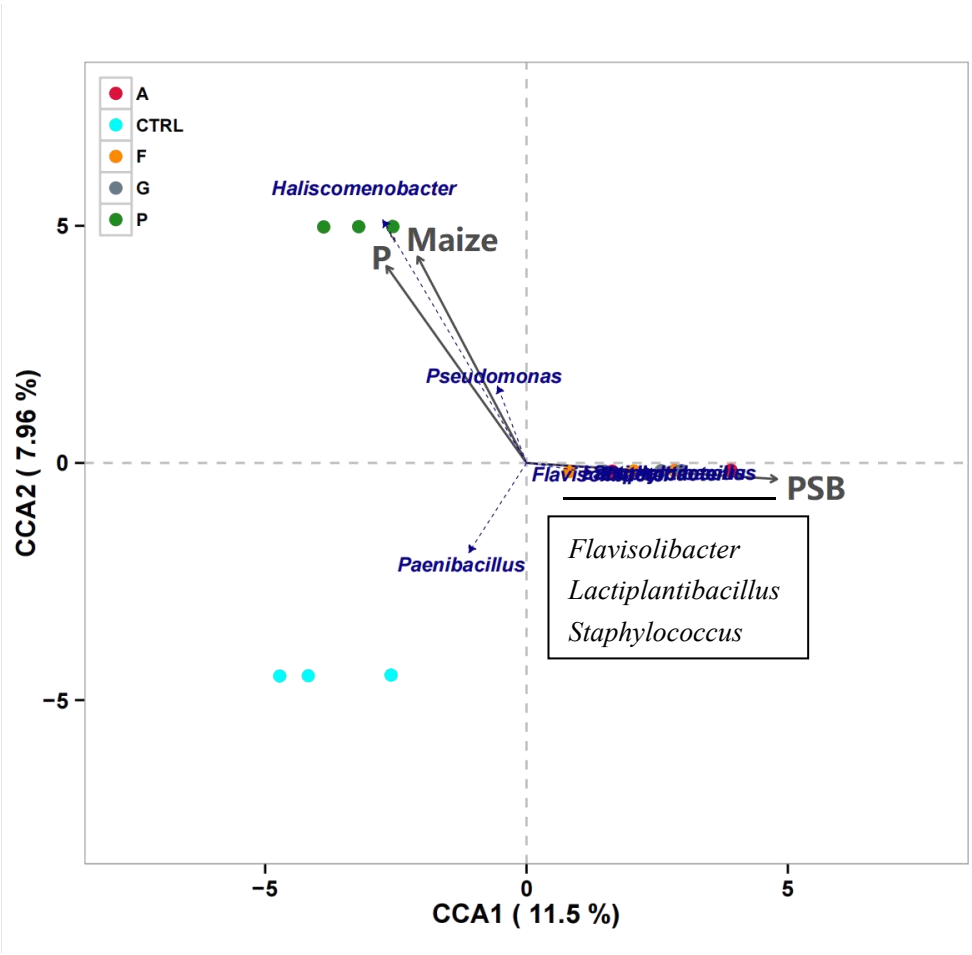


Fig. 5.2.8 Canonical Correspondence Analysis of Environmental Factors and Maize Seedling P accumulation.

CCA1 and CCA2 show low explanatory power, suggesting weak influ-ence of the selected environmental factors on bacterial composition. To further investigate the relationship between exogenous PSB and indigenous rhizobacterial communities, consider expanding environment-

al factors or increasing the sample size.

5.3 Discussion

In this chapter, three strains (A, F, and G) were employed to inoculate maize roots in order to investigate their effects on the maize rhizobacterial community. The results were then compared with non-inoculated maize roots (control) and well-nourished maize roots (P treatment).

Based on the results of soil and maize seedling physiological indicators, it is evident that the changes in soil indicators were not as pronounced as observed in Chapter 4. Additionally, the maize seedling physiological indicators did not exhibit a significant increase in the PSB treatment, suggesting that the PSB strains, especially strain A, did not effectively enhance the planting effects compared to the soluble inorganic P nutrient in this experiment.

To investigate the reason why the PSB strains did not exhibit similar growth-promoting efficiency as observed in sterilized soil, an analysis of the rhizobacterial community was conducted. The results of the species annotation statistics revealed that the strains inoculated in the soil (A: *Enterobacter chuandaensis*; F: *Klebsiella aerogenes*; G: *Pseudomonas hunanensis*) did not become dominant species in the rhizobacterial community (they did not even rank among the top 30 abundant species).

