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Diversity of Fungal Community in Rhizosphere Soil of Tomato with Different Diseases

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Abstract

As an indispensable part of rhizosphere microorganisms, fungi participate in the processes of soil and rhizosphere element cycle and the degradation of pollutants in soil through self metabolism, and have huge potential in protecting tomato from abiotic and biological stresses. Tomato is one of the main varieties in the global trade of vegetables and fruits. In recent years, the planting area of tomato has continued to expand. The simple and intensive planting combined with high temperature and wet environmental conditions has facilitated the growth and breeding of diseases and pests, which has led to a sharp decline in tomato production. In addition, excessive use of chemicals and fertilizers has led to environmental pollution, which has led to a decline in tomato quality, and has caused huge losses to China's vegetable economy. In this experiment, the rhizosphere soil of several common tomato diseases was used as research materials, and ITS sequencing was carried out for rhizosphere soil fungi by using high-throughput sequencing analysis. The operational taxon units (OTUs), alpha diversity, beat diversity and community structure were analyzed.

Keywords: tomato, rhizosphere soil microorganisms, high-throughput sequencing, fungal diversity

1. Introduction

Tomato is an extremely important cash crop and plays an important role in the world's fruit and vegetable trade. With large-scale planting, various soil borne diseases seriously affect crop quality and yield. In addition, due to the extensive use of chemicals and fertilizers in disease control and fertilization, soil microorganisms have been destroyed, resulting in the reduction of microbial diversity and the change of community composition. Fungi widely exist in the soil. As the link between the soil and the rhizosphere, they can transform the nutrients in the soil to accelerate the absorption of plants, thus improving the acidity and alkalinity of the soil. Healthy soil environment makes microorganisms in plant rhizosphere in a balance of mutual constraints. The analysis of soil diversity and community composition is of great significance to the industrialization of tomato production (Alebel, 2022).

In this experiment, the root soil of tomato

common diseases was used as materials to analyze the diversity and community composition of soil fungi. The effects of fungal diversity and community composition in soil on environmental change, pathogen inhibition, tomato quality improvement and habitat stability were discussed. It provides reference for scientific planting of tomato and other crops, improving the quality of crop products, preparing compound microbial agents, and selecting beneficial strain resources (Jessica, 2021).

2. Experimental Materials and Methods

2.1 Test Design

Set 4 groups of soil sample treatment, with 3 replicates for each group. They are healthy tomato rhizosphere soil (JK), wilt rhizosphere soil (KW), mosaic rhizosphere soil (HY), gray mold rhizosphere soil (HM).

2.1.1 Experimental Materials

Respectively taken from grey mold, fusarium wilt, mosaic and healthy rhizosphere soil and stored in - 80℃ refrigerator.

2.1.2 Sample Soil Collection

Adopt the five point sampling method, and take three samples from each group. The soil around the rhizosphere shall be preserved without harming the root. After mixing, it is a sample, and each sample is about 5g. The centrifuge tubes in the laboratory were packed separately and sent to the company for high-throughput sequencing.

2.1.3 Experimental Instruments and Reagents

Ultra clean bench of test instrument BSC-1304 Ⅱ A2; Electrophoresis instrument DYY-6D; PCR instrument ABI GeneAmp * 9700; Gel imaging ChampGell 5000; Vortex oscillator IKAGenius3.

Experimental reagent TrasStart Fastpfu DNA Polymerase; E. Z.N.A. SoilDNA extraction kit; Tri_HCl eluent TruseqTM DNA Sample Prep Kit; AxyPrepDNA gel recovery kit

2.2 Experimental Methods

2.2.1 Fungal OTU Analysis

Operational taxons (OTUs). After comparison, a group of sequences with data similarity greater than 97% is usually a biological population. Cluster the samples to obtain OTU. Taxonomic annotation of OTU. Obtain the number of OTUs of four groups of samples and draw the OTU Venn diagram. The figure can visually display the similar level of OTU composition among samples. The number of OTUs owned respectively and jointly by different samples can be obtained.

2.2.2 Fungal Diversity Analysis

2.2.2.1 Fungal Alpha Diversity Analysis

Choose Mothur software under three measurement indicators, namely Chao1, Shannon and Simpson. The alpha diversity of tomato rhizosphere soil samples under different diseases was analyzed.

