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Suppression of Fusarium Wilt of Banana with Application of Bio-Organic Fertilizers*[1]

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ABSTRACT

Fusarium wilt is one of the most serious diseases of banana plants caused by soil-borne pathogen *Fusarium oxysporum* f.sp. *cubense* (FOC). In this study a pot experiment was conducted to evaluate the effects of different bio-organic fertilizers (BIOs) on Fusarium wilt of banana, including the investigations of disease incidence, chitinase and β-1,3-glucanase activities of banana plants, and FOC populations as well as soil rhizosphere microbial community. Five fertilization treatments were considered, including chemical fertilizer containing the same N, P and K concentrations as the BIO (control), and matured compost mixed with antagonists *Paenibacillus polymyxa* SQR-21 and *Trichoderma harzianum* T37 (BIO1), *Bacillus amyloliquefaciens* N6 (BIO2), *Bacillus subtilis* N11 (BIO3), and the combination of N6 and N11 (BIO4). The results indicated that the application of BIOs significantly decreased the incidence rate of Fusarium wilt by up to 80% compared with the control. BIOs also significantly promoted plant growth, and increased chitinase and β-1,3-glucanase activities by 55%–65% and 17.3%–120.1%, respectively, in the banana roots. The population of FOC in the rhizosphere soil was decreased significantly to about 10^4 colony forming units g^{-1} with treatment of BIOs. Serial dilution plating and denaturing gradient gel electrophoresis analysis revealed that the application of BIOs increased the densities of bacteria and actinomycetes but decreased the number of fungi in the rhizosphere soil. In general, the application of BIOs revealed a great potential for the control of Fusarium wilt disease of banana plants.

Key Words: biocontrol, denaturing gradient gel electrophoresis, fungal disease, manure compost

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INTRODUCTION

Fusarium wilt of banana (*Musa* spp.), commonly known as Panama disease caused by *Fusarium oxysporum* f.sp. *cubense* (E.F. Smith) Snyder & Hansen (Snyder and Hansen, 1940), is one of the most serious fungal diseases and the major limiting factor for banana production worldwide (Lin *et al.*, 2009). There are no effective chemical control measures for panama disease and the currently practiced corm injection procedure with fungicide carbendazim is tedious and environmentally unfriendly (Getha and Vikineswary, 2002). Other control methods such as field sanitation, soil treatments with fumigants, flood fallowing and crop rotation with nonhosts of the fungus have rarely provided long-term control in any production area (Ploetz *et al.*, 1990). Currently, the selection of resistant cultivars is generally accepted as a disease control method and several resistant cultivars (*e.g.*, Farmosona) are grown in larger areas in Taiwan for the management of Fusarium wilt (Ploetz, 1990). As an alternative approach, the effectiveness of some plant growth-promoting and antagonistic bacteria against soil-borne pathogens has been widely evaluated (Saravanan *et al.*, 2003). Unfortunately, directly inoculation of biocontrol agents into soil may lead to poor activity of these microbes (Alabouvette *et al.*, 2006). To overcome this problem, some integrated approaches, including enhancement of the activity of biocontrol agents by adding organic amendments, were attempted to achieve better effects (Saravanan *et al.*, 2003).

Hoitink *et al.* (1975) first put forward that compost can be used as a peat substitute to control root pathogens. Later, the biocontrol research was increasingly focused on developing the right combination of compost and antagonistic microbes. *Trichoderma as-*

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perellum and sewage sludge compost were utilized to suppress Fusarium wilt of tomato (Cotxarrera *et al.*, 2002). Trillas *et al.* (2006) also reported the significant biocontrol of rhizoctonia disease of cucumber by the application of a mixture of agricultural compost and *Trichoderma* spp. It was thought that compost can provide nutrients for microbes, thus increasing the antagonist's viability and facilitating their survival in rhizosphere and colonization on plant roots (Boehm *et al.*, 1993).

