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The Effects of *Trichoderma viride* T23 on Rhizosphere Soil Microbial Communities and the Metabolomics of Muskmelon under Continuous Cropping

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Abstract: The continuous cropping can restrict the large scale and intensive cultivation of muskmelon, and the use of *Trichoderma* preparation to alleviate the negative effects is an effective mean. Although the impact on rhizosphere soil microbial communities and metabolites after applying *Trichoderma* are still unclear. In this study, we applied the fermentation broth of *Trichoderma viride* T23 to muskmelon under continuous cropping, collected rhizosphere soil samples at 60 days after transplantation, and investigated the changes in the microbial communities and metabolites of muskmelon by using high-throughput sequencing and metabolomic analysis, respectively. The results showed that *T. viride* T23 could effectively reduce the disease index of muskmelon wilt (65.86 to 18) and significantly increase the soil pH value (6.06 to 6.40). *Trichoderma viride* T23 induced drastic shifts in the richness, structure, and composition of rhizosphere microbial communities, and Proteobacteria, Bacteroidetes, and Actinobacteria were the dominant bacterial phyla. Bioactive substances such as scopoletin, erythronic acid, and palmitic acid were significantly upregulated in the rhizosphere soil, which enhanced soil activity. Overall, *T. viride* T23 resolves the continuous cropping limitation in muskmelon by improving soil physicochemical properties, elevating the biomass and diversity of soil microbial communities, and stimulating the production of soil active substances.



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Keywords: *Trichoderma viride*; muskmelon; continuous cropping obstacle; microbial community structure; metabolites

1. Introduction

Muskmelon (*Cucumis melon* L.), as an important vegetable crop from *Cucurbitaceae*, is widely cultivated worldwide [1]. The fruit of muskmelon is rich in protein, carbohydrates, carotene, vitamin B, niacin, calcium, phosphorus, iron, and other nutritional elements that are beneficial for people in the summer [2]. As the demand for muskmelon continues to grow, its planting area and its yield in China are also increasing. In 2020, the planting area of muskmelon in China was 387,826 hectares, with an annual yield of 13,865,368 tons (www.fao.org, accessed on 1 March 2023). At the same time, the obstacle of continuous cropping in the process of melon industrialization and intensive planting (leading to a decline in the quality and yield of muskmelon) is becoming increasingly serious [3]. Compared with the soil without muskmelon planting, the microbial diversity in the soil has changed; the diversity of bacteria and actinomycetes has decreased and the number of fungi has increased significantly, while *Fusarium oxysporum* and ammonifying bacteria have become the dominant taxa in the soil [4]. Due to the long-term continuous cropping of muskmelon, several changes have occurred in the soil, including an increased availability of N in the soil, a decreased availability of P and K, nutrient imbalances in the soil, a substantial reduction in organic matter, soil acidification and salinization, and a remarkable degradation of the physical and chemical properties of the soil [5]. In addition, the process causes the accumulation of autotoxins in the soil, changes the soil microenvironment,

affects physiological processes such as the enzyme activity and photosynthesis of crops, and inhibits the root activity of the crops [6]. The phenolic acid autotoxic substances in the root exudates of muskmelon can inhibit the growth of muskmelon and can also promote the growth of *Fusarium oxysporum*, leading to the occurrence of muskmelon wilt [7].

Trichoderma, as a common biological control agent, can effectively alleviate the continuous cropping obstacle in muskmelon production [8,9]. The application of Trichoderma can improve the activity of beneficial microbes in the soil and can inhibit the *Stagonosporopsis cucurbitacearum* gummy stem blight disease pathogen [8]. The study shows that the application of *Trichoderma harzianum* and *Trichoderma viride* in the field can degrade autotoxic substances, effectively reduce the occurrence of muskmelon wilt, and then prevent the negative effects accompanied by muskmelon continuous cropping [10].

Trichoderma viride T23 is an effective biocontrol agent that alleviates the continuous cropping obstacle in muskmelon; however, its impact on soil microbial communities and soil metabolites is yet to be elucidated. This experiment was conducted in a greenhouse inside the experimental base of Shenyang Agricultural University (N 41°49′51.66″, E 123°34′15.67″, continuously planted with muskmelon for 9 years). *Trichoderma viride* T23 fermentation broth was applied after muskmelon transplantation and the muskmelon rhizosphere soil in the continuous cropping field was sampled and analyzed after the entire growing season ended. The objective of this study was to determine the changes in microbial communities and metabolites in the rhizosphere soil of muskmelon after a *Trichoderma viride* T23 application. We performed high-throughput sequencing based on 16S rDNA and ITS genes to identify the bacterial and fungal community composition and metabolomic profiling to explore the metabolites in the rhizosphere of muskmelon. Then, we used statistical differences and correlation analyses to clarify the role of *Trichoderma viride* T23 in alleviating the negative effects of continuous cropping on muskmelon.

2. Materials and Methods

2.1. Experiment Sites and Design

The tested muskmelon variety was a thin-skinned melon named “Super Sweet” (produced by Xin Shuo Seed Industry Co., Ltd., Changchun, China). The tested strain was *Trichoderma viride* T23, which was preserved by the Institute of Plant Immunology of Shenyang Agricultural University. In the greenhouse, there were 10 plots, to which 3 kg of organic fertilizer (N, P, and K $\geq$ 12 g/kg) was applied. Then, they were covered with mulch film to prevent water loss. Then, muskmelon seeds were disinfected with 75% ethanol for 1 min and then rinsed twice in sterile water and kept for sprouting at 30 °C for 24 h. Afterwards, the sprouted seeds were sown in the plates, with 1 grain per point and 72 points per plate. Thirty days after sowing, muskmelon seedlings with a consistent length were selected for transplantation, with twenty plants per plot. Five plots were randomly selected as the treatment group (numbered from T23-1 to T23-5); the other five plots were the control group (numbered from CK1 to CK5). At 0 d, 10 d, 30 d, and 50 d after transplantation, for each plant in the treatment group, *T. viride* T23 fermentation broth was applied, which was cultured in potato dextrose (PD) medium with 100 mL of 10^6 cfu/mL; an equal volume of water was applied to the plants in the control group. All the plants were grown in continuous cropping soil without artificial inoculation but with *Fusarium oxysporum*, which naturally causes wilt disease.

