Original article

Palmitic acid mediated change of rhizosphere and alleviation of Fusarium wilt disease in watermelon

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ABSTRACT

Palmitic acid (PA) in root exudates or decaying residues can reduce the incidence of soil-borne diseases and promote the growth of some crop plants. However, the effects of PA on soil-borne pathogens and microbial communities are poorly understood. Here, we investigate the effects of PA on overall watermelon microbial communities and the populations of *Fusarium oxysporum* f.sp. *niveum* (Fon). The effects of PA on the mycelial growth and spore production of Fon were tested *in vitro*, while its effects on Fon, total bacteria and total fungi populations, and microbial communities were evaluated in a pot experiment. The results revealed that all test concentrations of PA inhibited Fon mycelia growth and spore production. The pot experiment showed that 0.5 mM and 1 mM PA reduced Fon but increased total bacteria populations, and 0.5 mM and 1 mM PA 0.5 mM and 1 mM PA promoted the change to a soil type of bacteria soil. Meanwhile, 0.5 mM PA and 1 mM PA altered the community composition of the rhizosphere microorganisms and reduced the relative abundance of two bacterial operational taxonomic units (OTUs) and the two fungal OTUs that were significantly ($p < 0.01$) related with disease severity and increased that of four bacterial OTUs and the two fungal that were highly significantly ($p < 0.01$) negatively correlated with the disease severity. These results suggest that application of PA decreased the populations of Fon, changed the rhizosphere microbial composition, reduced the disease severity of Fusarium wilt, and promoted the growth of watermelon.

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1. Introduction

Watermelon is one of the most important horticultural crops with high economic value, and hence widely cultivated throughout the world. However, continuous monoculture has disrupted soil microbial balance, rapidly increasing the incidence of Fusarium wilt disease. Due to this disease, the yearly yield of watermelon crop is reduced by approximately 35.1–58.5 million tons world-wide (Tang et al., 2019). Fusarium wilt of watermelon is caused by the soil-borne fungal pathogen *Fusarium oxysporum* f.sp. *niveum* (Fon) which can rest in the soil for up to 20 years making it very difficult to control (Larkin et al., 1996; Xu et al., 2015). For the past 20 years, several measures have been taken to mitigate the occurrence of this disease, such as soil fumigation (Xue et al., 2019), chemical agents (Everts et al., 2014), biological control (Liu et al., 2019; Wu et al., 2019), and other agricultural methods (Li et al., 2019; Tang et al., 2019). But, an effective biological measure to curb this disease is still lacking, and hence more studies are required.

Fatty acids are essential molecules that play crucial roles in almost all organisms, and act as signaling agents in plant-plant, plant-microbe and plant-environment interactions (Tumlinson and Engelberth, 2008; Upchurch, 2008; Raffaele et al., 2009). Several studies have revealed that fatty acids and their derivatives directly inhibit the growth of plant pathogens within the rhizosphere, and improve the surrounding environment of plant rhizosphere to reduce the occurrence of crop diseases and promote

crop growth (Davis et al., 1997; Liu et al., 2008; Liu et al., 2012). Palmitic acid (PA) is a saturated long-chain fatty acid compound that is widely found in the seeds of beans, sunflowers and cotton (Lukonge et al., 2007; Perez-Vich et al., 2016; Zhao et al., 2019). Also, it is found in high levels in many plant root exudates and residues (Guo et al., 2010; Pan et al., 2013). Previous studies have shown that PA can inhibit the growth of plant soil-borne pathogens and promote the growth of seedlings (Davis et al., 1997; Abdel-Naime et al., 2019; Ding et al., 2019). However, the effects of PA on watermelon growth and its rhizosphere microbial community have rarely been explored.

Previous studies have reported that root exudates of different wheat varieties exhibit different inhibitory abilities on pathogens, and PA isolated by gas chromatography-mass spectrometry has the ability to inhibit the growth of Fon mycelium in vitro (Pan et al., 2013). Therefore, we hypothesize that application of PA at different concentration would alter soil microbial communities and reduce the Fon populations in watermelon rhizosphere. The changes in absolute abundances of Fon and changes in microbial communities in watermelon rhizosphere were studied using quantitative PCR and high-throughput sequencing. Our results provide new insights and a theoretical reference for treating and alleviating the soil-borne disease of watermelon wilting.

2. Materials and methods

2.1. Fungal strains

Fon was isolated from the infected roots of watermelon in Xiangfang farm of Northeast Agricultural University, Harbin, China (45° 72′ N, 126° 76′E) and identified as physiologically race No. 1 via 16S rRNA gene sequencing analysis and light microscopy observation. All the strains were preserved at 4 °C in refrigerator on potato dextrose agar (PDA) slants by regular transfer, which consists of potato 200 g, dextrose 20 g and agar 20 g per liter (pH = 7).

