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Variation of rhizosphere bacterial community in watermelon continuous mono-cropping soil by long-term application of a novel bioorganic fertilizer

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ABSTRACT

The application method for a novel bioorganic fertilizer (BIO) was developed to improve its biocontrol efficacy of Fusarium wilt (Ling et al. 2010). However, its efficacy on controlling Fusarium wilt and the variations of microbial community after long-term application for watermelon production had not been elucidated. To clarify, a 4-years pot experiment of mono-cropping watermelon was conducted. The results revealed that though the disease incidences were increased in all treatments with the increase of continuous cropping years, the treatment of BIO application both in nursery and pot soil always maintained the lowest disease incidence. The real-time PCR results showed that the population of *Paenibacillus polymyxa* was decreased with continuous cropping years, but in all seasons, the treatment with BIO application both in nursery and pot soil had a highest population of *P. polymyxa* than the other treatments. On the other hand, the abundance of the pathogen FON was increased with the increase of continuous cropping years and the lowest rate of increase was found by BIO application in both nursery and pot soil. DGGE patterns showed that the bacterial diversity was weakened after mono-cropping of watermelon for 4 years, but the consecutive applications of BIO at nursery and transplanting stage resulted in the minimal change of bacterial diversity. More detailed differences on bacterial diversity between control and double application of BIO treatment after 4-years monoculture were analyzed by 454 pyrosequencing, which showed the dominant phyla found in both samples were *Firmicutes*, *Proteobacteria* and *Actinobacteria*, and the consecutive applications of BIO recruited more beneficial bacteria than control, such as *Bacillus*, *Paenibacillus*, *Haliangium*, *Streptomyces*. Overall, these results, to a certain extent, approved that the consecutive applications of BIO at nursery and transplanting stage could effectively suppress watermelon Fusarium wilt by regulating the rhizosphere bacterial diversity. These results could give some clues that how to regulate the soil microbial community to an appropriate level which can keep the plant healthy and thus control the soil-borne diseases.

1. Introduction

Farmers growing plants in a continuous mono-cropping system often encounter problems such as reduced production and quality (Zhang et al. 2013). This problem, described as the continuous cropping obstacle in this paper, includes plant stunting and leaf yellowing in an uneven pattern across the field. While the problem occurs worldwide at varying severity, but there are locations and regions, where it does not occur.

Continuous mono-cropping on watermelon production is very prevalent in China, and results in serious continuous cropping obstacle of the crop. In the continuous mono-cropping system, watermelon is generally suffered by the *Fusarium* wilt. This disease is caused by *F. oxysporum* f. sp. *niveum* (FON) (Joffe 1986), which is considered to be the most important soil-borne facultative pathogen, causing economically important losses of watermelon crop and limiting watermelon production in many areas of the world (An et al. 2011). Currently, biological control represents an alternative for the protection of plants against *Fusarium* wilts. In our lab, we have developed a novel bioorganic fertilizer (BIO) by fermenting mature compost with the antagonistic microbe, *Paenibacillus polymyxa* SQR-21 (SQR-21), which showed very good effect on biocontrol of watermelon *Fusarium* wilt disease (Wu et al. 2008; Ling et al. 2010). To improve the biocontrol efficacy of BIO, we also

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developed a nursery application way (Ling et al. 2010). In brief, the BIO was applied at plant seedlings grown in nursery pots with healthy soil (i.e. never planted with watermelon before) until the seedlings had 3–4 true leaves. The seedlings were then transplanted by transferring all of the nursery soil to infested fields. We proposed that this mode is more consistent with the ecological requirements of bio-products, because the application way can make the beneficial microbes effectively colonized in the rhizosphere before the roots is challenged by the pathogens. This proposal was demonstrated by a rhizobox experiment in our previous work (Ling et al. 2012). But it still needs further studies for evaluation especially in continuous mono-culture system.

P. polymyxa has been identified as a potential biological control agent (Beatty and Jensen 2002; Haggag and Timmusk 2008). *P. polymyxa* SQR-21 was isolated by Provincial Key Lab of Organic Solid Waste Utilization, Jiangsu, China, and its characteristics and some action modes against *Fusarium* are already published (Raza et al. 2010, 2011; Raza and Shen 2010; Ling et al. 2011a, 2011b). However, the BIO, as a bio-product which was combined organic fertilizer with *P. polymyxa* SQR-21, to affect the soil ecology especially the soil microbial community in watermelon mono-culture system should be further investigated.

As we all known, soil microbial community is thought to be responsible for biological processes that is necessary for maintaining a healthy soil and suppressing plant diseases (Garbeva et al. 2004; Raaijmakers et al. 2009). It has been shown that a decrease in soil microbial diversity was responsible for the development of soil-borne plant diseases (Mazzola 2004). An et al. (2011) even addressed that the differences in the rhizosphere microbial community may contribute to the differences in resistance to *F. oxysporum* f. sp. *niveum*. Some decades ago organic amendments were proposed to control soil-borne diseases by increasing the soil suppressiveness (Lumsden et al. 1983). The mechanisms involved, however, are not fully understood yet and subject of ongoing studies. Therefore, considerable interest was paid on monitoring the changes in activity and composition of soil microbial communities after the application of the novel organic fertilizer, BIO, employing our developed application way in the continuous mono-culture system.

