Study on the Microbial Diversity of Rhizosphere Soil of Different Tobacco Varieties

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Abstract: The aim of this paper is to study the microbial diversity of rhizosphere soil of tobacco. The research objects are rhizosphere soil samples of four tobacco varieties, Yunyan 85, Yunyan 87, Yunyan 202 and K326. High throughput sequencing technique is used to sequence DNA genome in 16S rDNA-V4 and ITS1 regions of rhizosphere soil of four tobacco varieties; the species abundance, Alpha diversity and Beta diversity are analyzed. The results show that the sequence of bacterial diversity from high to low was Yunyan 202, Yunyan 87, K326 and Yunyan 85; the order of fungal diversity from high to low was Yunyan 202, K326, Yunyan 87 and Yunyan 85. Bacteria were mainly distributed in Proteobacteria and Actinobacteria. The dominant bacteria were Kaistobacter and Rhodanobacter. Fungi were mainly distributed in 5 phyla, include Ascomycota and Zygomycota. The dominant fungi were Pseudogymnoascus. The rhizosphere soil of Yunyan 202 had Pseudomonas, which was not found in the soil of other 3 species. Marinobacter and Alcanivorax were abundant in Yunyan 202 and Yunyan 85, but extremely rare in other 2 species. Pseudonocardia, Janthinobacterium, Sphingomonas, Burkholderia and Rhodanobacter could be found in the soil samples of all species, of which the abundances of Janthinobacterium, Sphingomonas and Burkholderia varied greatly, while the abundances of Pseudonocardia and Rhodanobacter differed slightly. For the fungi diversity, Retroconis and Tremellomycetes sp could only be found in Yunyan 202. The abundances of Penicillium and Sordariomycetes SP were similar in Yunyan 202 and Yunyan 85; in the other two varieties almost no Penicillium or Sordariomycetes SP were detected. Fungi of Myrothecium, Chaetomium, Fungi SP and Pseudogymnoascus could be found in the soil of all species, but their abundances differed greatly. For instance, the abundances of Myrothecium were 1.73% in Yunyan 202, 2.06% in Yunyan 87, 1.44% in Yunyan 85 and 3.76% in K326.

1. Introduction

Various kinds of microorganisms distributed in the rhizosphere soil of tobacco. In order to study the species and structure of these microorganisms, and further understand the relationship between tobacco species and microorganism distribution, the rhizosphere soil of different tobacco species were studied in this paper. Shi-wei Zhang and Qi-ding Zhong used the terminal restriction fragment length polymorphism (T-RFLP) technique to study the bacterial communities of rhizosphere and phyllosphere of grape, and analyzed the diversity of bacterial communities; it was found that the dominant bacteria of rhizosphere of different grapes were Mycetozoan, while the dominant bacteria

of phyllosphere of grapes were Actinomycetes. [1] Si-yuan-Zhu, Qing-ming Tang and their coworkers studied the microorganisms in rhizosphere and non rhizosphere soil of 6 different ramie varieties, and found that the number of bacteria was largest in main microbes; the number of bacteria was 10 times of fungi and actinomycetes, and the sequence of biomass was bacteria > fungi > actinomycetes; similar to other crops, there were significant differences in the microorganisms of rhizosphere soil of different species. [2] At present, the main research method of analyzing microbial diversity of rhizosphere of different tobacco varieties is the first generation sequencing method. [3-5] The usage of the second generation sequencing method has not been reported. In this study, the second generation high-throughput sequencing technology which has been developed in recent years was used to analyze the community structure of microbes in rhizosphere soil of different varieties of tobacco, hoping to lay a foundation for further research and development of microbial resources, as well as the breeding of tobacco species with high disease resistance.

2. Research Materials and Methods

2.1 Soil sample collection

The tested tobacco varieties were Yunyan 87, Yunyan 202, K326 and Yunyan 85. In May of 2017, the samples were taken from Xuanwei test field located at Luoshui Town, Xuanwei County of Qujing in Yunnan Province. The test field was used to cultivate tobacco for consecutive 3 years. 5 sampling points were selected. 1kg soil sample was taken from each point at the depth of 0-20cm. After uniform mixing at the site, the four division method was used to discard superfluous soil samples. Then the samples were put into the polyethylene bags and sealed. Labels were attached inside and outside the bags, indicating the sampling number, name, depth, location, date and gatherer. Then the samples were taken back to the laboratory for low temperature preservation.

2.2 Extraction of total DNA

According to the instruction book of genomic DNA extraction kit produced by E.Z.N.A. Soil DNA Kit, the soil DNA was extracted. Then the purity and concentration of DNA were examined by 8g/L agarose gel electrophoresis.