2.2. Soil sampling

Monoculture watermelon soil with sandy loam texture was collected in the spring from the top layer (0–15 cm) of a greenhouse at the Xiangfang farm of Northeast Agricultural University, Harbin, China (45° 72′ N, 126° 76′E). Watermelon has been planted in this greenhouse for five years and was infected by Fon. After removing the plant residues and large stones by using a <6 mm sieve, the soil was thoroughly mixed and transported to a greenhouse. The physicochemical properties of the soil were as follows: Organic matter, 34.65 g·kg^{-1}; Inorganic N, 82.09 g·kg^{-1}; Available P, 73.37 mg·kg^{-1}; Available K, 137.25 mg·kg^{-1}; EC, 0.23 mS cm^{-1} (1:2.5, w/v); pH, 6.89 (1:2.5, w/v), and were determined by the method described by Zhou et al. (2018).

2.3. In vitro evaluation of antifungal ability

To determine the effects of PA on Fon mycelial growth and spore production in vitro, 5 different concentrations of PA were used according the previous study by Pan et al. (2013). PA (0, 0.25, 0.5, 1, and 2 mmol) was added to 40 ml acetone, transferred to the PDA medium and potato dextrose broth (PDB) medium (potato 200 g and dextrose 20 g per liter), and the pH of the solution was adjusted to 7 using 0.1 M NaOH solution. Finally, the volume was fixed to one liter with distilled water and configured into PDA medium and potato dextrose broth (PDB) containing different concentrations of PA, respectively; And 0 mmol L^{-1} PA (including 4 ml acetone) was used as control. The plugs (5 mm in diameter) was taken from the Fon mycelium edge of growth after growing

for five days, inoculated separately in PDA (20 ml) plate and PDB (100 ml) with different concentrations of PA. All the plates with PDA medium were placed in an incubator at 28 °C for 5 days in dark conditions, and all the flasks with PDB medium were placed in shaking table at 28 °C, 150 rpm for 5 days in dark conditions. The mycelial diameter and spore production were measured using a ruler and a hemacytometer, respectively, and each treatment was repeated five times as described previously (Bardia and Rai, 2007; Williamson and Richardson, 1988). The effective concentration (EC$_{50}$) values were calculated by estimating the regression of colony growth inhibition and spore production inhibition probability against the logarithmic value of PA concentration (Liu et al., 2019).

2.4. Greenhouse pot experiment

The experiment was carried out in the greenhouse of the Horticultural experiment Center of Northeast Agricultural University. Watermelon seeds (Cv. Cuiyu) were soaked in 2.5% sodium hypochlorite solution for 20 min to perform disinfection and germinated at 28 °C in the hole trays (one seed per hole) containing vermiculite. When the seedlings sprouted, they were kept in a greenhouse (30 °C day/20 °C night, relative humidity of 60% and 16 h light/8 h dark).

PA was added to distilled water and the pH was adjusted to 7 using 0.1 M NaOH solution, which was chosen to not change the existing soil pH (Fierer and Jackson, 2006). The monoculture soil of watermelon was homogenously poured with PA solution at concentrations of 0 mM, 0.25 mM, 0.5 mM, 1 mM, 2 and 4 mM. The watermelon seedlings during the four-leaf stage were transplanted into the pots (24 cm × 18 cm) containing 3 kg of soil, with each pot containing a single seedling. Watermelon was harvested after 60 days of planting. Watermelons were subjected to typical management and water content of the soil was regulated with distilled water every 2 days (about 60% of its moisture holding capacity). In total, 360 pots were used for this experiment (6 treatments × 20 pots × 3 replicates). Harvesting was done after 60 days of planting and samples were collected.

2.5. Plant weight, yield and disease severity

To determine the Plant fresh weight and dry weight of the whole watermelon plant, 5 watermelon plants from each replication were selected and carefully removed from the soil on days 60 after transplanting. These plants were weighed after cleaning and drying for 72 h at 70 °C to investigate the plant fresh weight and dry weight. The ripened fruit was harvested to investigate the fruit yield. Twenty plants from each replicate were used to calculate the disease severity of Fusarium wilt by using the method prescribed by Chu et al. (2014). The scores were provided such that 0 represents no obvious yellowing symptoms, all leaves green; 1 represents leaf yellowing area of 1–25%; 2 represents leaf yellowing area of 26–50% and slight wilting; 3 represents leaf yellowing area of 50–75%and wilting; And 4 represents yellowing area greater than 75% and severe wilting.

2.6. Soil sample collection

The rhizosphere soil samples of watermelon were collected from 5 plants from each replication and uniformly mixed to form a composite soil sample for each replicate on days 20, 40 and 60 after transplanting as described previously by Zhou and Wu (2012).