2.2.2.2 Diversity Analysis of Fungi Beta

Compare the specific differences between each pair of samples to generate a distance matrix between all pairs of samples. The similarity of soil diversity among four groups of different diseases was compared. Principal component analysis (PCA) was used.

2.2.3 Analysis of Soil Fungal Community Structure

By comparing the representative sequence of OTU with the microbial reference database, the species classification information corresponding to each OTU can be obtained, and then the community composition of each sample at each level (family, genus) can be counted, and the species abundance table at different classification levels can be generated using QIIME software.

3. Experimental Results and Analysis

3.1 Evaluation of Sequencing Quality of Soil Fungal Samples

Table 1. Four groups of fungal samples were sequenced

Sample	Sequence number	Base number	Average length	Shortest sequence length	Maximum sequence length
JK1	70247	17582052	250	141	524
JK2	70105	17303735	247	143	520
JK3	69587	16759616	241	142	509
KW1	62784	14942755	238	145	512

KW2	71670	17179795	240	142	505
KW3	69463	16952094	244	144	535
HY1	71338	16330430	229	141	497
HY2	70533	16375919	232	142	518
HY3	61695	14454267	234	140	524
HM1	51603	12567928	244	141	523
HM2	47981	11362727	237	140	497
HM3	70870	16584554	234	143	511

A total of 787876 sequences were obtained from four groups of tomato soil fungal samples under different disease conditions, and the average sequence number of each sample was 69980 (JK), 67972 (KW), 67855 (HY), 56818 (HM), respectively. The longest average length of the measured sample is 250bp (JK1), and the shortest average length is 229 (HY1). The longest sequence length is 535 (KW3), the shortest sequence length is 140 (HY3 and HM2), the most alkali base is 17582052bp (JK1), and the least alkali base is 11352727bp (HM2). Data evaluation by statistical (Table 1) parameters

3.2 Fungal OTU Analysis

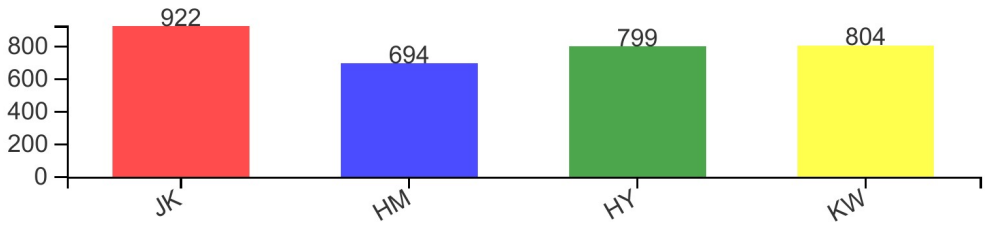

Figure 1. OTU Number of Fungi in Four Groups of Soil Samples

The number of fungi in rhizosphere soil samples of different diseases varies greatly. In Figure 1, there are 3219 OTUs for the four groups of samples, and the maximum number of OTUs for JK is 922. The minimum number of OTUs of HM is 694. The number of OTUs of other HY and KW is not much different (about 802). Compared with fungi in diseased soil, the number of JK was far greater than that of OTU in other three groups. The results showed that the fungal diversity in tomato soil was destroyed and the number of OTU was significantly reduced under the action of different disease fungi.