In present study, we conducted a pot experiment by monocropping watermelon for 4-year, and employed some techniques (real-time PCR, PCR-DGGE, 454 pyrosequencing) to monitoring variation of rhizosphere bacterial community in watermelon continuous cropping soils after BIO application. The objectives of this study were to evaluate whether the developed application way accord with its ecological requirements, to detect the dynamic change of *P. polymyxa* and FON number and determine the impact of the BIO on diversity of soil bacterial communities, in the continuous mono-culture system.

2. Materials and methods

2.1. Bioorganic fertilizer preparation

The antagonistic microbe *P. polymyxa* SQR21 was used to prepare bioorganic fertilizer (BIO). The SQR21 was isolated and identified by our laboratory and found to be highly efficient against *Fusarium oxysporum* causing cucumber and watermelon wilt diseases (Zhang et al. 2008; Ling et al. 2010). The antagonist was grown in beef extract and peptone liquid culture on a shaker at 170 rpm at 30 °C for 2–3 days. This culture was then used directly to prepare the BIO product as described below.

Organic fertilizer (OF), used for the BIO product, was composed of amino acid fertilizer and pig manure compost (1:1, w/w). Amino acid fertilizer was made from oil rapeseed cakes that were enzymatically hydrolyzed by aerobic microbial fermentation at <50 °C for 7 days. This amino acid fertilizer was containing of 44.2% organic matter and 12.9% of amino acids, small molecular peptides and oligo peptides. The nutrient contents were 4.4% nitrogen (N), 2.3% P_2O_5, and 0.7% K_2O. Pig manure compost was made by Tianniang Ltd. in Suzhou by composting pig manure at a temperature range of 30–70 °C for 25 days. This compost was contained of 30.4% organic matter, 2.0% N, 3.7% P_2O_5, and 1.1% K_2O.

The novel organic fertilizer which was enriched with *P. polymyxa* SQR21 was named bio-organic fertilizer (BIO). The BIO product used in this experiment was obtained by aerobically fermenting OF with the SQR21 for 6 days at <45 °C. The density of SQR21 was 5×10^9 CFU g^{-1} dry weight (DW) BIO at the end of fermentation. The BIO product was stored at less than 25 °C prior to use in experiments.

2.2. Experimental designs

To ensure effective colonization of microbes in the plant rhizosphere, the BIO was applied to the nursery soil (Ling et al. 2010). In brief, watermelon seedlings were grown in the nursery pots until the seedlings had 3–4 true leaves, and then transplanted to pots with infected soil. Transplanting was conducted by transferring all of the nursery soil with the plant seedlings to new locations.

2.2.1. Soils

The nursery soil was collected from a paddy field without a history of watermelon cultivation, which contained 25.4 g/kg organic matter, 2.04 g/kg total N, 13.0 mg/kg extractable P, 110.2 mg/kg exchangeable K and the pH of the soil was 7.0. The soil used in pot experiments was collected from the surface of continuous mono-cultivated watermelon plots located in Jiangyin, Jinagsu province, China, with pH 7.6, 22.9 g/kg organic matter, 2.34 g/kg total N, 10.7 mg/kg extractable P, 97.2 mg/kg exchangeable K. This plot had a two-year history of continuous watermelon cultivation and watermelon plants grown in this soil were infected with *F. oxysporum* f. sp. *niveum*.

2.2.2. Seedling incubation in nursery pots

Watermelon seeds, the cultivar Zaojia 84-24,were surface-sterilized in 2% NaClO for 3 min, rinsed for three times in sterile water, and then germinated in 9 cm plates covered with sterile wet filter paper at 30 °C. The germinated seeds were grown in nursery pots with 300 g nursery soil, and one seedling was maintained in each nursery pot. Three treatments of the nursery soil were used: (i) C, no organic or bio-organic fertilizer (control), (ii) O, amended with the organic fertilizer as described above at a rate of 10 g/kg, and (iii) B, amended with the BIO as described above at a rate of 10 g/kg.

2.2.3. Pot experiment description

The seedlings with 3–4 true leaves were transplanted into pots (12 L in volume and 25 cm in height). Approximately 10-kg fresh soil from the diseased field was filled into each pot, and transplanting was conducted by transferring all of the nursery soil with the plant. In addition to the three treatments during the nursery stage, another three treatments were designed during the pot experiment: the soil in the pots was amended (i) with BIO at a rate of 5 g/kg (wet weight basis) or (ii) OF at a rate of 5 g/kg (wet weight basis) or (iii) without both of OF and BIO. All five treatments in this pot experiment are listed in Table 1. The treatment CC without application of OF or BIO both in nursery and pot was served as the control.

The same pot experiment was repeated for four growing seasons from 2009 to 2012 in a greenhouse. The pot soil used in the later season was same as the soil used in the former season with the same treatments. In each season, three blocks were randomly laid