2.7. DNA extraction and quantitative PCR amplification

To assess the effectiveness of PA, the populations of soil microorganisms including total bacteria, total fungi and Fon were determined. A 0.250 mg watermelon rhizosphere soil was used for extraction of soil DNA using the PowerSoil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, USA) following the manufacturer's protocol. DNA was extracted thrice from each soil sample replicate and the product was combined to make a composite DNA sample. Quantitative PCR was used in an IQ5Real-time PCR system (Bio-Rad Lab, LA, United States) to detect the population of bacteria, fungi and Fon in the rhizosphere soil of watermelon. The specific primer pair sets used were as follows: bacteria (338-F: 5′-ACTCCTACGGGAGGCAGCAG-3′/518-R: 5′-ATTACCGCGGCTGC TGG-3′), fungi (ITS1-F: 5′-CTTGGTCATTTAGAGGAAGTAA-3′/ITS4-R : 5′-TCCTCCGCTTATTGATATGC-3′), and Fon (Fon-1: 5′-CGATTAGC GAAGACATTCACAAGACT-3′/Fon-2:5′-ACGGTCAAGAAGATGCAGGG TAAAGGT-3′) (Lin et al., 2010). The quantitative PCR reaction system (20 ul) was as follows: For bacteria/fungi, template DNA (2 μl), primers (1 μl), 2 × Taq PCR MasterMix (10 μl; Tiangen Biotech, Beijing, China) and sterile water (6 μl); For Fon, template DNA (2 μl), primers (0.5 μl), 2 × Taq PCR MasterMix (10 μl) and sterile water(7 μl). The quantitative PCR reaction conditions were as follows: for bacteria/fungal, (1) 94 °C for 5 min (2) 26 cycles of 94 °C for 60 s, 65 °C for 90 s and 72 °C for 90 s (3) 72 °C for 10 min; For Fon, (1) 94 °C for 5 min (2) 35 cycles of 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s (3) 72 °C for 3 min. A target gene from bacteria, fungi and Fon genomic DNA were used to create the plasmid standard for their quantification. The bacteria, fungi and Fon DNA copy concentration was calculated based on the method previously described by Jin et al. (2019). PCR assays were conducted in triplicates.

2.8. Illumina Miseq sequencing and raw data processing

To further determine the effects of PA on soil microbial community composition, DNA from all composite soil samples at 40 days extracted by Illumina Miseq were sequenced. The V4-V5 regions of the bacterial 16SrDNA gene and the ITS1 regions of the fungal rRNA gene were amplified using the primer sets of 515F/907R and ITS1F/ITS2R, respectively (Fazzini et al., 2011; Derakhshani et al., 2016). Both the forward and reverse primers also had a 6-bp unique barcode to each sample. The amplification products of the three PCR reactions were combined and purified using the Agarose Gel DNA purification kit (TaKaRa), and were then mixed accordingly to achieve the same molar concentration of the final mixture.

The mixture was subjected to sequencing in an Illumina MiSeq instrument (USA) at Major Bio-Pharm Technology Co., Ltd., Shanghai, China. The raw sequence reads were de-multiplexed, quality-filtered, and processed using FLASH as described previously (Magoc and Salzberg, 2011; Zhou et al., 2017). Briefly, the chimeric sequences were processed in QIIME (Caporaso et al. 2010). The sequences were clustered into operational taxonomic units (OTUs) at 97% sequence similarity with UPARSE against an agglomerative

clustering algorithm (Edgar, 2013). Taxonomic assignments to OTUs were carried out using BLAST against the SILVA (Quast et al., 2013) (bacterial) and Unite (Koljalg et al., 2013) (fungal) databases. All sequence data have been archived in the NCBI Sequence Read Archive (SRA) with the Accession Number SUB7295634. A total of 24219 16SrDNA gene sequences and 31594 ITS gene sequences were randomly selected for further analysis.

2.9. Statistical analysis

SPSS 13.0 software (SPSS, Inc., Chicago, IL) was used for data analysis. One-way ANOVA and Duncan's multiple range tests were used for detecting statistical significance. Correlation analysis was carried out using bivariate correlation and Pearson correlation coefficients. $P < 0.05$ and $P < 0.01$ was considered to be statistically significant.

3. Results

3.1. In vitro antifungal ability evaluation

The results of the antifungal ability evaluation revealed that all concentrations of PA had significantly inhibitory effects on mycelial growth and the spore production ability of Fon when compared with control, and the inhibitory effects increased with increasing concentrations of PA after incubating for 5 days (Table 1 and Table 2), ($P < 0.05$). A concentration of 2 mM PA showed the strongest antifungal ability, and the inhibition of mycelial growth and spore production reached 37.2% and 71.7% as compared to control, respectively.

3.2. Plant weight and yield

Watermelon fresh weight, dry weight and fruit yield with 1 mM PA treatment were highest among rest of the treatments (Table 3). when compared with control, 1 mM PA treatment showed a significant increase in the seedling fresh weight, dry weight and fruit yield by 43.8%, 46.0% and 20.2%, respectively (Table 3), ($P < 0.05$).