2.2. Muskmelon Wilt Investigation and Sample Collection

At 60 d after transplantation, the disease index of all the muskmelon plants and the relative control effects were calculated. Based on the wilt status of the whole plant, disease severity was classified into five grades [11,12]: 0 = no disease symptoms; 1 = the lower part of stem and leaves are wilted <20% and the upper plant grows normally; 3 = the stem and leaves are wilted up to 50%, with the upper plant affected slightly; 5 = the stem and leaves are mostly wilted more than 80% and only the top grows normally; 7 = the whole plant is

severely wilted or dead. The disease index and control effect on muskmelon wilt formulas are as follows:

$$\text{Disease index} = \frac{\sum(\text{Plant number in the grade} \times \text{Grade number})}{\text{Total plant number} \times \text{The highest grade number}} \times 100$$

$$\text{Control effect(\%)} = \frac{\text{Disease index in the control group} - \text{Disease index in the treatment group}}{\text{Disease index in the control group}} \times 100$$

After the investigation, a sampling point was randomly set within each plot and the rhizosphere soils of five muskmelons around the sampling point were collected and kept as one sample in a sealed bag after thorough mixing and labeled with the names of the corresponding plots. All the samples were decontaminated through a 40-mesh sieve and kept at -80°C for later use. In addition, five muskmelon plants collected at each sampling point were subjected to the disease investigation as described above.

2.3. DNA Extraction and High-Throughput Sequencing

DNA extraction from the soil samples was performed using a DNA extraction kit (dneasy powersoil kit), following the manufacturer's instructions. After the DNA was extracted, the concentration of DNA was examined by 1% agarose gel electrophoresis and nanodrop2000. The genomic DNA was used as a template for PCR amplification with barcoded primers and Tks Gflex DNA polymerase (Takara Biomedical Technology Co., Ltd., Beijing, China) [13]. For bacterial diversity analysis, the V3-V4 variable regions of 16S rDNA genes were amplified with universal primers 343 F (5'-TACGGRAGGCAGCAG-3') and 798 R (5'-AGGGTATCTAATCCT-3') [14]. For fungal diversity analysis, the ITS I variable regions were amplified with universal primers ITS1 F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS2 R (5'-GCTGCGTTCTTCATCGATGC-3') [15]. The amplification was performed with 30 μL of PCR mixture containing 15 μL of $2\times$ Gflex PCR buffer, 1 μL of each primer (5 pmol/ μL), 50 ng of template DNA, and 0.6 μL of Tks Gflex DNA polymerase (1.25 U/ μL). The PCR reaction program was as follows: pre-denaturation at 94°C for 5 min; 26 cycles (denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and amplification at 72°C for 20 s); final extension at 72°C for 10 min; hold at 4°C (PCR Instrument: Bio-rad 580BR10905, Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR products were purified and sequenced on Illumina NovaSeq 6000 from Shanghai Oebiotech Biomedical Technology Co., Ltd., Shanghai, China.

2.4. Sequence Processing and Data Analysis

After trimming, paired-end reads were assembled using the FLASH software [16]. Then, reads with chimera were detected and removed by using the QIIME software (version 1.8.0). The primer sequences were removed from the clean reads and clustering was performed to generate operational taxonomic units (OTUs) using the Vsearch software with a 97% similarity cut-off [17]. All the representative reads of the bacteria and fungi were annotated using the Silva 138 and UNITE database.

Statistical analysis was performed using the cloud platform of Shanghai Oebiotech Biomedical Technology Co., Ltd., Shanghai, China (<https://cloud.oebiotech.cn/task/>, accessed on 1 March 2023). The alpha diversity was derived from the Chao1 and observed species index (richness) and the Shannon and Simpson index (richness and evenness) [18]. The beta diversity was plotted for principal coordinate analysis (PCoA), using the Bray–Curtis distance matrix [19].

2.5. Soil Physicochemical Properties' Measurements

The soil pH was measured by using a pH meter. The soil organic matter content (g/kg) was determined by the potassium dichromate method. The total nitrogen content (g/kg) was determined by the Kjeldahl nitrogen method. The available phosphorus content (mg/kg) was determined by the colorimetric method. The content of available potassium

(mg/kg) and slowly available potassium (mg/kg) was determined by the flame photometry technique [20,21].

2.6. Soil Metabolomics Analysis

The Agilent 7890 gas chromatograph coupled with a 5977A mass spectrometer with a DB-5MS column (30 m length $\times$ 0.25 mm i. d. $\times$ 0.25 μ m film thickness, Agilent J&W Scientific, Folsom, CA, USA) was employed for GC-MS/MS analysis of the soil samples. Helium (>99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min through the column. The temperature of the inlet was 260 °C and the injection volume was 1 μ L, with split mode and solvent delay for 5 min. The initial oven temperature was 60 °C for 0.5 min, ramped to 125 °C at a rate of 8 °C/min, to 210 °C at a rate of 4 °C/min, to 270 °C at a rate of 5 °C/min, to 305 °C at a rate of 10 °C/min, and finally held at 305 °C for 3 min. Mass data were acquired in full scan mode with a range of 50–500 m/z [22].

2.7. Statistical Analysis of Metabolome Data

Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to distinguish the overall differences in metabolic profiles between the groups, with their variable importance in projection (VIP) scores [23]; the threshold of the VIP was set to 1. Those with a p -value for the t -test < 0.05 and a VIP score $\geq$ 1 were considered differential metabolites between the two groups. Pearson correlation coefficients were measured between the microorganisms and the differential metabolite samples [23].

