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# Effect of biofertilizer for suppressing *Fusarium* wilt disease of banana as well as enhancing microbial and chemical properties of soil under greenhouse trial

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ABSTRACT

Biofertilizer application has been proposed as a strategy for the management of banana *Fusarium* wilt disease, which has severely decreased banana production. In this study, a 4-season pot experiment was conducted to evaluate the effects of sustainable biofertilizer application at low and high levels on banana *Fusarium* wilt disease suppression, soil chemical properties and microbial communities. The results showed sustainable biofertilizer application effectively controlled the disease, especially at a high level. Next-generation sequencing of the 16S rRNA and internal transcribed spacer (ITS) genes using the MiSeq platform showed that the soil bacterial and fungal communities in the treatment amended with a high level of biofertilizer (HBIO) were significantly different from a low level biofertilizer treatment (LBIO) or chemical fertilizer control (CF). Moreover, the abundance of *Firmicutes* and *Bacillus* was significantly increased, while the abundance of *Acidobacteria*, *Bacteroidetes* and *Ascomycota* was significantly decreased in the HBIO treatment compared with the CF control. Furthermore, the abundance of *Fusarium* was significantly reduced in the HBIO treatment compared with CF control and was slightly reduced (not significant) compared with the LBIO treatment. Redundancy analysis and Spearman correlation showed that *Bacillus*, *Spartobacteria_genera* and *TM7_genera* dominated in the HBIO treatment and they were positively correlated with the soil pH and the contents of total nitrogen and carbon and available phosphorus, which were negatively correlated with disease incidence. In conclusion, sustainable biofertilizer application suppressed the *Fusarium* wilt disease might through improving soil chemical condition and manipulating the composition of soil microbial community, including specific enrichment of *Firmicutes* (*Bacillus*), *Anoxybacillus*, *Spartobacteria_genera*, *TM7_genera*, *Cantharellus*, *Pateramyces* and *Synchytrium*.



## 1. Introduction

Maintaining soil health is one of the most vital requirements for crop production in agricultural systems (Oros-Sichler et al., 2007). Soil microorganisms play important roles in organic carbon mineralization, nutrient cycling, and disease transmission and resistance, which are associated with soil health (Hollister et al., 2013). It is well established that agricultural inputs, such as organic amendments and mineral fertilizer, can affect soil microorganisms in different ways (Bünemann et al., 2006). Given that the microbial community is one of the main drivers of disease suppression that responds to soil health, determining the responses of the soil microbial community to different agricultural inputs is particularly important (Garbeva et al., 2004).

The Cavendish banana cultivar, which accounts for 90% of banana production in China, is the most widely planted and important cash crop in south China, which is the second largest

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banana producer worldwide (Chen et al., 2013; Li et al., 2013). However, banana production is severely threatened by Fusarium wilt disease, which is caused by the soil-borne fungus Fusarium oxysporum f. sp. cubense (FOC) (Ploetz, 2006; O'Donnell et al., 1998). Moreover, the disease has recently become a serious threat to Cavendish banana production in China because the main cultivated specie is susceptible to FOC Tropical Race 4, a new fungal pathogen strain of Fusarium oxysporum f. sp. cubense (Butler, 2013; Chen et al., 2013).

Compared with other effective management measures for controlling this disease, such as the screening of resistant cultivars (Hwang and Ko, 2004), the application of soil fungicides and fumigation (Meldrum et al., 2013; Nel et al., 2007) and crop rotation (Zhang et al., 2013), biocontrol is the most promising strategy due to presenting advantages related to safety, longevity, environmental conservation and low cost-effectiveness with a high return (Wang et al., 2013). However, direct inoculation of biocontrol strains into the soil without a suitable organic substrate may result in unsuccessful and unstable control of *Fusarium* wilt disease because of nutrient-poor conditions (El-Hassan and Gowen, 2006). Applying biofertilizer after secondary solid fermentation from organic material and biocontrol agent has been reported to be a timely method for controlling many soil-borne diseases (Zhao et al., 2014; Wang et al., 2013; Qiu et al., 2012). Biofertilizer application is becoming popular in China, as increasing numbers of Chinese farmers are recognizing the effects of biofertilizer in protecting plant roots from soil-borne pathogens, improving soil fertility and increasing crop production (Zhang et al., 2014a).