3.3. Watermelon Fusarium wilt disease severity

The watermelon began to develop symptoms of Fusarium wilt gradually after 30 days of transplantation, while no disease severity was observed in watermelons treated with 1 mM PA after 30 days. During the harvest period, the watermelon treated with 1 mM PA demonstrated the lowest disease severity, which decreased by 71.8%, when compared with the control treatment (Fig. 1), ($P < 0.05$).

Table 1
Effects of PA on Fon mycelium growth after incubating for 5 days.

Treatment	Mycelial growth inhibition			
	Colony diameter/mm	Toxicity regression equation	R^2	EC_{50}/mM L^{-1}
0 mM PA(Control)	74.9 ± 1.27a	y = 1.31x-0.68	0.968	3.357
0.25 mM PA	69.6 ± 0.55b			
0.5 mM PA	64.7 ± 1.30c			
1 mM PA	54.7 ± 2.21d			
2 mM PA	47.0 ± 2.10e			

Letters indicate values (means ± SE, n = 3) with significant differences within the same column (P < 0.05, Duncan's multiple range test).

Table 2
Effects of PA on Fon Spore production.

Treatment	Spore production inhibition			
	Number of spore (10^6 cfu·ml^{-1})	Toxicity regression equation	R^2	EC$_{50}$/mM L^{-1}
0 mM PA (Control)	1.84 ± 0.15a	Y = 0.64x-0.34	0.864	0.296
0.25 mM PA	0.92 ± 0.08b			
0.5 mM PA	0.85 ± 0.04b			
1 mM PA	0.71 ± 0.04c			
2 mM PA	0.52 ± 0.06d			

Letters indicate values (means ± SE, n = 3) with significant differences within the same column (P < 0.05, Duncan's multiple range test).

Table 3
Effects of PA on plant weight and yield of watermelon.

Treatment	Whole plant fresh weight/g	Whole plant dry weight/g	Fruit yield/g plant^{-1}
0 mM PA(Control)	116.54 ± 7.53c	15.78 ± 2.19b	406.4 ± 22.2cd
0.25 mM PA	135.66 ± 11.86b	16.78 ± 1.46b	437.3 ± 18.2bc
0.5 mM PA	142.76 ± 11.11b	18.22 ± 1.22b	454.6 ± 30.5bc
1 mM PA	167.57 ± 11.70a	21.46 ± 0.98a	488.3 ± 26.1a
2 mM PA	140.95 ± 7.59b	16.96 ± 1.54b	411.2 ± 16.4d
4 mM PA	104.16 ± 6.22c	10.85 ± 1.22c	364.2 ± 34.8e

Letters indicate values (means ± SE, n = 3) with significant differences within the same column (P < 0.05, Duncan's multiple range test.
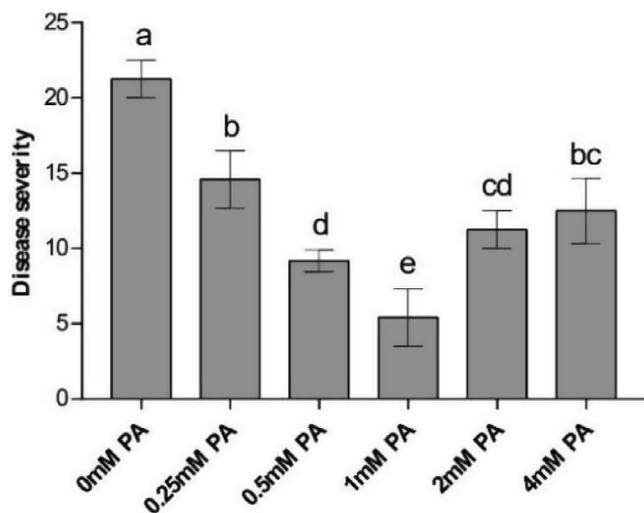

Fig. 1. Effects of PA on the disease severity in watermelon. Letters indicate values (means ± SE, n = 3) with significant differences within the same group (*P* < 0.05, Duncan's multiple range test).

3.4. Absolute abundances of total bacteria, total fungi and Fon

The total bacteria in the soil initially showed an increasing trend and then a decreasing trend in each period with increasing concentrations of PA (Fig. 2a). Among different concentrations of PA, the total bacteria after treatment with 1 mM PA concentration was the highest and significantly increased in each period (Fig. 2a), (*P* < 0.05). Compare with control, the total bacteria increased by 33.3%, 70% and 66.7% on days 20, 40 and 60 after planting, respectively (Fig. 2a). As for the total fungi in soil, the addition of 2 mM PA and 4 mM PA concentrations significantly reduced the total fungal population when compared with the control, and no significant difference was found from the remaining treatments (Fig. 2b), (*P* < 0.05). With respect to the Fon population present in the soil, all treatments, except the one with 0.25 mM PA concentration, significantly decreased the population and the inhibitory ability

increased with increasing concentrations of PA during each period (Fig. 2c), (*P* < 0.05). The reduction of Fon population by treatment with 4 mM PA concentration remained the lowest during all stages, which decreased by 59.4%, 50.6% and 42.6%, respectively, when compared with control treatment. The bacterial to fungal ratio of the group treated with 1 mM PA concentration on day 20 after planting treatment and the ones treated with 1 mM PA, 2 mM PA and 4 mM PA concentrations on days 40 and 60 after planting were significantly higher when compared to control (Fig. 2a), (*P* < 0.05).