Furthermore, the inoculation of exogenous PSB did not significantly alter the bacterial abundance of maize roots (α Diversity), but it did affect the community composition (β Diversity).

The results of Anosim also confirmed the effectiveness of sample grouping, indicating that the inter-group difference was greater than the intra-group difference, and the sample grouping was statistically significant. Additionally, Marker specifications between groups demonstrated that the inoculation of exogenous PSB increased the relative abundance of bacteria in uncultivated soil. Functional gene prediction revealed that the hydrocarbon degradation capability in the G treatment was stronger compared to the control and P treatments.

Table 5.3 Pearson correlations within control and PSB treatment

	Fresh weight	Dry weight	P accum.	pH	ACPase	Oxalic
PSB	.676*	.603*	—	—	.707*	-.894**
Fresh weight		.961**	.866**	—	.603*	-.656*
Dry weight			.955**	—	.630*	-.591*
P accum.				—	.749**	—
pH					—	—
ACPase						—

Pearson correlation analysis was conducted to investigate the relationship between PSB inoculation and various indicators of maize and soil (Table 5.3). The results revealed that PSB inoculation had a positive influence on the fresh weight and dry weight of maize plants. It also positively affected the soil acid phosphatase activity and exhibited a negative correlation with soil oxalic acid content. Although there was no direct correlation observed between maize seedling phosphorus accumulation and PSB inoculation, it was observed that maize seedling P accumulation was positively correlated with soil acid phosphatase activity. Therefore, it can be inferred that:

$$\text{P accum.} \propto \text{ACPase} \propto \text{PSB}$$

In other words, PSB inoculation established an indirect positive connection between soil acid phosphatase activity and maize seedling P accumulation.

In general, based on the results of maize seedling physiological indicators, it can be observed that the PSB strains used in this study did not improve maize yield as effectively as soluble inorganic P nutrients. Additionally, strain A did not significantly enhance maize seedling P accumulation as observed in the sterilized soil co-cultivation. As mentioned earlier, the soil environment influences the release of soil phosphorus by PSB, thereby affecting maize seedling P accumulation. In this experiment, PSB strains should coexist or compete with the

indigenous microbial community in the niche, further influencing their ability to release soil phosphorus and promote maize seedling P accumulation.

Through amplicon analysis, it was found that the strains (A, F, G) inoculated into the soil did not become dominant bacteria, except for the relatively higher abundance of strain A in A2 samples. Furthermore, while PSB inoculation showed a positive correlation with the abundance of dominant bacteria in groups A, F, and G, these dominant bacteria did not exhibit a positive correlation with maize seedling P accumulation. However, if soluble P supply is not considered and only Pearson correlation analysis is conducted among the three groups (control and PSB treatments), it can be observed that PSB inoculation establishes an indirect positive correlation with maize seedling P accumulation through soil acid phosphatase activity. The potential reason for this discrepancy is likely due to the limited inclusion of environmental factors or a small sample size (indicated by low CCA1 and CCA2 values). Further investigation is needed to clarify and explore the relationship between these factors more comprehensively and explicitly.

Chapter VI

General Discussion

6.1 PSB P release ability

In this research, PSB demonstrated high potential for dissolving TCP, but they had weak effects on $FePO_4$ and $AlPO_4$, which aligns with the findings of Chung *et al.* (2005) and Jiang *et al.* (2020). These results may be attributed to the isolation method, where TCP was used as the P source for the isolation medium. Furthermore, these PSB altered the P fractions of Ci and La. Previous studies have shown that PSB isolates belonging to the same genus exhibit different phosphorus release abilities in various soils. For instance, *Pseudomonas* sp. in yellow cinnamon soil (Liu *et al.*, 2019), *Klebsiella* sp. in soil from a subtropical moso bamboo forest (Liu *et al.*, 2022), and *Pantoea rodasii* in sandy loam (Lee *et al.*, 2016) enhanced the available P content. Additionally, *Enterobacter* sp. reportedly increased P uptake in sugarcane, which is consistent with the findings of strain A in this study, showing increased P accumulation in maize (Safirzadeh *et al.*, 2019).

