Full Length Article

Changes and Diversity in Bacterial Community Structure in Soybean Rhizosphere at Different Growth Stages

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Abstract

Composition and quality of soil are influenced by rhizosphere microbial number and species, with direct impact in plant growth and development. In order to evaluate alterations in bacterial communities and diversity in soybean rhizosphere at different growth stages soil samples were collected in June (early growth period) and September (late growth stage). Illumina high-throughput sequencing studies were used to determine the change of 16S rRNA V3+V4 sequence of bacterial communities. ANOVA was used to analyze the composition of species, and the alpha index to study bacterial diversity. Results showed differential bacterial abundance and diversity at different development stages; particularly, abundance and diversity indexes of bacteria at later growth stages were higher than those at early ones. Furthermore, *Proteobacteria, Actinobacteria,* and *Acidobacteria* represented the dominant bacteria in soybean root, but bacterial composition significantly varied depending on the development stage of the plant. © 2019 Friends Science Publishers

Key words: Bacterial flora; Development phase; Illumina high-throughput sequencing; Soybean rhizosphere soil

Introduction

Rhizosphere microorganisms play an important role in soil ecology, and contribute to the soil fertility index (Chen *et al.*, 2003). Composition and transformation of soil biochemical activity and nutrients are directly influenced by the rhizosphere microbial number and species (Preston *et al.,* 2002; Xu *et al.,* 2014). There are a number of factors influencing the structure of rhizosphere microorganisms, including type and development stages of plants and their individual genotypes (Marschner *et al*., 2001). Fan (2010) reported that during plant development the number of bacteria and fungi increased at the beginning, but it decreased with time; however, *Actinobacteria* number increased with plant development. Xu *et al.* (2008), using PCR-DGGE technology, found that soybean genotypes did not contribute to the bacterial community structure. Furthermore, Zhang (2008), using traditional culture methods, showed that lily rhizosphere bacteria rapidly increased during vegetative growth, but decreased during bud development. At present, there is marginal information about the effects of different growth stages on rhizosphere microorganisms load and type. Therefore, the aim of the present study was to investigate alterations in soybean rhizosphere bacterial communities and diversity at different plant growth stages. Soybean is an important crop of economic importance worldwide; about 10% is directly used for human consumption, 20% for its oil, and 70% in livestock feed (Susser and Uzzell, 1991; Li *et al*., 2016).

Research on microbial diversity is limited due to the extent of cultivable microorganisms (Kamagata and Tammaki, 2005), which can be overcome using 16S rDNA molecular analysis techniques, such as PCR-RFLP, DGGE/TGGE, and SSCPP; however, disadvantages of such technologies include low detection limits, large workloads, and low sensitivity (You *et al*., 2014). In contrast, high-throughput sequencing technology (HTST) has the advantages of producing massive sequence data, in less time, low costs, and high accuracy (Quail *et al*., 2012). HTST is the sequencing platform developed by Illumina, Roche454, and Life Technologies (Logares *et al*., 2012; Lou *et al*., 2014). In particular, Illumina HiSeq 2500 can produce 600G data in 7 days (Quail *et al.*, 2012). The present study was carried out to evaluate alterations in bacterial communities and diversity in soybean rhizosphere at different growth stages.

Materials and Methods

Soil Samples

Soybean rhizosphere soil samples were collected in June (DWA sample) and September (DYA sample) of 2016, in

Xianyang City, Shaanxi Province by the S-shaped sampling method. Samples were then thoroughly mixed, divided into three sets, and kept in plastic bags at -20°C, until use (Table 1).

Soil DNA Extraction

Soil DNA was extracted using a PowerSoil® DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, C.A.), following manufacturer's instructions. DNA concentration and purity were measured on 1.8% agarose gels.

High-throughput Sequencing

Primers 338F/806R amplified the V3 + V4 region within the 16S rDNA gene (Zhang *et al.,* 2015). PCR reactions were carried out in 50 μL reactions containing 0.2 *μ*L Q5 high-fidelity DNA polymerase, 10 *μ*L high GC enhancer, 10 μL buffer, 10 μM forward and reverse primers, and 40 ng of template DNA under an initial denaturation at 95°C for 5 min, followed by 15 cycles for 16S rDNA. Each cycle involved denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, with a final elongation step at 72°C for 7 min. Pyrosequencing was performed on an Illumina HiSeq 2500 at Biomarker Technologies (Beijing, China).

Bioinformatics Analysis

After the sequencing was completed, FLASH v1.2.7 software was used to splice the reads of each sample by overlap, resulting in raw tags. Clean tags were obtained by filtering raw tags using the Trimmomatic v0.33 software. UCHIME v4.2 software was used to identify and remove chimera sequences and obtain effective tags (Edgar *et al.*, 2010). Tags were then clustered at 97% similarity using UCLUST (Wang *et al.,* 2012) in QIIME (version 1.8.0) software to obtain operational taxonomic units (OTUs) and taxonomical annotations based on Silva taxonomy databases.