3.5. Relative abundance of most influential bacteria and fungi

To further clarify the effect of PA on the composition of microbial community in the rhizosphere of watermelon, the relative abundance of bacterial and fungal OTUs of ≥0.1% in the rhizosphere of watermelon and significantly correlation with watermelon Fusarium wilt disease severity were analyzed (Table S1 and S2), (*P* < 0.05). At OTU level, there were 3 bacterial OTUs (i.e., OTU1708, OTU134 and OTU1677) with significant positive correlation with watermelon Fusarium wilt disease severity, which were all unclassified members at the genus level (S1), (*P* < 0.01). The relative abundance of the bacteria OTU1708 and OTU1677 within 0.5 mM PA and 1.0 mM PA treatments, and OTU134 within 1 mM PA treatment were significantly decreased when compared to control treatment (Fig. 3a), (*P* < 0.05). The relative abundance of the 3 bacterial OTUs treated with 1.0 mM PA decreased by 32.6%, 50.0% and 55.5%, respectively, as compared to control treatment. However, there were four bacterial OTUs, OTU2982, OTU2779, OTU3125 and OTU974, that showed a significantly negative correlation with watermelon Fusarium wilt disease severity, were belonging to *Hahella*, *Pseudomonas*, *Bacillus* and *Lysobacter* genera of bacterial genera, respectively (S1), (*P* < 0.01). The relative abundance of these four bacteria were all significantly increased within 0.5 mM PA and 1.0 mM PA treatments and the relative abundance treated within 1.0 mM PA increased by 30 times, 13.35 times, 0.62 and 0.71 times, respectively, when compared with control (Fig. 3a), (*P* < 0.05).

Meanwhile, there were five fungal OTUs, OTU395, OTU97, OTU90, OTU768 and OTU69, that showed a significantly positive correlation with watermelon Fusarium wilt disease severity, and these belonged to *Guehomyces*, *Mortierella*, *Guehomyces*, *Mortierella and unclassified* of fungal genera, respectively (S2) (*P* < 0.01). The relative abundance of the fungi OTU395 and OTU97 within 0.5 mM PA and 1.0 mM PA, and OTU90, OTU768 and OTU69 within 1.0 mM PA were significantly decreased when compared to control treatment, and their abundance within 1.0 mM PA were decreased by 45.5%, 74.8%, 78.8%, 60.6% and 64.4% (Fig. 3b), (*P* < 0.05). However, there were also two fungal OTUs, OTU825 and OTU535, that showed a significantly negative correlation with Fusarium watermelon wilt disease severity, were belonging to *Mortierella* and *Humicola* spp., respectively (S2), (*P* < 0.01). The relative abundance of the two fungal OTUs were all significantly increased within 0.5 mM PA and 1.0 mM PA treatments and the relative abundance
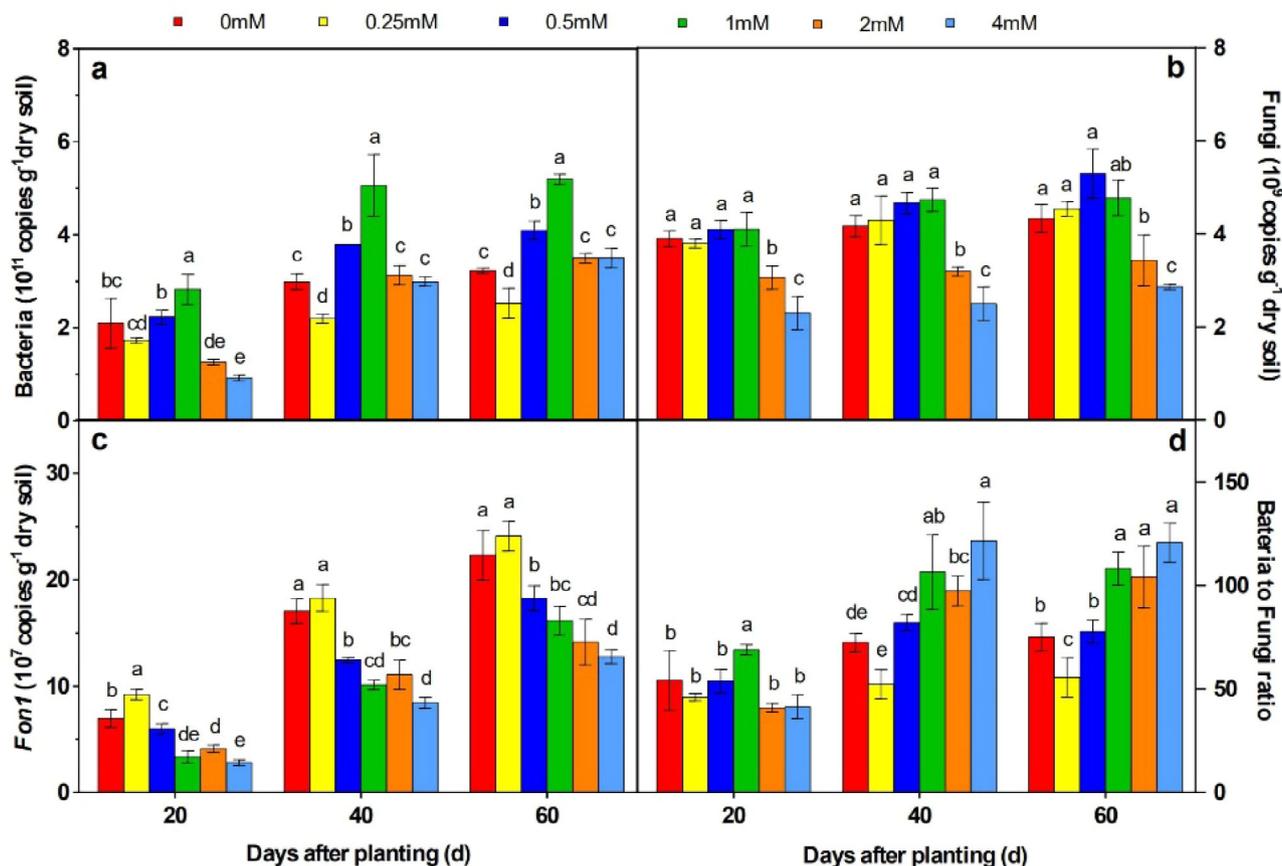
Fig. 2. Effects of PA on soil bacteria (a), fungi (b), Fon (c) populations and the bacteria to fungi ratio (d) in watermelon, and these results were detected using quantitative PCR. Letters indicate values (means ± SE, n = 3) with significant differences within the same group (P < 0.05, Duncan's multiple range test).