3. Results

3.1. Muskmelon Wilt Disease Index and the *T. viride* T23 Control Effect on Muskmelon Wilt

In the investigation of muskmelon wilt within all the plants from the 10 plots (Table 1), the average disease index of muskmelon wilt in the *T. viride* T23 treatment group was 18, with the lowest value being 5.71 and the highest value being 48.57; the average disease index in the control group was 65.86, with the lowest value being 23.57 and the highest value being 88.57. The average control effect of *T. viride* T23 on muskmelon wilt was 72.67%. The incidence of muskmelon wilt in the samples was similar to that on the whole. In the investigation of the samples, the average disease index of muskmelon wilt in the *T. viride* T23 treatment group was 20.86 and the average disease index of the control group was 70.29, with the average control effect being 70.32%.

Table 1. Incidence and control effect of muskmelon wilt.

Treatments	The Whole Disease Index	Samples' Disease Index
<i>T. viride</i> T23	20.86 $\pm$ 23.98 b	18.00 $\pm$ 15.33 b
CK	70.29 $\pm$ 25.95 a	65.86 $\pm$ 17.68 a
Control effect on wilt	70.32%	72.67%

Different lowercase letters in rows indicate significant differences at $p < 0.05$.

3.2. Effect of *T. viride* T23 on Microbial Diversity in the Muskmelon Rhizosphere

High-throughput sequencing was performed on 10 samples of muskmelon rhizosphere microorganisms. There were 691,765 sequences and 46,843 OTUs in the V3-V4 variable regions of 16S rDNA, with 1,833,991 sequences and 5187 OTUs obtained by ITS sequencing. The analysis and comparison of microbial diversity was based on the sequencing results.

3.2.1. The Alpha Diversity of the Microbial Communities

After the application of *T. viride* T23, a series of changes occurred in the microbial community of the muskmelon rhizosphere. Figure 1a shows the bacterial diversity indexes; each index in the treatment group is higher than that in the control group. The Chao1 and observed species indexes in the *T. viride* T23 treatment group were highly significant in comparison with the control group ($p < 0.01$), while the Shannon and Simpson indexes displayed no significant differences. What was similar to the changes in the bacterial

diversity indexes was the fact that the diversity indexes of fungi in the treatment group were higher than that in the control group. The fungal diversity indexes in Figure 1b show that the Shannon and Simpson indexes in the *T. viride* T23 treatment group were significant in comparison with the control group ($p < 0.05$). The observed species index displayed an extremely significant difference ($p < 0.01$) and the Chao1 index displayed no significant difference. This indicates that *T. viride* T23 significantly increased the number of microbial communities in the rhizosphere soil of muskmelon and changed the diversity of soil fungal communities. In addition, the results highlight that *T. viride* T23 had a greater impact on the diversity of the soil fungi.

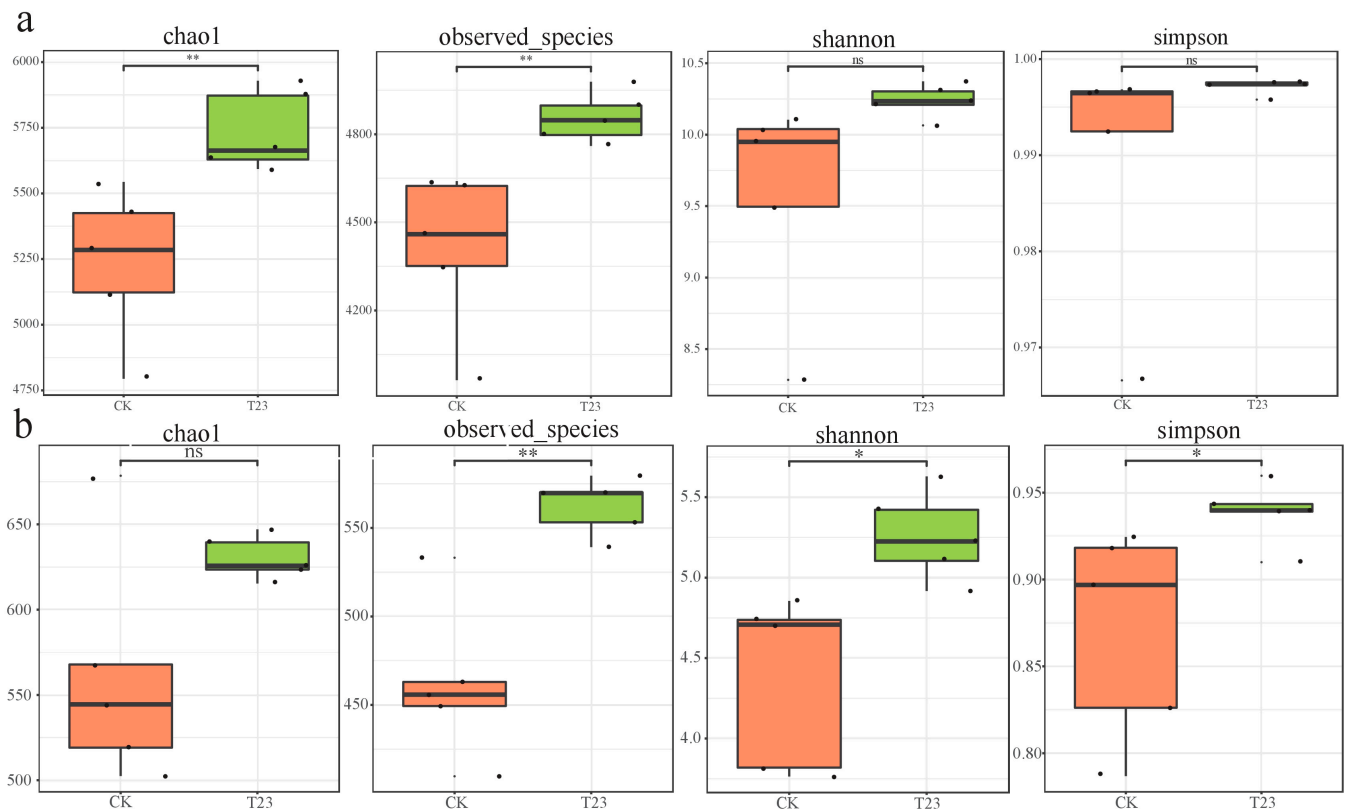


Figure 1. Effects of *T. viride* T23 on the microbial diversity of muskmelon rhizosphere soil. (a) Diversity index of the bacterial community. (b) Diversity index of the fungal community. * indicates that the two groups of data have significant differences, ** indicates that the two groups of data have extremely significant differences, and ns indicates that there are no significant differences.