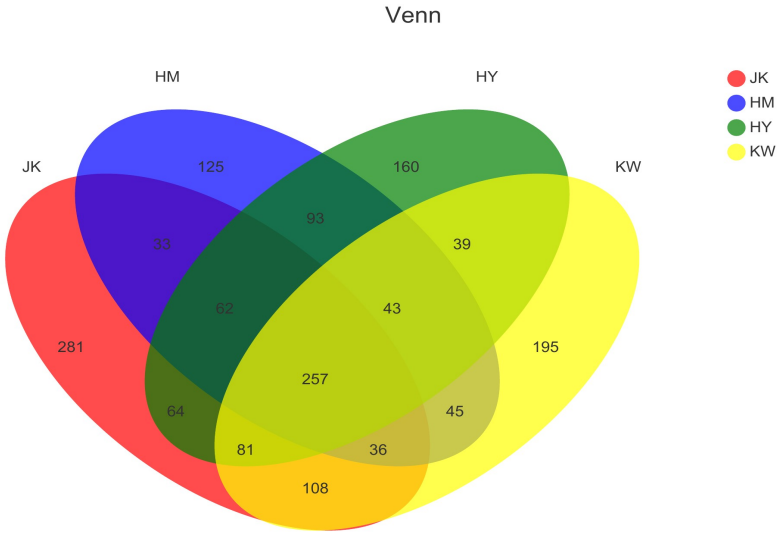

Figure 2. OTU Venn Diagram of Four Groups of Soil Samples

The OTU Venn diagram of samples shall be prepared according to the number of OTUs of different disease soils. Compared with OTU of soil fungi of different disease groups, the total number of JK, HM, HY and KW is 257. The number of OTUs unique to JK is 281, the number of OTUs unique to HM is 125, the number of OTUs unique to HY is 160, and the number of OTUs unique to KW is 195. In addition, it can be seen that the number of OTUs shared by JK, HM, HY and KW is 33, 62 and 36 respectively, which are relatively small. In addition, the number of OTUs in the mating part of HM, HY and KW was only 43. Compared with the above four groups of soil samples, JK has the largest number of OTUs, which indicates that the diversity of soil fungi is significantly different under different diseases.

3.3 Diversity Analysis of Soil Fungi with Different Diseases

3.3.1 Fungal Alpha Diversity Analysis

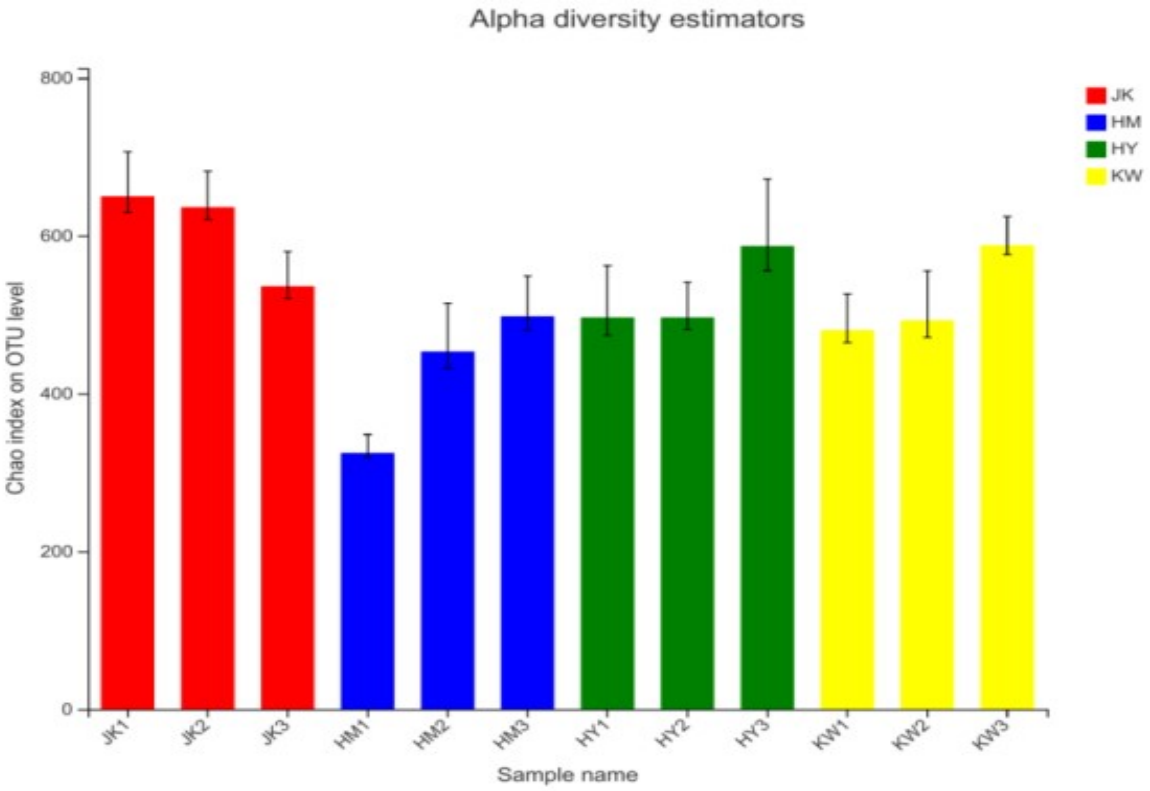

Figure 3. Chao1 Index of Fungi in Four Groups of Soil Samples

In Figure 2, JK's Chao1 index is far larger than other samples, with the highest value of 673 for JK and the lowest value of 418 for HM. Therefore, JK has a high abundance and number of fungal species. Compared with JK, the Chao1 index of HM1 sample is the lowest 323. It indicates that HM has the least microbial abundance and microbial number in the four groups of sample soils.