treated within 1.0 mM PA increased by 787.4 times and 2.2 times, respectively, when compared with control (Fig. 3b), (P < 0.05).

4. Discussion

Many studies have reported the excellent antifungal potential of PA (Liu et al., 2008). Previous study showed that PA is the main component of wheat root exudates, which inhibited the growth of Fon mycelium in vitro, and the current study further confirmed the findings (Pan et al., 2013). Also, the spore production capacity of Fon was strongly inhibited by all PA treatments. Previous studies have shown that fatty acids can be transfer to the fungal membrane, which exacerbates the disorder of the plasma membrane, and further alters the protein conformation and increases the membrane permeability, eventually disintegrating the cytoplasm (Bergsson et al., 2001). Moreover, some studies have shown that acetyl fatty acids interfere with the biosynthesis of Fon sphingolipids and affect the normal synthetic metabolism of the fungi (Li et al., 2003). These evidences may be vital reasons of antifungal mechanism of PA, we need to further verify this mechanism.

As an exogenous carbon source material, PA inevitably caused changes in soil microbial composition. In this experiment, the low concentrations of PA (0.25–1 mM) increased the levels of watermelon rhizosphere bacteria when compared with the control, which was similar to the results of the study conducted by Zhang et al. (2017). Notably, the relatively high concentrations of PA (2–4 mM) decreased the total bacteria and fungi in the soil, and this might be due to the formation of osmotic pressure by high concentrations of PA that either destroyed or disintegrated some micro-

bial plasma membranes and tissues (Avis and Belanger, 2001). Controlling the growth of pathogens might be an important measure to alleviate soil-borne diseases (Zhao et al., 2017). The results of our study showed that all treatments except at the concentration of 0.25 mM PA reduced the Fon population of soil, indicating the inhibitory ability of PA on Fon and in potentially managing the disease. More interestingly, the population of Fon gradually increased with the growth of watermelon at all treatment concentrations. This might be due to the fact that some compounds, such as cinnamic acid, ferulic acid and p-hydroxybenzoic acid released by the roots of watermelon during continuous monocropping, which in turn promoted the growth of Fon (Lv et al., 2018). PA was shown to reduce the population of Fon during the harvest stage when compared to the control, indicating the positive effect of PA. The ratio of bacteria to fungi is also considered as important indicators for assessing soil health (Janvier et al., 2007). Compared with the control, the concentration of 1 mM PA increased the bacteria to fungi ratio in the rhizosphere of watermelon and improved the health of the soil. Zhou et al. (2010) have also shown that exogenous PA could increase the bacteria to fungi ratio of the rhizosphere, improve soil microbial composition and promote the growth of eggplant.