3.2.2. Beta Diversity of Microbial Communities

Principal coordinate analysis (PCoA) showed that the bacterial communities under different treatments were divided into different clusters and that the two clusters were far apart (Figure 2a); the same was seen in the fungal communities (Figure 2b). The application of *T. viride* T23 significantly changed the microbial community structure of muskmelon rhizosphere soil, accounting for 38.96% of the variation (PC1 26.74% and PC2 12.22%) and 40.78% of the variation (PC1 25.9% and PC2 14.88%) of the changes in the bacterial and fungal community structure, respectively.

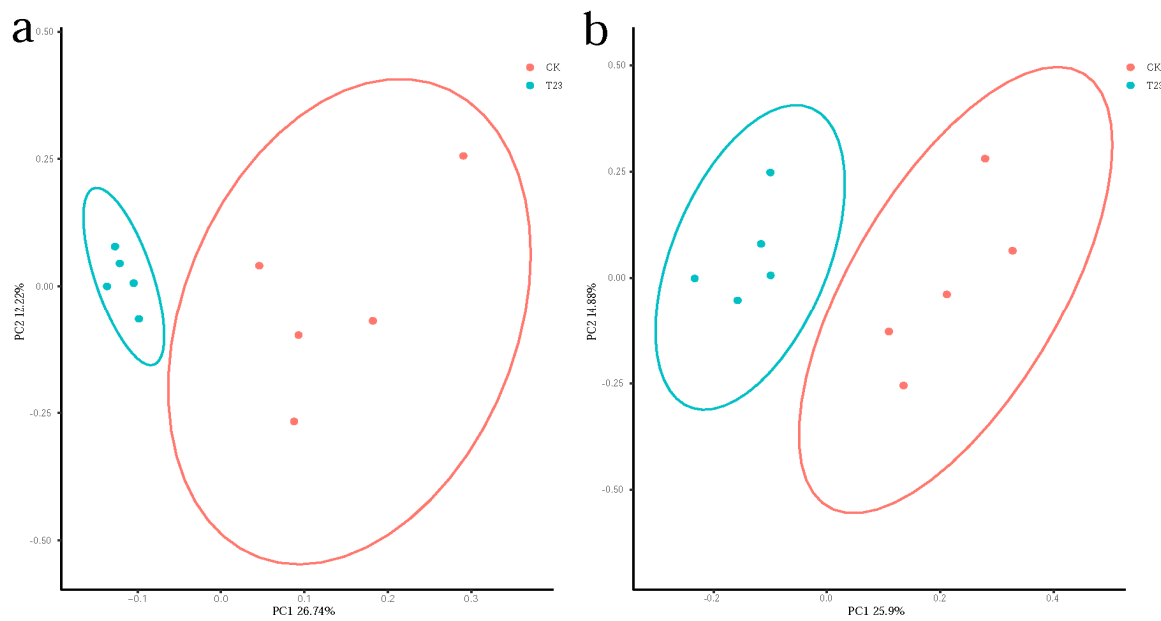


Figure 2. Effect of *T. viride* T23 on the beta diversity of soil bacterial (a) and fungal (b) communities, respectively, using principal coordinate analysis (PCoA). Blue dots represent the *T. viride* T23 group and orange dots represent the control group.

3.3. Relative Abundance of Microbial Communities

The high-throughput sequencing data of the samples were annotated and summarized at different taxonomic levels. In the bacterial communities, 28 phyla, 74 classes, 172 orders, 305 families, and 629 genera of species were identified. At the phylum level (Figure 3a), mainly Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Gemmatimonadetes, and Acidobacteria were abundant, and the relative abundance accounted for 98.18% and 97.69% of the control and treatment groups, respectively. Among them, the most abundant phylum was Proteobacteria, which accounted for 48.19% and 49.53% in the control and treatment groups, respectively. In the top 15 most abundant phylum, the relative abundances of Epsilonbacteraeota, Elusimicrobia, and Cyanobacterium in the treatment group were significantly higher than those in the control group ($p < 0.05$).

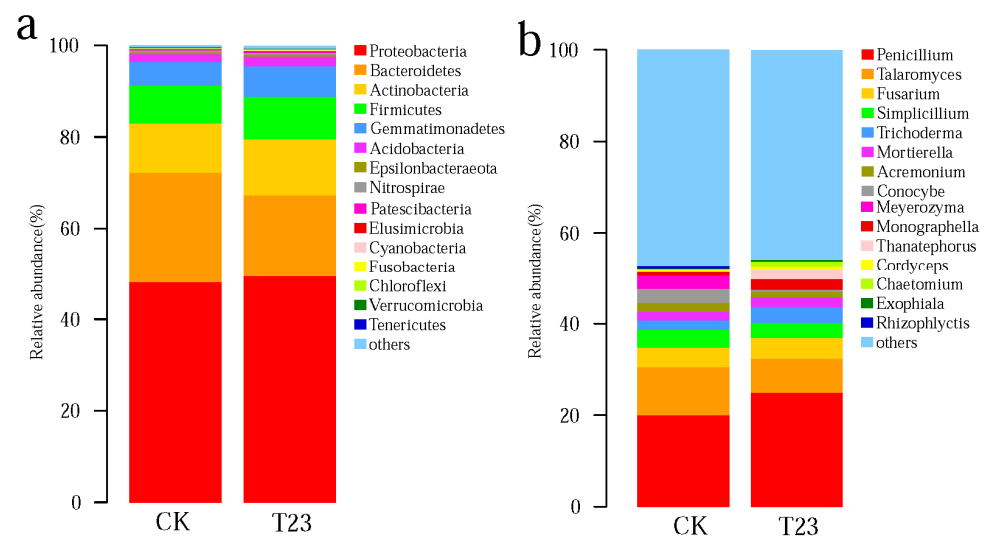


Figure 3. Composition of soil microbial communities in the muskmelon rhizosphere (top 15 in relative abundance). (a) Communities' structure at the level of bacterial phylum. (b) Communities' structure at the fungal genera level.