The five PSB used in this research exhibited different abilities to release phosphate sources (Figure 2.3.1), which may be attributed to their distinct P release mechanisms (Wang *et al.*, 2014; Zhu *et al.*, 2009), consequently leading to variations in their efficiency to release P from

soils. Moreover, the efficiency of these PSB in releasing P from TCP in shake flask cultures did not correspond to their efficiency in releasing HCl-P in soils. For example, strains F and G released more abundant P from TCP than the other three strains (Figure 2.3.1), but they did not cause a stronger decrease in sterilized soils compared to the other strains (Table 3.3.3b and 3.3.3b). One possible reason for this discrepancy is that the environment provided by the culture medium in shake flasks greatly differs from that of the soil. It has been reported that the buffering capacity of soils can limit the solubilization of soil phosphates by microorganisms (Cabala-Rosand and Wild, 1982; Gyaneshwar *et al.*, 1998), and PSB organic acid production can be influenced by different nitrogen and carbon conditions in the soil (Cuningham and Kuiak, 1992). Some studies have also indicated that PSB with high solubility to TCP in the medium did not enhance P accumulation in plants (Poonguzhali *et al.*, 2008; Collavino *et al.*, 2010). Bashan *et al.* (2013) argued that TCP is not a suitable selection factor for PSB testing. From this research, we also believe that evaluating the release ability of PSB to soil P solely based on their release ability to TCP is inaccurate.

This study revealed that although different strains may cause varying changes in soil P fractionation, the trend of changes in the same soil remains consistent. For La, a soil with pH 5.0-5.5 and high PRP, its soil P fractionation are predominantly composed of moderately labile P. With

the inoculation of PSB, regardless of TCP supply, La primarily increased moderately labile P, with a small amount of released P contributing to labile P. Delfim *et al.* (2020) also reported significant increases in NaOH-Pi levels due to changes in the P pools of Andisol and Ultisol with pH 5.5 and 5.8, induced by B. thuringiensis, which is consistent with our findings. For Ci at pH 8.0 with high HCl-P content, PSB primarily increased labile P. Thus, the changes in soil P fractionation caused by PSB can be considered soil-type dependent. Our research results suggest that the varying phosphate release abilities of PSB result in different changes in soil P fractionation, but these changes do not significantly alter the original soil P fractionation.

In the inoculation experiment, Pearson correlation analysis was conducted for two soils. Both soils have four treatments: with TCP supply (control and PSB treatment) and without TCP supply (TCP and combination treatments). No significant correlation was found between labile P and HCl-P (data not shown). Additionally, it was observed that when the content of calcium phosphate in the soil was high (combination treatment of La, PSB, and combination treatments of Ci), the decreases in HCl-P caused by the inoculation of these five PSB exceeded the increases in labile P. A study by Liu *et al.* (2022) also demonstrated that inoculating *Klebsiella* in soil from a subtropical moso bamboo forest (HCl-P approximately 65 mg-P kg^{-1} soil) resulted in a greater decrease in HCl-P

compared to the sum of the increases in H_2O-P, $NaHCO_3$-Pi, and $NaHCO_3$-Po.

6.2 Maize seedling P accumulation

The effects of PSB on improving plant P uptake and promoting biomass have been widely reported (Biswas *et al.*, 2022; Dasila *et al.*, 2022; Liu *et al.*, 2022; Sabra *et al.*, 2022). The results of our study also indicated that PSB promoted the maize seedling P accumulation: the maize seedling P accumulation of the PSB treatment and the combination treatment were greater than the control.

All treatments of the two soils were found to have significantly increased maize seedling P accumulation, but the difference between the PSB treatment and the TCP treatment was not significant. Maize seedling P accumulation of all treatments in La was less than that in Ci. Regarding the increase rate of maize seedling P accumulation, LaA was found to be less than CiA, and LaP was similar to CiP, but LaAP was much higher than CiAP. Although P accumulation is low, it is apparent from the increase rate that the combination effect for La is strong. For Ci, PSB inoculation alone worked well, but TCP did not bring much benefit.

Findings from linear regression incorporating the soil type, PSB inoculation, and TCP supply on maize seedling P accumulation indicated that the soil type, PSB inoculation, and TCP supply had significant

positive effects on maize seedling P accumulation, and soil type had the greatest effect on maize seedling P accumulation. For La only, PSB inoculation and TCP supply had significant positive effects on maize seedling P accumulation, and TCP supply's effect was slightly higher than that of PSB inoculation. But in Ci, the effect of PSB inoculation is greater than that of TCP supply. Although the soil type has a great influence on plant P accumulation, its selectivity in agricultural production is minimal. Therefore, PSB and P fertilizer are anticipated as effective means to increase crop yield.

6.3 Maize rhizosphere bacteria community

Whether exogenous PSB have impact on indigenous microbial communities is the result of multiple factors. For example, the competition of exogenous strains for niche, that is, the colonization of exogenous strains in the environment. The success of colonization is not only related to the strain, but also to the soil environment and soil microbial environment (Liu J. *et al.*, 2020).