Soil microbial community composition directly or indirectly affects the growth and disease occurrence in plants (Mendes et al., 2011). As a result, many researchers have sought to optimize soil health and facilitate plant growth by changing the composition of soil microbial communities, such as by using soil fumigation combined with organic fertilizers and the use of intercropping instead of continuous monoculture (Ren et al., 2016; Xue et al., 2019). In this study, the results of Miseq sequencing indicated that
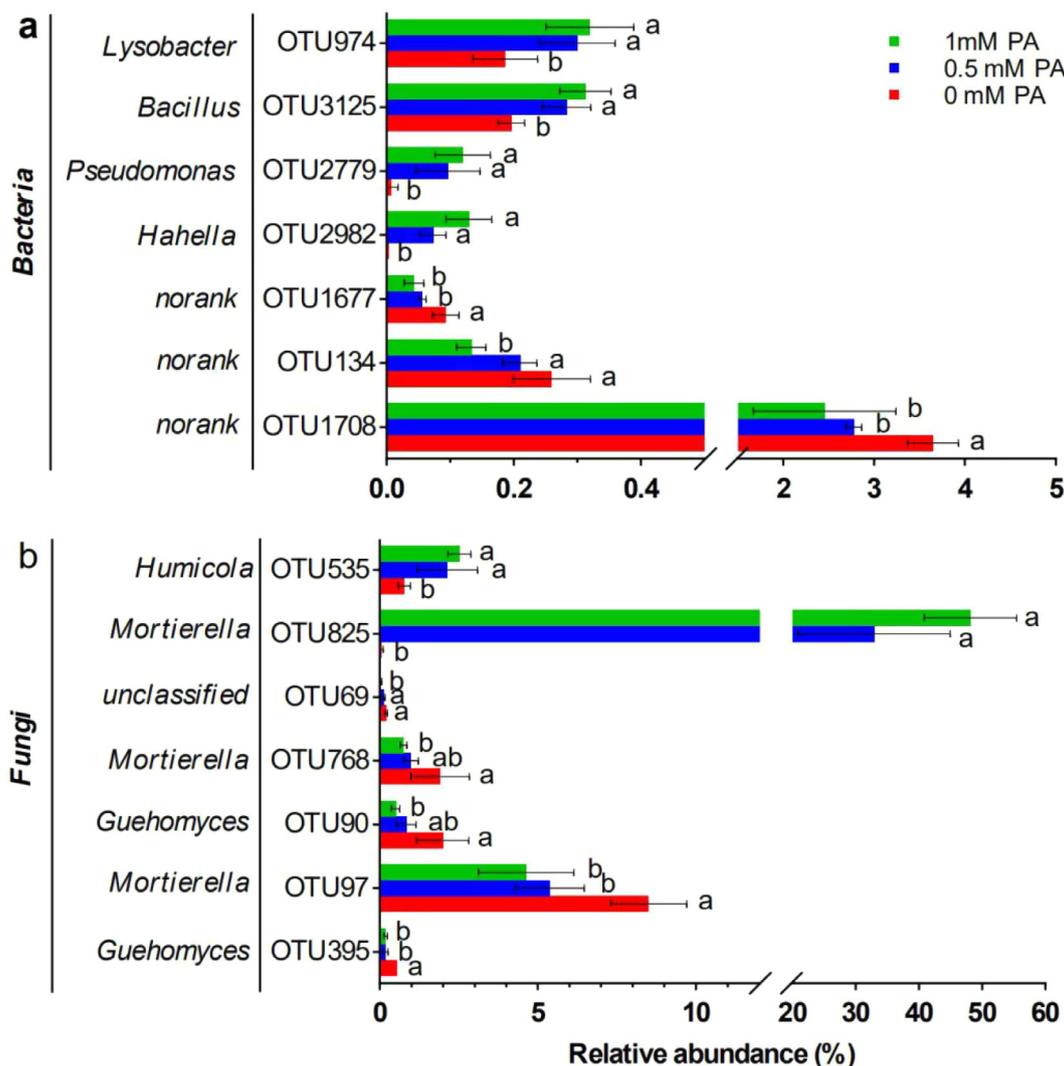
Fig. 3. Comparison of relative abundance of most influential bacteria and fungi. The bacteria in (a) showed significant positive or negative correlation with the disease severity, the fungi in (b) showed significant positive or negative correlation with the disease severity ($P < 0.01$). Letters indicate values (means ± SE, n = 3) with significant differences within the same OTU ($P < 0.05$, Duncan's multiple range test).