In the fungal communities, 10 phyla, 27 classes, 68 orders, 125 families, and 209 genera were identified. At the genera level (Figure 3b), *Penicillium*, *Talaromyces*, *Fusarium*, *Simplicidium*, *Trichoderma*, *Mortierella*, *Acremonium*, *Conoxybe*, and *Meyerozyma* exhibited a relative abundance of more than 1%. *Penicillium* had the highest relative abundance, accounting for 20.03% and 25.04% in the control group and treatment groups, respectively. The relative abundance of *Trichoderma* in the treatment group (3.84%) was 78.46%, which was higher than that in the control group (1.95%). Among the top 15 genera with relative abundance, the relative abundance of *Monographella* and *Exophylla* in the treatment group was significantly higher than that in the control group ($p < 0.05$).

3.4. Soil Physicochemical Properties

Table 2 shows that the contents of organic matter, available phosphorus, available potassium, and slowly available potassium in the *T. viride* T23 treatment group were lower than those in the control group and that the content of total nitrogen in the soil was higher than that in the control group. However, there was no significant difference between the above groups. Only the average soil pH in the treatment group (6.40) was significantly higher compared with the control group (6.06). This suggests that *T. viride* T23 did not affect the nutrient composition of soil but significantly increased the soil pH and changed it to an alkaline state.

Table 2. Effects of *T. viride* T23 on soil physicochemical properties.

Treatments	pH	Organic Matter (g/kg)	Total N (g/kg)	Available P (mg/kg)	Available K (mg/kg)	Slow Acting K (mg/kg)
CK	6.06 b	22.04 a	1.105 a	13.27 a	126.3 a	600.6 a
<i>T. viride</i> T23	6.40 a	21.46 a	1.216 a	12.9 a	120.1 a	570 a

Different lowercase letters in rows indicate significant differences at $p < 0.05$.

3.5. Effect of *T. viride* T23 on Rhizosphere Metabolism

In the metabolomic data, we identified a total of 250 metabolites, predominantly including aromatic compounds, hydrocarbons, fatty acids, nucleotides, organic acids, and organic nitrogen oxides. We obtained a metabolite score map (Figure 4) by applying orthogonal partial least squares discriminant analysis (OPLS-DA); the score map can distinguish the entirety of the difference in the metabolite profile between the groups. The model parameters of this scoring map were R^2X (cum) = 0.583, R^2Y (cum) = 0.966, and Q^2 (cum) = 0.791. R^2X (cum) and R^2Y (cum) showed the cumulative interpretation rate of the model in the X-axis direction and the Y-axis direction during multivariate statistical analysis modeling. Q^2 indicated the predictive power of the model and $Q^2 > 0.5$ showed that this model had a good interpretation and prediction ability. The abscissa showed the predicted principal components (15%), showing the gap between the groups; the ordinate represented the orthogonal principal components (27.9%), showing the gap within the groups. The cluster separation effect of T23 and CK indicated that the application of *T. viride* T23 significantly changed the metabolic structure of the rhizosphere soil of muskmelon.

3.6. Differential Metabolite Analysis

According to the analysis of the metabolomic data, we obtained a total of 48 differential metabolites (Figure 5). Compared with the control group, there were 20 upregulated differential metabolites, which contained urea, erythronic acid, scopolamine, palmitic acid, etc.; the largest increase (184.33%) was recorded for urea. In the treatment group, there were 28 downregulated differential metabolites, including citrulline, capric acid, 4-hydroxymandelic acid, and aconitic acid; the largest decrease in content was recorded for 4-hydroxymandelic acid, which decreased by 85.18%. Among these differential metabolites, compounds such as scopoletin, palmitic acid, and aconitic acid have certain biological

activities, which can affect the composition of microbial communities in soil and the growth of plants.

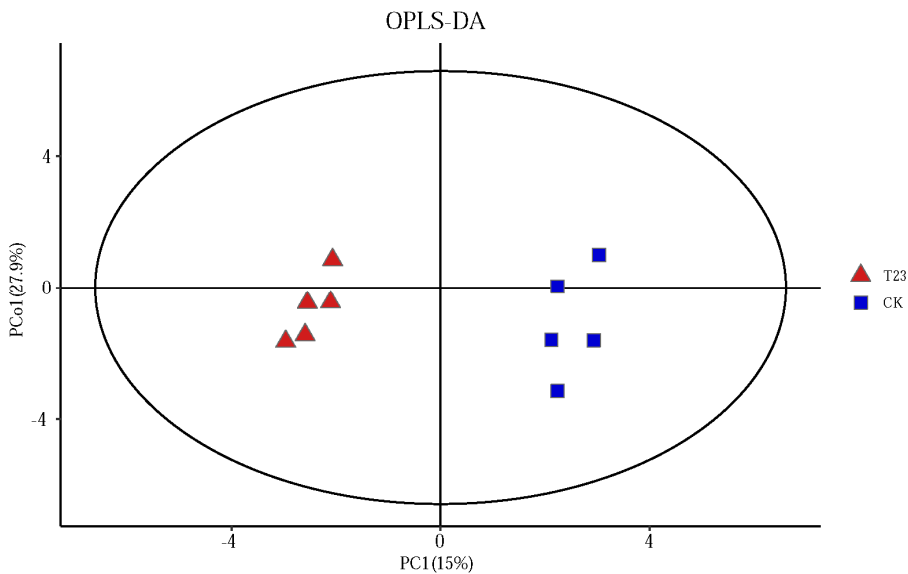


Figure 4. OPLS-DA score map of muskmelon rhizosphere soil after *T. viride* T23 application. Red triangles represent the *T. viride* T23 group and blue dots represent the control group.