Results

Sample Sequence Number Characteristics

Sequence information and OTU statistical analyses of samples are shown in Table 2. The sequencing was 280–460 bp in length. A total of 109803 bacterial sequences were obtained from the soil samples; 57996 and 51807 bacterial sequences were respectively obtained from DWA and DYA samples. A total of 1919 OTUs was clustered in the two samples. The curve tends to be flat, indicating that the amount of sequencing data is reasonable and that more data volume has a marginal contribution to finding a new OTU (Fig. 1).

Bacterial Community Structure

The species composition analysis reflects the community structure of the sample at different taxonomic levels. The relative abundant bacterial sequences were classified in the following phyla: *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia*, *Nitrospirae*, *Planctomycetes*, and *Armatimonadetes*. Differential abundance of these bacterial phyla in DYA and DWA samples can be observed in Fig. 2. There were statistically significant differences between most phyla in DWA and DYA samples, except for *Actinobacteria*, *Planctomycetes*, and *Armatimonadetes* (Table 3).

Microbial Diversity Indexes

Bacterial alpha diversity in DWA and DYA samples was determined by the Shannon, Simpson, Ace, and Chao indexes, as shown in Table 4. According to Shannon and Simpson indexes, bacterial diversity was shown to be higher in DYA than in DWA soil samples (Table 4).

Nonmetric Multidimensional Scaling (NMDS) Analysis

NMDS analysis is often used to compare the differences between sample groups. Samples NMDS diagram is shown in Fig. 3, where distribution of sample points represents the degree of similarity between samples. As seen in Fig. 3, DYA and DWA samples are placed in different quadrants, indicating low similarity.

Discussion

Bacteria represent an important part of soil microorganisms; their species and load have a direct impact on soil biochemical activity and nutrients. In addition, Illumina HTST is fast, convenient, accurate, and is widely used in the study of soil microbial diversity. In the present study, bacterial community structure and diversity in soybean rhizosphere soils at two different growth stages were evaluated. Results showed that the coverage index of each sample was above 99%, which proves that the sequencing structure can reflect the real situation of the sample.

In this study, 25 phyla were obtained, being the predominant phyla *Proteobacteria, Acidobacteria,* and *Actinobacteria*; *Proteobacteria* was the most abundant bacterial group. Yuan *et al*. (2015) the bacterial community structure in paddy soils and found that *Proteobacteria, Acidobacteria,* and *Chlorophyta* were the dominant bacteria in, followed by *Actinomycetes.* In addition, Jangid *et al*. (2008) showed that *Proteobacteria* was the dominant genus in different types of cultivated soils, followed by *Acidobacteria*, and Yang *et al*. (2015) reported that the main flora in different mixed forest soil bacteria were *Proteobacteria*, *Acidobacteria*, *Actinomycetes*, and *Chlorophyta*. Furthermore, Niu *et al*. (2017) observed that *Proteobacteria* was the dominant (28.56%), followed by *Actinomycetes* (17.2%), and *Bacteroidetes* (12.3%)

microorganisms found in saline-alkali soil in Hexi Corridor. In the present study, it was observed that *Proteobacteria* was the dominant, followed by *Acidobacteria, Actinomyces*, and *Chlorophyta* microorganisms in the rhizosphere soil, which may be due to factors such as geography, ecological diversity, soil nutrition, and crops type.

Alpha diversity reflects the species diversity within a single sample. The Chao1 and Ace indexes show the number of species in the community, but do not indicate their abundance, whereas the Shannon and Simpson indexes were used to measure community diversity. Species abundance and species evenness in the sample community were affected by Chao1, Ace, and Shannon indexes. The smaller the Simpson index, the higher the species diversity of samples. Chao1, Ace, and Shannon indexes showed that DWA was higher than DYA, whereas Simpson index showed that DWA was lower than DYA. It was observed that the Chao1 index trend in all samples was different from that of Simpson index; Haegeman *et al.* (2013) suggested that adding the low abundance sequence to the Chao1 index leads to an undervaluation of diversity, and this underestimation will increase with the number of species in the population, which may be different in our study. Diversity index might be the main reason for the inconsistent results.

NMDS is a non-linear model that better reflects the differences between samples, as compared with linear models such as PCA / PCoA. It was observed that stress value was cero, which indicates that NMDS analysis is highly reliable. Our results showed that DWA and DYA were placed in different quadrants, indicating a low similarity between the two samples, and that there was a certain variability in the bacterial community structure at different growth stages. In addition, a number of studies have confirmed that the amount of rhizosphere microbes and community structure will change at different growth stages. Duineveld *et al.* (2001) found a significant variation in young and mature plants rhizosphere soil microbial community structure. Furthermore, Farina *et al.* (2012) reported that the rhizosphere microbial community structure of rape seeds varied at different growth stages, and that the rosette stage had the highest bacterial abundance. Using the DGGE technique, Zhang (2014) studied bacterial communities in soybean rhizosphere, finding that the bacterial diversity in full flowering stage was higher than that in mature stage. In addition, Xu *et al.* (2009) and others based on the DGGE map showed that rhizosphere bacteria diversity first increased and then decreased, beginning from the early-flowering stage to the maximum drumming stage, the lowest maturity. The present study found that the late growth stage of soybean showed higher microbial diversity than that at the early growth stage. Diversity and abundance of soilborne bacteria are influenced by abiotic and biotic factors; however, few studies have evaluated these bacterial characteristics at early and late stages of plant development and growth. Factors such as soil sampling and nutrition, crop varieties, geography, and