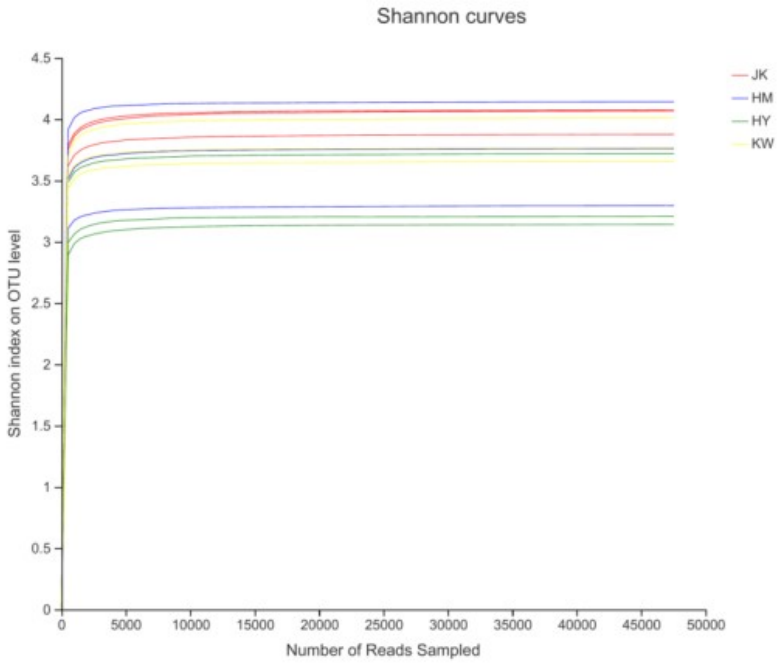

Figure 4. Shannon index curve of fungi in four groups of soil samples

In Figure 4, JK is higher than the other three groups HY, HM and KW. The highest Shannon index of JK is 4.1, and the lowest one of HY is 3.38. According to the Chao1 index, under the attack of different pathogenic microorganisms, the diversity of soil fungi was destroyed and the original microbial balance could not be maintained. The Shannon index of KW is slightly higher than that of HM, and the Shannon index of HM is slightly higher than that of HY. Compared with the above three groups of sample soils, the HY index is the lowest and the diversity is the most damaged.