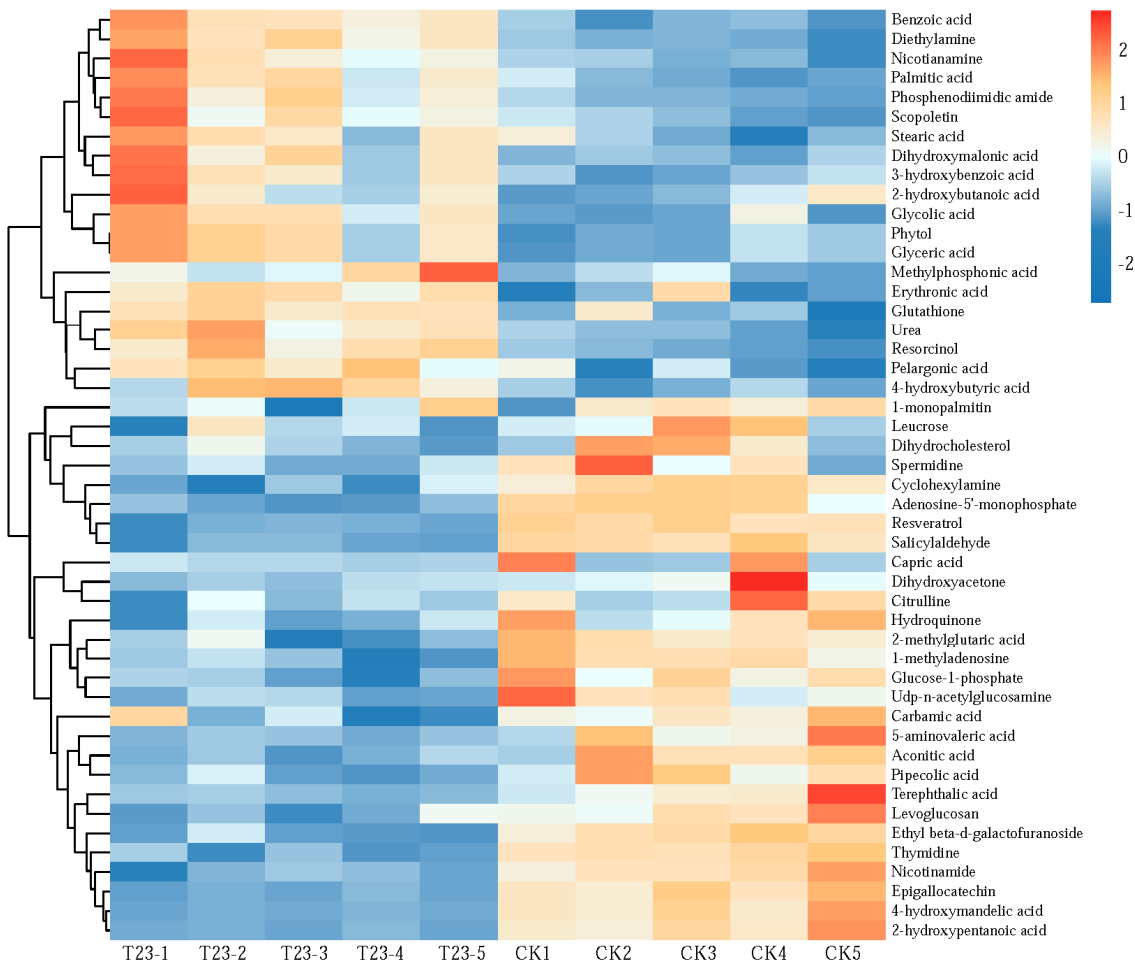


Figure 5. Heat map of metabolite contents in the rhizosphere of the muskmelon. Yellow represents high content and blue represents low content.

3.7. Correlations of Soil Microbial Communities with Differential Metabolites

Figure 6 shows the correlations of soil microbial communities with differential metabolites. The results indicate that nine bacterial phyla and six fungal genera had a significant correlation, with 31 and 41 differentially expressed metabolites, respectively. There were eight, seven, five, two, and one differential metabolites, which showed significant positive correlations with Epsilonbacteraeota, Elusimicrobia, Firmicutes, Acidobacteria, and Gemmatimonadetes, and six, seven, three, two, and six differential metabolites with a significant negative correlation, respectively. There were 2, 17, 1, 1, and 14 kinds of differential metabolites that were significantly positively correlated with Conoxybe, Meyerozyma, Trichoderma, Mortierella and Monographella and 4, 7, 1, 4, and 21 kinds of differential metabolites with a significant negative correlation, respectively. Among the different metabolites, 2-hydroxyxanthanoic acid, urea, 2-methylglutaric acid, resorcinol, and scopoletin were significantly correlated with multiple fungal genera or bacterial phyla.

