別紙４

学 位 論 文 の 要 旨

論文題目　　The role of phosphate solubilizing bacteria in soil phosphorus dynamics and phosphorus accumulation of maize seedlings (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)

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1. Introduction and Aim

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 Lateritic red earths 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.

2. Isolation and Screening of PSB Strains

This chapter presents the procedure for isolating PSB strains from soil and subsequently screening them for further experiments. The processes involved in this study encompassed 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.

3. PSB release P from sterilized soils (inoculation experiment)

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

It was revealed that the patterns of change by PSB inoculation within the same soil remained consistent, while different PSB strains might cause variations in P fractionation. For La soil, 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 P availability in soils 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.

4. 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 chapter. 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 and 4.86 mg-P kg^{-1} soil in La and Ci, respectively, with combination of TCP and strain A application, 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. PSB strain 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 P accumulation of maize seedlings 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 properties, was observed.

5. 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 strains 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. It was conducted for further analyses, such as alpha diversity, beta diversity, differential analysis between groups, correlation analysis, function prediction.

PSB strains did not enhance maize seedling P accumulation in natural soil (non-sterilized La) as significantly as soluble P did. 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.

6. Conclusion and Perspectives

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 La soil and labile P for Ci soil. Furthermore, this research clarified that PSBs had a direct or indirect influence on enhancing maize P accumulation by impacting soil physicochemical properties. The efficiency of this enhancement varied among different soil types. Additionally, it was 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. 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.