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Inoculation with Phosphate-Solubilizing Fungi Diversifies the Bacterial Community in Rhizospheres of Maize and Soybean*[1]

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ABSTRACT

Application of phosphate-solubilizing microorganisms (PSMs) has been reported to increase P uptake and plant growth. However, no information is available regarding the ecological consequences of the inoculation with PSMs. The effect of inoculation with phosphate-solubilizing fungal (PSF) isolates *Aspergillus niger* P39 and *Penicillium oxalicum* P66 on the bacterial communities in the rhizospheres of maize (*Zea mays* L. 'Haiyu 6') and soybean (*Glycine max* Merr. 'Heinong 35') was examined using culture-dependent methods as well as a culture-independent method, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). Compared with the control, the number of culturable microbes for soybean was significantly greater with P39, whereas for maize, the same was significantly greater with P66. In addition, a greater number of microbes were found in the rhizosphere of maize compared with soybean. The fingerprint of DGGE for 16S rDNA indicated that inoculation with PSF also increased bacterial communities, with the P66 treatment having higher numbers of DGGE bands and a higher Shannon-Weaver diversity index compared with P39; the composition of the microbial community was also more complex with the P66 treatment. Overall, complex interactions between plant species and exotic PSMs affected the structure of the bacterial community in the rhizosphere, but plant species were more important in determining the bacterial community structure than the introduction of exotic microorganisms.

Key Words: bacterial community, diversity, PCR-DGGE, phosphate-solubilizing fungus, rhizosphere

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Phosphorus (P) is an essential macronutrient for plant growth. Despite phosphorus being widely and abundantly distributed in the soil in both its inorganic and organic forms, many soils throughout the world are deficient in P. Phosphorus can be tightly bound with calcium, iron, or aluminum, leading to precipitation of P (Li *et al.*, 2003). Although large amounts of soluble phosphate are applied to soil as fertilizers, plants are able to use only a small portion of the applied phosphate, and the remainder is rapidly immobilized and becomes unavailable to plants (Han *et al.*, 2005a, b). Use of P fertilizers has become an expensive practice. Therefore, the use of cheap, alternative sources of P, such as rock phosphate (RP), has received considerable attention in recent years (Rajan *et al.*, 1996). However, the P in RP is not plant-available in soils with pH greater than 5.5. Even when the soil conditions are optimal, plants grown in soils to which RP was added recorded lower yields compared with those grown in soils to which soluble phosphate was added (Khasawneh and Doll, 1978).

The rhizosphere is the region in the soil surrounding a plant's root system and is affected by the excretions of the plant roots. It is a region of high microbial activity, where microbes carry out fundamental processes that contribute to nutrient cycling, plant growth, and root health. Soil microbes,

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especially those isolated from the rhizosphere, are capable of solubilizing inorganic phosphate materials (Kucey *et al.*, 1989). In pot experiments and field trials, the inoculation with phosphate-solubilizing microorganisms (PSMs) in the soil, seed, or seedlings has increased P uptake and total plant biomass (Chabot *et al.*, 1996). However, the effect of PSMs on microbial communities in the rhizosphere is not known.

Traditional techniques that are used to elucidate the soil microbial community are based on cultivation methods. However, culture-dependent techniques cannot detect more than 90% of the microorganisms existing in nature (Ward *et al.*, 1990). Therefore, culture-independent methods, such as PLFA (phospholipid fatty acids analysis) (Zelles, 1999) and polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.*, 1993; Teng *et al.*, 2004), are increasingly being used for the analysis of microbial communities. Therefore, the objective of this study was to investigate the effects of inoculation with phosphate-solubilizing fungal (PSF) isolates on bacterial and fungal numbers in the rhizospheres of maize and soybean using the plate counting method and on the bacterial community using the PCR-DGGE method.

MATERIALS AND METHODS

Phosphate-solubilizing fungi

From previous studies, two fungal isolates, *Aspergillus niger* P39 (P39) and *Penicillium oxalicum* P66 (P66), were identified as the most effective PSMs (Wang *et al.*, 2005) and were used throughout this study. Spores obtained from a 7-d growth in potato dextrose agar were suspended in sterile distilled water containing 0.1% Tween-80. Final spore concentration of 1×10^7 spores mL^{-1} water suspension was used as the inoculant.

Pot experiment

The sand culture technique was used in this experiment (Marschner *et al.*, 2004). Eight seeds of maize (*Zea mays* L. 'Haiyu 6') or soybean (*Glycine max* Merr. 'Heinong 35') were placed on the sand surface of each pot and covered with an additional 2 cm of sand. A completely random design was used, with three inoculation treatments, *i.e.*, no inoculation (as a control, CK), inoculation with P39, and inoculation with P66, and four replicates.

To each pot, 1 500 g of sand mixed with 2 g of rock phosphate (Yichang, Hubei Province, China; P, 105 g kg^{-1}; 100-mesh) was added. The pots were watered daily with 0.1 × Hoagland's nutrient solution without phosphorus to maintain nutrient and water supply for plant growth. In May, the pots were placed in a greenhouse with natural light. After emergence, the seedlings were thinned to 4 per pot. Each seedling of maize and soybean was inoculated either with 1 mL water (for the control) or with spore suspension.

After 50 d of growth, plant shoots were harvested, oven-dried, and their dry weights were measured. Samples of rhizosphere were collected by shaking off the sand particles adhering to roots and used for microbial numeration, and the mixed samples were used for PCR-DGGE analysis.

Plate counts

The numbers of culturable bacteria, fungi, and PSMs were determined using the agar plate method. The culture medium for bacteria was beef extract peptone agar and that for fungi was rose bengal streptomycin glucose agar. The PSMs were grown in the Pikovskaya medium containing 5.0 g L^{-1} tricalcium phosphate (Pikovskaya, 1948). Plates were incubated at 25–28 °C for 2–3 d with bacteria, 5 d with fungi, and 7–10 d with PSMs. Counts of culturable microbes were expressed as CFU (cell-forming units) per gram of fresh rhizosphere sand.