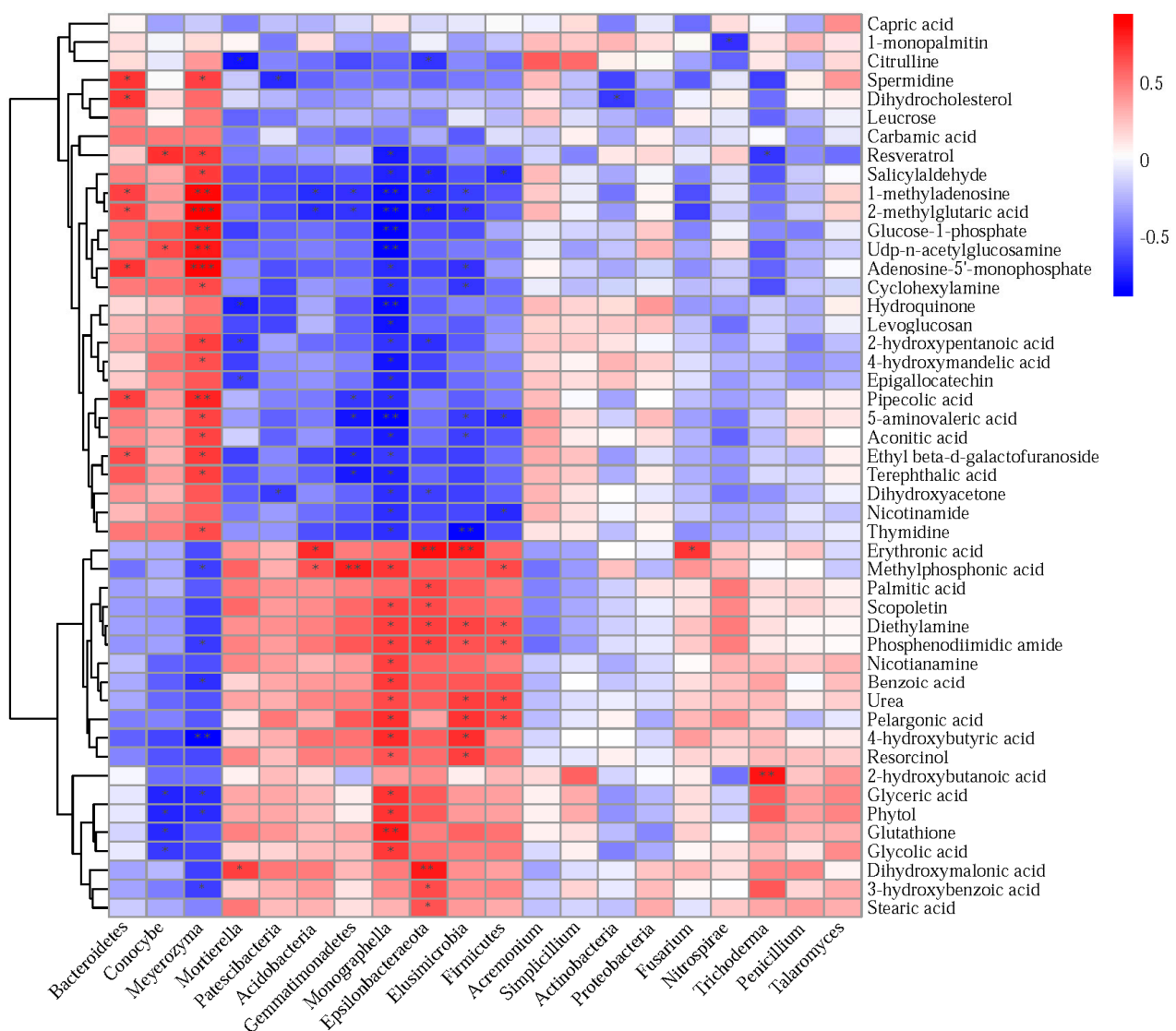


Figure 6. Heat map of the correlation analysis between soil microbial communities and differential metabolites. Red represents a positive correlation and blue represents a negative correlation; the darker the color, the greater the correlation. ***, **, and * represent significance at $p < 0.001$, $p < 0.01$, and $p < 0.05$.

4. Discussion

4.1. Control Effect of *T. viride* T23 on Muskmelon Wilt

Trichoderma spp. belongs to the phylum of Ascomycota, the class of Sordariomycetes, the order of Hypocreales, the family of Hypocreaceae, and the genus of *Trichoderma*. At present, more than 250 species of *Trichoderma* have been reported in the world [24]. *Trichoderma* is widely distributed in nature, generally exists in the soil, and is an important biological control agent. *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma acanthosporium*, and *Trichoderma koningii* have been reported to be used for the prevention and control of plant diseases [25]. In the greenhouse study, when used for potting, the control effect of *Trichoderma pseudokoningii* 886 on cucumber wilt reached up to 78.64% [11]. In another example, *Trichoderma harzianum* exhibited up to an 87.9% control effect on tomato wilt at the seedling stage [26]. In the present study, the control effect of *T. viride* T23 on muskmelon wilt was recorded to be 72.67%, which was similar to the previously reported control effect of various *Trichoderma* strains on fusarium wilt in different crops. The control effect of *T. viride* T23 on muskmelon wilt in the samples group was 70.32%, which was close to the value (72.67%) of the entire plant. This shows that the collected samples could accurately represent the overall plant.

4.2. Effects of *T. viride* T23 on Soil Physicochemical Properties and Soil Microbial Communities

Soil microorganisms are an important part of soil and their microbial community structure and diversity are the key factors that affect plant growth. A good soil microbial community structure is conducive to the healthy growth of plants and the more complex the microbial community structure is, the stronger its stability is. Some studies have shown that, as time goes on, the continuous planting of a single crop on the same plot will lead to a reduction in the physical and chemical properties of the soil and an imbalance in the soil microbial community, which makes it no longer suitable for the planting of the original crop. In soil with a continuous strawberry cultivation of 7 years, the soil pH value decreased by 10% and the content of soil organic matter and total nitrogen increased with the extension of continuous planting years [27]. With the increase in the continuous cropping years of *Codonopsis pilosula*, the soil pH value showed a downward trend, while the total nitrogen and total phosphorus in the soil increased with the extension of continuous cropping years [28]. In this study, among the soil physical and chemical indicators such as organic matter, total nitrogen, and soil pH value, only the pH value in the *T. viride* T23 treatment group was significantly higher than that in the control group and the other indicators were not significantly different. This showed that *T. viride* T23 can improve the soil pH value, reduce the soil acidification, and improve the soil environment for plant growth. This result was consistent with previous research. For example, after using different soil conditioners to improve the soil quality of continuous cropping cucumber, the soil pH and other physicochemical properties were significantly increased [29]. After the application of compost products to improve the soil quality of watermelon continuous cropping, the soil pH value increased significantly [30].