PA improved microbial community composition in the rhizosphere of watermelon. The relative abundance of the OTU1708 and OTU1677 in the rhizosphere soil of 0.5 mM PA and 1 mM PA were decreased when compared to control and they have positive correlation with the disease severity, which indicates the two bacteria might be harmful bacteria to the health of watermelon. On the contrary, the relative abundance of the OTU2982, OTU2779, OTU3125 and OTU974 in the rhizosphere soil of 0.5 mM PA and 1 mM PA, belonging to the genus *Hahella*, *Pseudomonas*, *Bacillus* and *Lysobacter*, respectively, were increased and showed a negative correlation with the disease severity. *Hahella* are more commonly found in petroleum sediments and can degrade hydrocarbons (Abed et al., 2014), and some studies reported that some species of *Hahella* can produce antifungal compounds, such as prodiginines (Kim et al., 2007). Besides, *Bacillus* and *Pseudomonas* are the two most common biocontrol bacteria that can inhibit Fon, and manage Fusarium wilt and promote plant growth (Zhang et al., 2020). *Lysobacter* play a key role in controlling soil-borne pathogens and there was a higher relative in the rhizosphere of vanilla and apple with amending of bio-fertilizers (Wang et al., 2016; Xiong et al., 2017).

For fungi, the relative abundance of OTU395 and OTU97 in the rhizosphere soil of 0.5 mM PA and 1 mM PA was decreased when

compared with control and showed positive correlation with the disease severity. OTU395 were assigned to *Guehomyces*, which can produce ligninase and have strong ability to degrade some carbon sources that are difficult to be degraded, which indicates that exogenous fatty acids carbon source, such as PA, can reduce microorganisms that feed on carbon source (Zhang et al., 2020). Interestingly, both *Guehomyces* and *Fusarium* were increased in the fields of strawberry for many years, which might exist as a harmful fungal genus in the rhizosphere of watermelon (Li and Liu, 2019). Most notably, the relative abundance of OTU825 and OTU535 in the rhizosphere soil of 0.5 mM PA and 1 mM PA were dramatically increased, which belonged to the genus *Mortierella* and *Humicola*, and demonstrated a negative correlation with the disease severity. Previous studies have shown that *Mortierella* and *Humicola* are usually recognized as beneficial fungi, *Mortierella* was negatively correlated with *Fusarium* in watermelon crop system and chili pepper-banana rotation system, and *Humicola* is also found in soils with high organic matter content (Hong et al., 2020; Lang et al., 2012; Meng et al., 2019; Wang et al., 2020). These evidences indicate that PA might directly or indirectly improve the rhizosphere environment of watermelon, reduce the population of Fon, suppress the Fusarium disease and promoting plant growth by enhancing the relative abundance of beneficial fungi. Moreover,

Mortierella has also been shown to use fatty acid carbon sources for metabolism to produce long-chain unsaturated fatty acids, such as linoleic acid and arachidonic acid, enhancing the systemic resistance of plants (Dye et al., 2020; Hao et al., 2014; Sumayo et al., 2014). However, a decrease of OTU97 from *Mortierella* was also observed in the rhizosphere soil of 0.5 mM PA and 1 mM PA, but the decrease was totally covered by OTU825. Therefore, we speculated that OTU825 was more sensitive to fatty acids to the carbon source response, and inter-genera competition inhibits OTU97 growth. Overall, these evidences suggested that PA can aggregate and increase bacteria or fungi that are capable of metabolizing fatty hydrocarbons. Not only do they play an important role in the metabolism of fatty acids, but most of them are regarded as beneficial bacteria or fungi which improved the rhizosphere of watermelon, reduced the disease severity of Fusarium wilt, and promoted the growth of watermelon. Nevertheless, the sustainable effects of PA to microbial community still warrants further investigation and we also need to pay more attention to whether other fatty acids have the similar effects.

5. Conclusion

Our results showed that PA, a kind of aliphatic compound that is seen in the root exudates of wheat, altered the amount of soil microorganisms, increased the amount of bacteria in the rhizosphere and reduced the populations of Fon. It also changed the rhizosphere microbial community composition, and increased the relative abundance of beneficial rhizosphere microorganisms. All these changes might promote watermelon growth and alleviate Fusarium wilt disease, but should be further verified by conducting studies in future. In summary, our results provided a novel treatment approach with PA, which promotes watermelon growth and alleviates wilt disease by inducing positive plant-microbial interactions.

Ethics approval

This paper has not been submitted elsewhere for publication. This research did not involve any human participants and/or animals.

Consent to participate

The authors declare their consent to participate.

Consent for publication

The authors declare their consent for publication.

Availability of data and material (data transparency)

The datasets generated and/or analyzed during the current study will be made available from the corresponding author on reasonable request.

Code availability

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

KXM, FZW, WHL and KP came up with the ideas and designed methodology; KXM, WTD and XYL collected the data; KXM, JMK analyzed the data. KXM, JMK and MKR did the writing of the manuscript. All authors contributed crucially to the drafts and gave final approval for publication.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.03.040.

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