2.3 PCR amplification

The diluted genomic DNA was used as a template. PCR was carried out through specific primers from 16S-V4 and ITS1 regions and equipped with Barcode [6]. PCR reaction conditions are: 94 degrees for 5 minutes; 94 degrees for 30 seconds, 56 degrees for 30 seconds and 72 degrees for 50 seconds repeated for 30 cycles; 72 degrees for 10 minutes. [7] PCR products were detected by agarose gel electrophoresis and then sequenced and analyzed by high throughput sequencing technique. Sequencing and bioinformatics analysis were commissioned to Beijing Novogene Corporation.

3. Research Results and Analysis

3.1 Microbial species diversity index

It can be seen that the Shannon index of bacteria was the highest in Yunyan 202, indicating that the diversity of bacteria was highest in Yunyan 202; the bacteria diversity of Yunyan 85 was the lowest. The Shannon index of fungi was the highest in Yunyan 202, indicating that the diversity of fungi was highest in Yunyan 202; the fungi diversity of Yunyan 85 was the lowest. The order of fungi diversity from high to low was Yunyan 202, Yunyan 87, K326 and Yunyan 85.

3.2 Distribution characteristics and abundances of microbial species

Bacteria distributed in the rhizosphere soil of 4 varieties of tobacco belonged to 2 phyla; the dominant groups were Proteobacteria (accounting for 95.39%) and Actinobacteria (accounting for 4.61%). Bacteria belonged to 4 classes: Alphaproteobacteria (accounting for 46.81%),

Gammaproteobacteria (accounting for 39.32%), Betaproteobacteria (accounting for 9.26%) and Actinobacteria (accounting for 4.61%). The top 10 genera were Pseudonocardia (accounting for 4.61%), Rhodoplanes (accounting for 15.68%), Kaistobacter (accounting for 25.19%), Sphingomonas (accounting for 5.94%), Burkholderia (accounting for 4.46%), Janthinobacterium (accounting for 4.79%), Marinobacter (accounting for 6.43%), Alcanivorax (accounting for 5.68%), Pseudomonas (accounting for 1.82%) and Rhodanobacter (accounting for 25.39%). The Pseudomonas bacteria accounted for 6.43% in Yunyan 202 and less than 0.05% in the other 3 varieties. Pseudonocardia accounted for 5.0%-6.5% in 4 species; the differences in abundances were not significant. The abundance of Janthinobacterium was highest in K326, accounting for 6.31%, but in Yunyan 202 it only accounted for 2.25%. The abundance of Sphingomonas was highest in Yunyan 87, accounting for 8.96%, while the proportions in other 3 varieties were 4.5%. The abundance of Burkholderia was highest in K326, accounting for 9.12%, while in Yunyan 202 the figure was only 1.78%. The proportions of Marinobacter were 12.48% in Yunyan 202 and 11.28% in Yunyan 85, but less than 1% in the other 2 species. The proportions of Alcanivorax were 14.32% in Yunyan 85 and 5.25% in Yunyan 202, but less than 0.1% in the other 2 species. The proportions of Rhodoplanes in 4 varieties were about 10%; the proportions of Kaistobacter were 28.61% and 29.76% in Yunyan 202 and K326 respectively, higher than the other 2 varieties. The abundance of Rhodanobacter was highest in Yunyan 85, accounting for 30.67%, and lowest in Yunyan 202, accounting for 12.86%. 5 genus of becteria, Pseudonocardia, Janthinobacterium, Sphingomonas, Burkholderia and Rhodanobacter can be found in all the 4 species, of which Pseudonocardia and Rhodanobacter had similar abundances in 4 species. The differences in species and abundance of dominant species were as following. In K326 and Yunyan 202, the dominant bacteria were Kaistobacter with the abundances of 29.76% and 28.61% respectively. The dominant bacteria in Yunyan 85 and Yunyan 87 were Rhodanobacter, with the abundances of 7.926% and 5.749% respectively. Pseudomonas could only be found in Yunyan 202. The abundances of Marinobacter were similar in Yunyan 202 and Yunyan 85, both around 11%, and less than 1% in K326 and Yunyan 87. The abundances of Alcanivorax were high in Yunyan 85 and Yunyan 202, but less than 0.1% in the other 2 varieties. Compared with the other 3 varieties, Yunyan 202 had Pseudomonas. The abundances of Marinobacter and Alcanivorax were similar in Yunyan 202 and Yunyan 85, while in the other two varieties these bacteria were scarcely.