3.3.2 Diversity Analysis of Fungi Beta

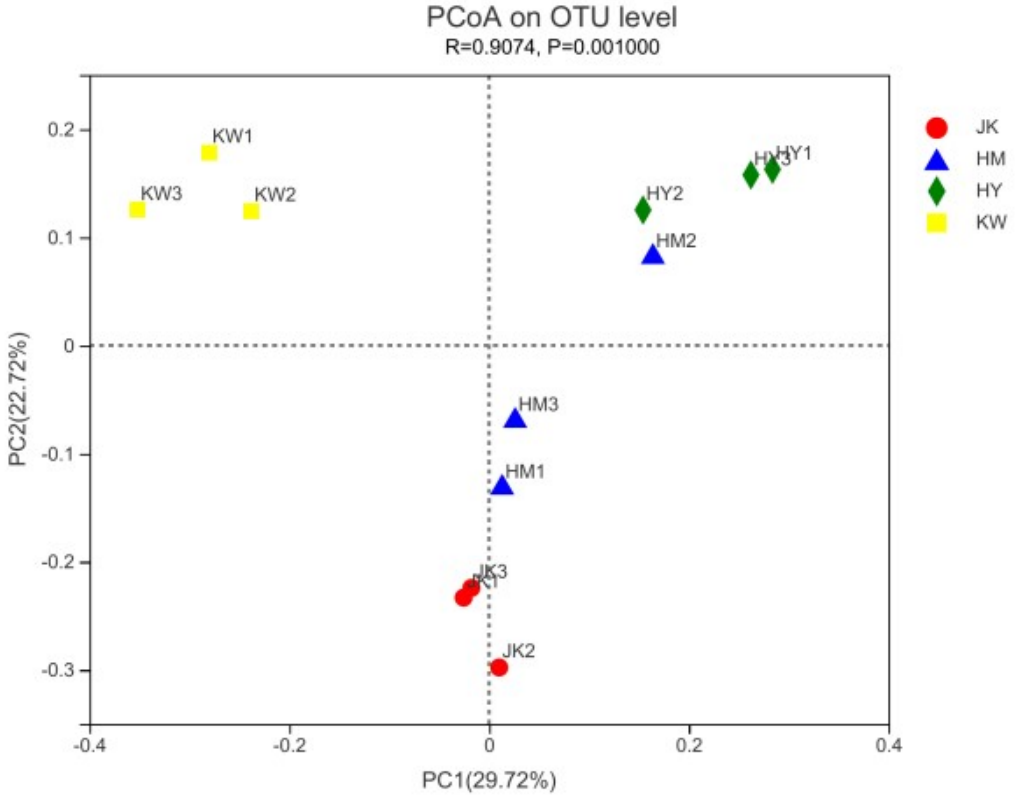

Figure 5. Principal Component Analysis (PCA) of Fungi in Four Groups of Soil Samples

The PCA analysis method was selected. The first principal component (PC1) was 46.07%, and the second principal component (PC2) was 21.4%. In Figure 5, the samples in JK group are relatively close, and the difference of fungal community composition in the group is small. The short distance between JK and HM indicates that there is little difference in community composition between them. The straight-line distance between JK and KW is far, which indicates that there is a large difference in community composition between them. The distance between JK and HY3 is the longest, and the difference of community composition between JK and HY is the largest. In addition, the distance between KW and HY3 (mosaic disease) and HM is far, which means that the community composition of KW and its two species is very different.

3.4 Horizontal Community Structure of Soil Fungi of Different Diseases

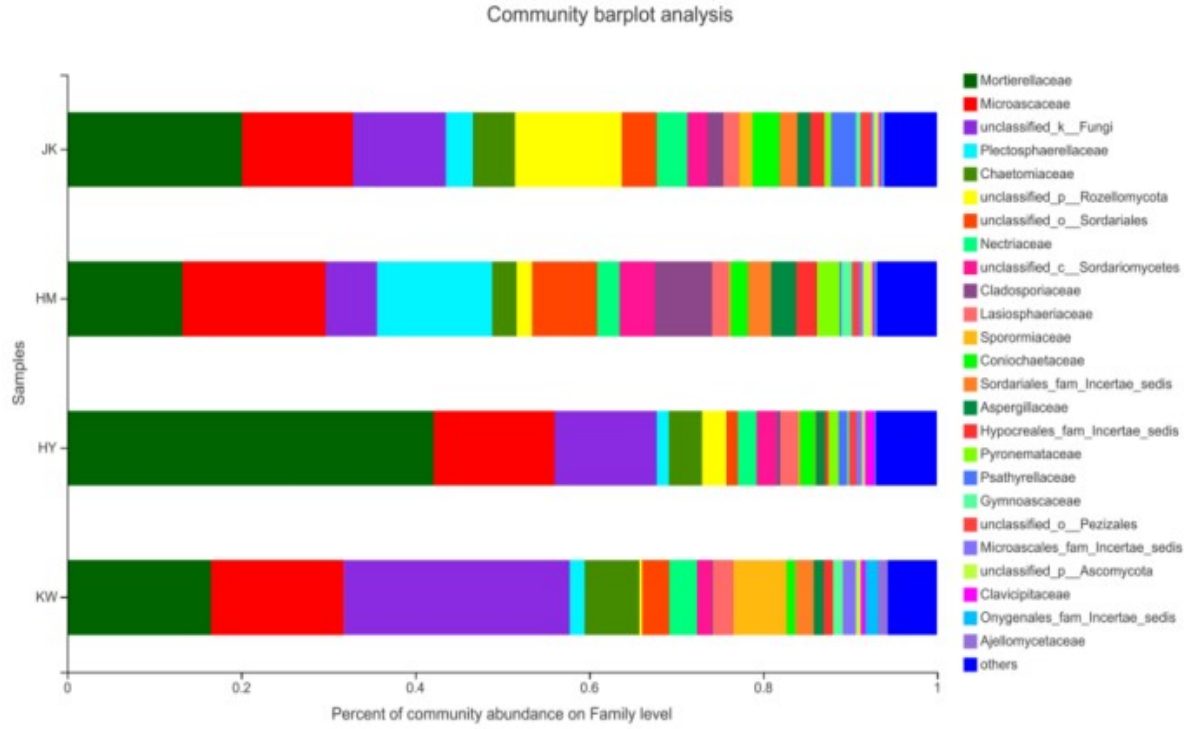

Figure 6. Relative abundance of fungal species in four groups of soil samples with different diseases

The results showed that Mortieriaceae grew more easily in HY and was inhibited in HM. The abundance of Microcystaceae in the samples is not different, and it is most distributed in JK, and few in the other three disease soils.