Wang *et al.* (2017) studied the colonization of *Bacillus* in the rhizosphere of Chinese cabbage by using GRP marker method, and found that the population of *Bacillus* remained in a stable range after 12 days of sliding, and successfully colonized. Liu H. *et al.* (2021) found a similar situation when studying the colonization of poplar rhizosphere by

Pseudomonas fluorescens. This study did not verify the colonization of the inoculated strains, but from the sequencing results, the PSB did not become the dominant population (except A2, where *Enterobacteriaceae* is the dominant population).

Although PSB did not become the dominant population and did not affect bacterial diversity, it still affected the structure of the rhizosphere bacterial community, which was consistent with what Huang *et al.* (2020) found when studying the growth promoting effect of Bacillus on peanuts.

In this study, the bacterial community structure of inoculated and uninoculated soil was different, and *Flavisolibactor*, which became the dominant species in F and G treatments, had a close relationship with soil ACPase activity. Liu *et al.* (2020) also found that although exogenous PSB did not become the dominant species in tomato rhizosphere, it changed the soil bacterial community structure, caused the decline of relative abundance of some indigenous bacteria, and also increased the abundance of several bacteria. That study also found that soil properties had a greater impact on soil microbial community. Liu believed that PSB changed soil properties and rhizosphere microbial communities by secreting organic acids. This is similar to our research results.

6.4 Conclusion and Perspectives

The diversity of global soils makes it appropriate to manage P

fertilization differently (Mengel, 1997). Results of our study supported this view. As findings obtained from our study suggest, a same strain's efficiencies in releasing soil P and improving labile P differ in different soil types (La and Ci). These differences might be partly attributable to the influence of the original soil P fractionation. P is assigned to different fractions during the conversion process. As for La, which is rich in iron and aluminum, is more likely to bind P to the NaOH-P fraction, leading to a low labile P situation. My study showed that the combination of TCP and PSB in La produced much higher labile P and maize seedling P accumulation than one treatment alone, whereas the combination of them in Ci did not produce a significant increase in the maize seedling P accumulation compared to PSB alone. In general, in La, additional P is beneficial for increasing the maize seedling P accumulation. Its use in combination with PSB can further optimize planting effects, whereas in Ci, using PSB to mobilize the soil P can produce good results.

As Barrow (2022) pointed out, long-term fertilized soils have already accumulated large amounts of P. What must be done is to transform it to useful nutrients for plants rather than to continue to add extra P. PSB is promising to mobilize accumulated P in soils. Study of P release by PSB in different soils will help to find PSB suitable for different soil type, thus promoting the development of targeted and efficient PSB fertilizer and providing possible help for the sustainable

development of agriculture.

Overall, this research examined the release of P by PSBs in two typical soil types in China. The results demonstrated that the effect of PSB inoculation on P release varied, with a primary increase in labile P for Soil Type La and labile P for Soil Type Ci.

Furthermore, this research clarified that PSBs had a direct or indirect influence on enhancing maize P accumulation by impacting soil physicochemical indicators. The efficiency of this enhancement varied among different soil types.

Moreover, this research investigated the impact of exogenous PSBs on maize P accumulation in natural soil. The findings revealed that PSBs not only influenced the soil rhizobacterial community but also established an indirect positive relationship with maize P accumulation.

These results contribute to a better understanding of the role of PSBs in P release and their effects on maize P accumulation in different soil environments. Further research is warranted to explore the mechanisms underlying these interactions and to optimize the application of PSBs for improved nutrient management in agricultural systems.

In conclusion, this research demonstrated that PSB is an effective means to mobilize P in soil. With the diverse soil profiles found globally, including significant P retention in long-term cultivated fields, further investigation into the dynamics of P release by PSB in various soil types

can contribute to the more efficient utilization of PSB. I hope that my research provides valuable insights for the enhanced utilization of PSB.

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Acknowledgment

My heart is overflowing with gratitude as I reflect on the completion of this research. Without the careful guidance and unwavering support of Professor Wasaki Jun, Professor Nakatsubo, and Professor Ueda, I would never have been able to achieve this milestone. Their mentorship has not only enriched my work, but also my personal life. My heartfelt thanks go out to each of them.

I am also deeply grateful to Ms. Christiana, whose invaluable assistance was instrumental in the completion of my doctoral dissertation. Her kindness and generosity will always be remembered.