The study showed that, compared with the soil without muskmelon planting, the soil microbial diversity changed, in that the diversity of bacteria and actinomycetes decreased, while the number of pathogenic fungi increased significantly, leading to the aggravation of muskmelon disease [4]. After long-term continuous cropping of cucumber, the number of fungi in the soil increased significantly and *Fusarium oxysporum* became the dominant fungi taxa in the soil [31]. The application of *T. viride* T23 effectively changed the microbial community structure and composition of continuous cropping soil. The results of PCoA showed that, in the analysis of the bacterial and fungal community structure, the samples of the treatment group and the control group were obviously separated (Figure 2). In the bacterial community composition, the treatment and control groups were mainly composed of Proteobacteria, Bacteroides, Actinobacteria, Firmicutes, Gemmatimonadetes, and Acidobacter. This was consistent with the results of previous studies on the bacterial communities of muskmelon [1], watermelon [32], and cucumber [33], indicating that the

composition of bacterial communities in the rhizosphere soil of Cucurbitaceae plants is relatively similar.

Some studies have shown that the continuous application of bio-organic fertilizers could inhibit the occurrence of banana wilt by improving soil physicochemical properties and improving the relative abundance of the Firmicutes (*Bacillus*) and the *Anoxybacillus* and *Spartobacteria* genera in the soil microbial community [34]. The application of *Paenibacillus polymyxa* NSY 50 could effectively reduce the abundance of *Fusarium*, improve the number of potentially beneficial groups such as the *Bacillus*, *Actinobacteria*, *Streptomyces*, *Actinospica*, *Catenulispora*, and *Pseudomonas* genera, and then reduce the occurrence of cucumber wilt and promote the growth of cucumber [33]. The number of *Bacillus*, *Pseudomonas*, and *Lysobacter* in rhizosphere microorganisms increased significantly after the application of a spent mushroom substrate to control cucumber wilt disease [35]. After applying *Bacillus subtilis* NCD-2 to control cotton verticillium wilt, the abundance of *Acidobacterium*, *Chloroflexi*, and *Planctomycetes* in the bacterial group increased significantly [36]. Using *Bacillus amyloliquefaciens* B1408 to inhibit cucumber wilt, the relative abundance of Proteobacteria, FBP, and Firmicutes was significantly increased within the treatment group [37]. The relative abundance of Proteobacteria, Actinobacteria, Firmicutes, Gemmatimonadetes, and *Acidobacterium* improved after the application of *T. viride* T23, which was in agreement with the previous studies. The relative abundance of Elusimicrobia in the treatment group was significantly higher than that in the control group ($p < 0.05$). Some studies have shown that Elusimicrobia plays an important role in the soil nitrogen cycle [38]. Some of the strains had the ability to both fix nitrogen and metabolize ammonium nitrogen in the soil, which is significantly related to soil fertility [39,40].

In fungal communities, the number and diversity of soil communities were significantly increased, indicating that the application of *T. viride* T23 restored the fungal community structure in the continuous cropping soil for healthy growth for muskmelon. Compared with CK, the relative abundance of Trichoderma in the treatment group was increased by 78.46% and Trichoderma was able to colonize the soil efficiently. Interestingly, *T. viride* T23 could effectively inhibit the occurrence of muskmelon wilt and reduce the disease index, but the relative abundance of *Fusarium* did not decrease significantly. It could be possible that *T. viride* T23 is able to change the soil microbial community structure and other factors to inhibit the activity of *Fusarium* and reduce its harm to muskmelon. This phenomenon is similar to the situation of using *B. subtilis* for the control of cotton verticillium wilt. The populations of *Verticillium dahliae* in the treatment group did not decrease significantly, but the incidence rate of cotton verticillium wilt decreased significantly [36].

4.3. Differential Metabolite Analysis

Soil metabolites are an important part of soil composition, which can affect both the growth of plants and the composition of soil microbial communities [41]. Therefore, it is of great significance to carry out research on soil rhizosphere metabolites. In this study, 250 metabolites were identified, mainly aromatic compounds, hydrocarbons, fatty acids, nucleotides, organic acids, and organic nitrogen oxides, which contained 48 differential metabolites. The content of scopolamine increased by 1.38 times and had various biological activities. At the concentration of 8.10 $\mu\text{g/mL}$, it could effectively inhibit the gray spot pathogen of soybean and significantly promote the germination of soybean seeds and the growth of seedlings and plants [42]. Scopolamine could also effectively inhibit the growth of *Fusarium oxysporum*, Fusarium rot, and Rhizopus mycelium [43]. Therefore, it is speculated that the application of *T. viride* T23 induced the synthesis of scopolamine in muskmelon, which helped muskmelon to overcome the infection of *Fusarium oxysporum* and promoted the growth of muskmelon plants. Among the different metabolites, the content of benzoic acid in the treatment group did not decrease significantly; it was generally considered to be an important autotoxin that affected the growth of muskmelon. This showed that *T. viride* T23 could alleviate the negative effects of continuous cropping on muskmelon. In differential metabolites and the microbial correlation analysis, 45 differential metabolites

had significant correlations with 11 bacterial phyla and four fungal genera. Among them, 11 bacterial phyla were closely related to the different metabolites, which reflected the quantitative advantages of each bacterial phyla.

5. Conclusions

In this study, *T. viride* T23 fermentation broth was applied to the continuous cropping soil of muskmelon; soil samples were collected 60 days after transplantation. The collected soil samples were tested by microbiome analysis, metabolomics, and other conventional testing methods. The results showed that *T. viride* T23 could increase the soil pH value and reduce the soil acidity, change the structure of the soil microbial community, increase the relative abundance of soil Proteobacteria, Actinobacteria, and Firmicutes, and improve the diversity of fungi. The application of *T. viride* T23 could also affect the production of scopolamine and other beneficial substances in soil metabolites, reduce the occurrence of muskmelon wilt, and promote the growth of muskmelon. Therefore, it can be considered that *T. viride* T23 is an effective agent to alleviate the negative effects of continuous cropping on muskmelon.

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