4. Discussion

By analyzing the OTU number of soil fungi in tomato disease, we can get that the OTU number of HM (gray mold) is at least 694. The number of OTUs of other HY (mosaic disease) and KW (fusarium wilt disease) is not significantly different (about 802). Compared with JK, it is 922. In the presence of disease, the number of fungal OTUs generally decreases.

In the alpha diversity analysis, JK has the highest Chao1 index of 673, and HM has the lowest Chao1 index of 418. HY and KW are both 533. It indicates that the number of species in three groups of samples is lower than JK, and the number of HM species is the least.

The minimum Simpson index JK is 0.0525, and the maximum HY is 0.18. The highest Shannon index JK is 4.1, and the lowest HY is 3.38. These two groups of indices can be observed together to reflect community diversity. In the data, the community diversity of three groups of disease samples is lower than JK. HY has the lowest community diversity. Different diseases have significant effects on the diversity of soil fungi. This is similar to Kumar Santosh (2022) and so on.

In Beta diversity analysis, the fungi in rhizosphere soil of tomato with different diseases were analyzed by principal coordinate analysis (PCoA) and principal component analysis (PCA), the composition of soil fungal

community of fusarium wilt and other two diseases is quite different.

At the level of family classification, the dominant family is Ascomycete, and the four families are Chaetochaceae, Microcystis, Erythrochecidae, and Chaetococcidae. Ascomycetes is the main driving force of carbon cycle and nitrogen cycle in ecosystems. In addition, the Mortierella family of Zygomycetes has a large proportion, which is similar to Thapa Magar Roniya (2022), etc. The research is consistent. It accounts for 13%~42% of the samples. Ascomycetes and Zygomycetes are important components of soil fungal community Xu Yaowen (2022) Ascomycetes and Zygomycetes have been proved to have the ability to decompose lignin and keratin, accelerating the alternation of soil components.

In the distribution data information of HY soil, Mortieriaceae accounts for the largest proportion compared with Xuan Wu (2021). It is believed that Mortierella fungi can strongly degrade cellulose. It can decompose organics, promote growth and increase the content of alkali. It plays a certain role. In addition, a large number of Mortierella may cause plant diseases.

Compared with JK control, the proportion of Fusarium in KW increased significantly, and the abundance of healthy plants increased, accounting for about 2% and so on. Among them, Fusarium oxysporum tomato specialized type is specially infected with tomato, which is the culprit of tomato wilt.

At the genus level, Mortierella is the dominant genus in 9.5%~31%. Among them, HM is the least. Mortierella can promote nutrient cycling and maintain soil fertility. Kernia is 2.5%~5%, classified in Ascomycetes, and has made outstanding contributions to promoting soil fertility. In sample KW, the content is only 2.5%. The white spot fungus accounted for 7% in HM and 2% in HY. Research shows that the white spot fungus can also cause plant diseases. Chaetomium and Aspergillus are the main antagonistic fungi against plant pathogens. The content in HM and HY is so small that the plant can not resist the pathogen and become infected.

To sum up, the composition of various microorganisms in the soil has an important impact on soil fertility and plant growth. The proportion of Ascomycetes and Zygomycetes is related to the absorption of nutrients by plants. In addition, natural antagonistic bacteria such as Chaetomium and Aspergillus are also indispensable in plant growth.

In addition, Ascomycetes, Unclassified and Others fungi occupy a large proportion in the soil, affecting soil nutrients and plant development. Among the family level JK, Unclassified p-Rozellomycota accounts for 19%, but there are few relevant studies at present. Whether the above fungi can decompose soil materials and increase element content needs further research.

5. Conclusion

In this study, under the conditions of different diseases of tomato HM (gray mold), HY (mosaic disease) and KW (fusarium wilt), JK (health) was compared by analyzing the number of soil fungal OTU, fungal diversity (alpha diversity, Beat diversity), community composition (family level, genus level) and other data. The results showed that disease microorganisms could significantly reduce the number of fungi and their community composition, and fungal diversity and community composition were significantly different among different diseases.

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