As I think back on the long hours spent in the lab, I am reminded of the guidance and support of Ms. Tateishi and Mr. Yamamura. Their expertise and dedication were crucial to the success of my experiments, and I am forever indebted to them.

I would like to express my thanks to Xiong Zilin, Aihara, Abiko, Yamada, Wang Ruixin, Saisako, and Lydia for their unwavering support. Your contributions have been immeasurable, and I could not have done it without you. Thank you from the bottom of my heart!

啥都不说了，我妈才是最伟大的！向我最亲爱的妈妈敬礼！妈您辛苦了！

毛小豆！妈妈爱你！你是最棒的！今后也要继续一起加油哦！

感谢我亲爱的姐姐！

Appendix

I. Abbreviation

Abbr.	Full form
ACPase	Acid phosphatase
AKPase	Alkaline phosphatase
Al-P	Aluminum phosphate
Ca-P	Calcium phosphate
Ci	Cinnamon soils
Fe-P	Iron phosphate
HCl-P	HCl extracted phosphorus
La	Lateritic red earths
$NaHCO_3$-P	$NaHCO_3$ extracted phosphorus
$NaHCO_3$-Pi	$NaHCO_3$ extracted inorganic phosphorus
$NaHCO_3$-Po	$NaHCO_3$ extracted organic phosphorus
NaOH-P	NaOH extracted phosphorus
NaOH-Pi	NaOH extracted inorganic phosphorus
NaOH-Po	NaOH extracted organic phosphorus
NPase	Neutral phosphatase
M labile P	Moderately labile phosphorus
Olsen-P	Available phosphorus in soil extracted via $NaHCO_3$ solution using the method proposed by Olsen in 1954
O-P	Occluded phosphate
P	Phosphorus
PRP	Phosphorus retention potential
PSB	Phosphate solubilizing bacteria
Resin-P	Resin exchangeable phosphorus

Abbr.	Full form
TCP	Tricalcium phosphate, $Ca_3(PO_4)_2$

II. Culture media and solution

• NBRIP g/L

Glucose,10; $MgCl_2 \cdot 6H_2O$, 5; $MgSO_4 \cdot H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1; $Ca_3(PO_4)_2$, 5; Mix components with distilled or deionized water.

Autoclave at 115°C for 15 minutes.

• LB g/L

Tryptone,10; Yeast Extract, 5; NaCl, 10; Dissolve components in distilled or deionized water.

For LB agar should be called LA add agar to a final concentration of 1.5%.

Autoclave at 121°C for 15 minutes.

• 50% glycerol

Make the 50% glycerol solution by diluting 100% glycerol in pure water.

Autoclave at 121 ℃ for 15min.

III. Equipment

• PH meter

PHS-3C Leici Shanghai China with electrode E-201.

IV. Measuring methods

• Molybdenum blue method (Murphy and Riley, 1962)

Reagent:

(1) Sulfuric acid (5N): Dilute 70 mL of concentrated sulfuric acid to 500 mL.

(2) Ammonium molybdate: Dissolve 20 g of high-purity ammonium molybdate in water and dilute to 500 mL. Store the solution in a brown glass bottle.

(3) Ascorbic acid (0.1 M): Dissolve 1.32 g of ascorbic acid in 75 mL of water. Ideally, this solution should be prepared on the day of use as ascorbic acid is easily oxidized. If the solution needs to be stored, add 25 mg of disodium ethylenediaminetetraacetate and 0.5 mL of formic acid per 75 mL of solution to stabilize the ascorbic acid.

(4) Potassium antimony tartrate (274.3 mg of rhodium per mL): Dissolve 0.2743 g of potassium antimony tartrate in distilled water and dilute to 100 mL.

(5) Reagent mixture: Mix 125 mL of 5 N sulfuric acid with 37.5 mL of ammonium molybdate, and then add 75 mL of ascorbic acid solution and 12.5 mL of potassium antimony tartrate solution. This reagent should be prepared as needed as it is stable for no more than 24 hours.

Stock phosphate solution: Prepare a solution containing 0.1757 g of potassium dihydrogen phosphate per liter. This solution contains 40 mg P

(as phosphate) per liter. Phosphate working solution: Dilute the stock solution to a solution containing 0.2 μg P (as phosphate) per mL.

Gradually dilute the phosphate use solution to prepare standard curve samples. Take 40 mL of the standard curve samples and the test samples, add 8 mL of the mixed reagent, make up to 50 mL in a volumetric flask, and mix well. After 15 minutes, measure the absorbance of the solution at 721 nm in a 1 cm cuvette.