Bacillus species have been successfully applied for the biocontrol of soil-borne pathogens (Ongena and Jacques, 2008). *Paenibacillus polymyxa* has been found not only to be a plant growth-promoting rhizobacteria, but also to be an effective biocontrol agent, which can produce a broad range of peptide metabolites with antibacterial and/or antifungal activities (Raza *et al.*, 2008) and form highly resistant endospores to both chemical and physical stresses. *T. harzianum* has also been used as an antagonistic fungal strain with reasonable effects against soil-borne pathogens (Wu *et al.*, 2009). A novel bio-organic fertilizer (BIO) by fermenting matured compost with biocontrol strains *P. polymyxa* SQR-21 and *T. harzianum* T37 was developed by our laboratory. This BIO was previously shown to suppress Fusarium wilt disease, and promote the growth of watermelon plants in greenhouse and field experiments (Wu *et al.*, 2009). Two other antagonistic strains, *Bacillus amyloliquefaciens* N6 and *Bacillus subtilis* N11, were also isolated from the banana continuously cropping soils and were fermented with matured compost to get specific BIOs for banana plants. In the present study, therefore, a pot experiment was carried out to assess the effects of the combinations of organic fertilizer and these antagonistic microorganisms on Fusarium wilt of banana, including the investigations of disease incidence, chitinase and β-1,3-glucanase activities of banana plants, and populations of FOC as well as soil rhizosphere microbial community.

MATERIALS AND METHODS

Bacterial and fungi strains

The pathogen fungal strain *Fusarium oxysporum* f.sp. *cubense* (FOC), as well as a tested antagonistic bacterial strain *P. polymyxa* SQR-21 and a tested antagonistic fungal strain *T. harzianum* T37, were provided by the Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, Nanjing Agriculture University, China (Wu *et al.*, 2009; Ling *et al.*, 2010). Two bacterial isolates showing the strongest antagonism against FOC (data not shown) were selected as antagonistic agents to fortify organic fertilizer. The 16S rRNA gene sequences of one bacterial isolate, designated as N6, showed 100% identity to *B. amyloliquefaciens* strain 16. The other isolate, designated as N11, shows 100% identity to *B. subtilis* strain DYJL26. Sequences of both strains were deposited into GenBank under accession Nos. GQ452909 and GQ452910, respectively. The bacterial strains were stored in Luria-Bertani medium at −80 °C in 20% glycerol while the fungal strain was maintained on potato dextrose agar plates and stored at 4 °C.

BIO preparation

Organic fertilizer used for BIOs preparation was composed of amino acid fertilizer and pig manure compost (1:1). Amino acid fertilizer was made from oil rapeseed cakes that were hydrolyzed by microbial enzymes for 7 d (Zhang, S. S. *et al.*, 2008). This amino acid fertilizer contained 442 g kg^{-1} organic matter, 129 g kg^{-1} sum of amino acids, small molecular peptides and oligo peptides, 44 g kg^{-1} nitrogen (N), 35 g kg^{-1} P_2O_5, and 7 g kg^{-1} K_2O. Pig manure compost was made by Tianniang Limited, China by composting pig manure at a temperature range of 30–70 °C for 25 d. This compost was composed of 304 g kg^{-1} organic matter, 20 g kg^{-1} N, 37 g kg^{-1} P_2O_5, and 11 g kg^{-1} K_2O.

For the BIOs preparation, 1 000 mL suspensions of the mixture of SQR-21 (1×10^9 colony forming units, CFU, mL^{-1}) and T37 (1×10^6 CFU mL^{-1}), N6 or/and N11 (1×10^9 CFU mL^{-1}) and organic fertilizer (5 kg) were thoroughly mixed in a 500 mm × 360 mm × 175 mm plastic case for secondary fermentation. The mixture was maintained at 40%–45% moisture under room temperature (20–31 °C) for 6 d and manually inverted every day. Then, the mixture was spread for air-drying in a ventilation room at room temperature for 2 d until water contents were less than 30%. During the secondary fermenting, temperature and bacterial density of the substrate were observed daily. The composts containing bacterial populations higher than 1×10^9 CFU g^{-1} DW or fungal populations higher than 1×10^5 CFU g^{-1} DW were defined as a BIO. The BIO was stored at 4 °C prior to use in the experiment.

Pot experiment

The properties of the soil used for pot experiment were listed as follows: pH of 5.4, organic matter of 7.3 g kg^{-1}, available N of 79 mg kg^{-1}, available P of 31 mg kg^{-1} and available K of 40 mg kg^{-1}. The soil was pre-inoculated with the spore suspension of FOC to obtain