Because the rhizosphere is where plants and microbes interact with each other and is critical for shaping disease suppression (Hadar and Papadopoulou, 2012), almost all current studies on the effects of biofertilizer application on the microflora are focused on the rhizosphere microbial community (Zhang et al., 2014b; Qiu et al., 2012). Considering that the rhizosphere microbial community can be influenced by the bulk soil composition and that biofertilizer is directly applied to bulk soil, understanding the shift in the bulk soil microbial community after the implementation of biofertilizer is particular important (de Ridder-Duine et al., 2005). On the other hand, almost all current studies on the effects of biofertilizer application have been performed using a single dose over a very short term (Zhao et al., 2014; Shen et al., 2013; Qiu et al., 2012). However, farmers seek to obtain perpetual economic benefits after biofertilizer application at an appropriate amount over a long time period and in a continual framework. Hence, it is necessary to monitor the effects of long-term biofertilizer application. As soil chemical properties can also be involved in the suppression on plant diseases (Garbeva et al., 2004), knowing the characteristics of soil chemical properties in disease-suppressive soils can also contribute to the management of soil-borne diseases.

A novel biofertilizer consisting of fermented *Bacillus amyloliquefaciens* strain NJN-6 and organic mixture of pig manure compost and amino acid fertilizer (2:3 w/w) has been shown to exhibit an excellent disease suppression ability against banana *Fusarium* wilt disease based on one seasonal pot experiment (Zhang et al., 2014b). Unfortunately, information regarding the long-term effects after biofertilizer application was lacking. Therefore, a 4-season pot experiment was conducted to investigate the long-term effects of this biofertilizer on the suppression of banana *Fusarium* wilt disease as well as soil chemical properties and the bulk soil microbial communities in this study. The objectives were to (1) evaluate the persistent ability of the biofertilizer to control banana *Fusarium* wilt disease; (2) determine the impact of this biofertilizer on soil chemical properties and the soil microbial communities using next-generation sequencing technology on the MiSeq platform; and (3) explore the potential correlation of disease suppression with soil properties and the microflora.

## 2. Materials and methods

### 2.1. Greenhouse experiment design

A 4-season greenhouse experiment was designed and conducted from November 2010 to November 2013 in a greenhouse at the WanZhong Agricultural Company, located in Jianfeng, Ledong County, Hainan Province, China. Two treatments, consisting of unsterilized soil amended with biofertilizer at a low level (150 g/pot, LBIO) and a high level (450 g/pot, HBIO), and a control consisting of unsterilized soil amended with only chemical fertilizer (CF) were designed with three replicates. All treatments were adjusted to the same amounts of N, P and K according to the nutrients of biofertilizer added in HBIO treatment if necessary by urea, calcium superphosphate, and potassium chloride, respectively (Table 1). Each replicate included 20 pots, and only one banana tissue culture seedling (*Musa acuminate* AAA *Cavendish* cv. Brazil.) was planted in each pot, which contained 15 kg of soil mixed with the corresponding fertilizers. Before each season, all the required fertilizers were applied to the pot soil once. After each season, the plants were removed, and the soil in each pot was left fallow for 6 months.

The biofertilizer with a pH value of 7.98, which contained 3.38% N, 2.80% $P_2O_5$ and 1.21% $K_2O$, was prepared by incubating *Bacillus amyloliquefaciens* strain NJN-6 into an organic mixture of amino acid fertilizer and pig manure compost (2:3, w/w) following the solid fermentation method described by Zhang et al. (2008). After fermentation, the biofertilizer contained approximately $1 \times 10^9$ CFU $g^{-1}$ dry weight of *B. amyloliquefaciens* strain NJN-6. The soil used in this experiment was collected from a banana orchard field with a 50% incidence of *Fusarium* wilt disease in 2010 located at WanZhong Agricultural Company (18°38′N, 108°47′E). The test soil exhibited a sandy loam texture developed from dry red soils with a pH value of 6.56 and contained 9.61 g/kg of total carbon (TOC), 1.27 g/kg of total nitrogen (TON), 18.6 mg/kg of available phosphorus (P) and 402 mg/kg of available potassium (K).

### 2.2. Disease incidence assay and soil sampling

Banana *Fusarium* wilt disease was monitored immediately after the seedlings were transplanted into the pots based on the observation of typical wilt symptoms (Jeger et al., 1995), including leaf yellowing, pseudostem splitting, dark brown discoloration of vascular tissues and plant death (Fig. S1). Disease incidence was calculated as the percentage of infected plants among the total number of plants. The disease incidence bioassay was performed until the disease incidence stabilized.

**Table 1**
The amounts of biofertilizer and necessary chemical fertilizer applied in the treatments amended with biofertilizer at a low level (LBIO) and high level (HBIO) and for the control amended with chemical fertilizer (CF).

| Treatment | Fertilizer amount (g/pot) | | | |
|---|---|---|---|---|
| | Biofertilizer | Urea | Calcium superphosphate | Potassium chloride |
| CF | 0 | 33.07 | 105 | 9.08 |
| LBIO | 150 | 22.04 | 70 | 6.05 |
| HBIO | 450 | 0 | 0 | 0 |