Fungi found in the rhizosphere soil of 4 varieties of tobacco could be divided into 5 phyla, namely Ascomycota (accounting for 84.06%), unknown group (accounting for 7.65%), Zygomycota (accounting for 4.78%), Basidiomycota (accounting for 2.72%), and Chytridiomycota (accounting for 0.79%). They can be divided into 12 classes. The top 5 classes with highest abundances were Dothideomycetes (accounting for 49.67%), Sordariomycetes (accounting for 25.70%), Eurotiomycetes (accounting for 3.94%), Leotiomycetes (accounting for 1.16%) and unknown group (accounting for 7.65%). The top 10 genera with highest abundance were Pseudogymnoascus (accounting for 49.67%), unidentified group (accounting for 7.65%), Sordariales (accounting for 5.81%), Corynascella (accounting for 3.72%), Myrothecium (accounting for 3.37%), Chaetomium (accounting for 3.03%), Tremellomycetes (accounting for 2.71%), Chaetomiaceae (accounting for 2.61%), Penicillium (accounting for 2.56%), Retroconis (accounting for 2%) and Mortierella (accounting for 1.87%). The abundances of Myrothecium in the rhizosphere soil of Yunyan 202, Yunyan 85, Yunyan 87 and K326 were 1.73%, 1.44%, 2.06% and 3.76% respectively, with the highest abundance in K326, followed by Yunyan 87, and lowest in Yunyan 85. The abundances of Chaetomium in the rhizosphere soil of Yunyan 202 and K326 were similar, accounting for 3.78% and 3.13% respectively, while the abundances in Yunyan 87 and Yunyan 85 were relatively low, accounting for 2.12% and 1.40%. The abundance of Retroconis was highest in Yunyan 202, accounting for 3.81%, but in the other 3 varieties the figures were less than 0.1%. The distribution of Tremellomycetes was similar to that of Retroconis in the 4 species, with higher proportion in Yunyan 202 and almost no distribution in the other 3 varieties. The abundances of Penicillium were significantly different among 4 varieties; the abundance in the rhizosphere soil of Yunyan 202 was up to 4.83%, but the figure was only 1.51% in the rhizosphere soil of Yunyan 87. The abundances of

Sordariomycetes in Yunyan 202 and Yunyan 85 were less than 0.1%, but the proportions were nearly 6.10% in the other 2 varieties. The abundance of Corynascella was as high as 6.22% in Yunyan 202, but in Yunyan 85 the number was only 0.75%. The abundance of Sordariales was less than 0.1% in the soil of Yunyan 85, but up to 11.13% in Yunyan 202; in the other two species, the abundances were similar around 1.5%. Pseudogymnoascus was the common dominant bacteria of Yunyan 85, Yunyan 87 and K326 with the abundances of 52.88%, 42.53% and 29.84%. But in Yunyan 202 the figure was only 7.65%. Myrothecium, Chaetomium and Pseudogymnoascus were distributed in 4 species with different abundances. For example, the abundances of Myrothecium were 1.73% in the soil of Yunyan 202, 2.06% in the soil of Yunyan 87, 1.44% in the soil Yunyan 85 and 3.76% in the rhizosphere soil of K326. The dominant fungi of K326, Yunyan 85 and Yunyan 87 were Pseudogymnoascus, but the dominant fungi of Yunyan 202 were Sordariales. Retroconis and Tremellomycetes abundant in Yunyan 202 were hardly detected in the other 3 varieties.

4. Conclusion and Discussion

In this paper, the Illumina MiSeq high-throughput sequencing technique was used to study the microbial diversity of rhizosphere soil of tobacco for the first time; the method can analyze the microbial community structure from the perspective of genome, overcome the defects of low flux of the first generation Sanger sequencing method, and break the technical bottleneck of isolated culture of microorganisms. [8] This study found that the effective sequence of bacterial OTU from high to low was Yunyan 85, Yunyan 202, Yunyan 87 and K326; the fungi sequence was Yunyan 202, Yunyan 87, K326 and Yunyan 85. The dominant bacteria, Kaistobacter, accounted for 6.112% and 5.489% in K326 and Yunyan 202 respectively, far exceeding the other genera. The dominant bacteria, Rhodanobacter, accounted for 7.926% and 5.749% respectively in Yunyan 85 and Yunyan 87, much more than other bacterial species. The dominant fungi of K326, Yunyan 85 and Yunyan 87 were Pseudogymnoascus, while the dominant fungi of Yunyan 202 were Sordariales. Pseudogymnoascus is a kind of cold resistant bacteria which can produce cellulose enzyme, and has strong tolerance to chilling. [9] Its abundances in Yunyan 85, Yunyan 87 and K326 indicate that the three tobacco varieties can resist cold weather better than Yunyan 202. From Shannon index, the bacterial and fungal diversities of rhizosphere soil of Yunyan 202 were higher than those of the other three species. Pseudonocardia, Janthinobacterium, Sphingomonas, Burkholderia and Rhodanobacter could be found in the soil of all species. Pseudomonas was found only in the rhizosphere soil of Yunyan 202. Marinobacter and Alcanivorax were abundant in Yunyan 202 and Yunyan 85, but extremely rare in other 2 species. The fungi of Myrothecium, Chaetomium and Pseudogymnoascus could be found in the soil of all species. There are no papers about the microbial diversity of rhizosphere soil of Yunyan 202. [10] This study found that the microbial diversity of rhizosphere soil of Yunyan 202 was higher than that of the other three varieties. That issue deserves further research.

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