Table 1: Classification of soil samples

Sample	Time
DWA	2016.6
DYA	2016.9

Table 2: Seg information and OTU statistical analysis of samples

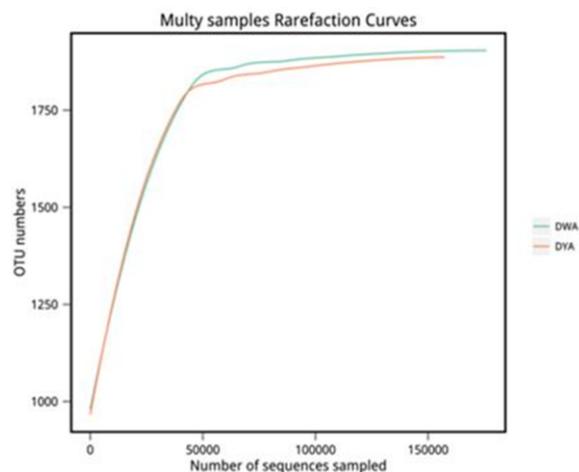| Sample ID	OTU	Seg
DWA	1847	57996
DYA	1804	51807
Total	1919	109803

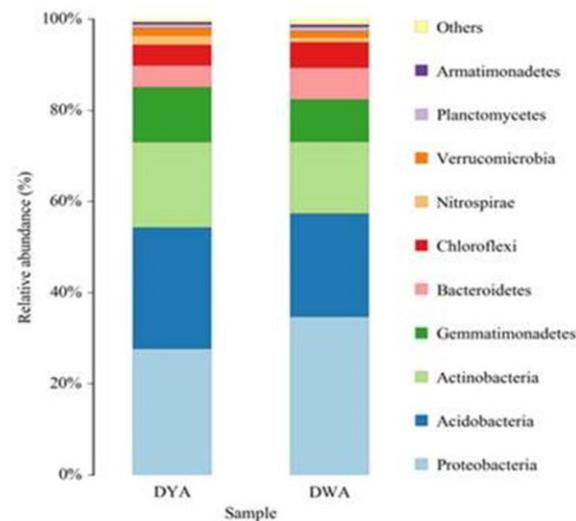**Fig. 1:** Bacteria sequencing in soybean rhizosphere soil samples.

Fig. 2: Relative abundances of microbial phyla in different soil samples

environmental factors may underestimate the size and diversity of microbial communities.

Conclusion

In this study, high-throughput sequencing was used to

investigate the bacterial community structure in soybean rhizosphere soil at two different growth stages. It was

Table 3: Comparison of the relative abundances of major flora at the phylum level

Taxon	*P* Value	Q	DWA:DYA（*P* value）
Actinobacteria	0.118938	0.156497	-
Proteobacteria	0.000881	0.00734	< 0.001
Acidobacteria	0.001117	0.006984	< 0.01
Gemmatimonadetes	0.001442	0.006009	< 0.01
Bacteroidetes	0.002821	0.007836	< 0.01
Chloroflexies	0.019238	0.036997	< 0.02
Verrucomicrobia	0.028427	0.044417	< 0.05
Nitrosipirae	0.002732	0.008537	< 0.01
Planctomycetes	0.128375	0.160468	-
Armatimonadetes	0.346699	0.393976	-

Table 4: Alpha diversity of samples

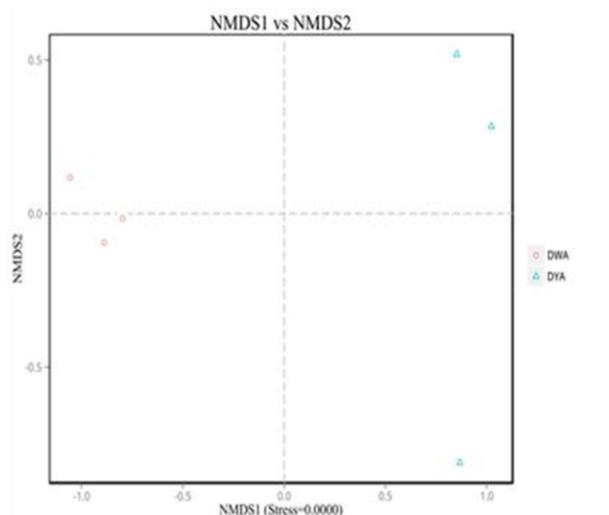| Sample ID	Shannon	Simpson	Ace	Chao	Coverage
DWA	6.289446	0.005808	1911.490588	1915.968750	0.9979
DYA	6.442155	0.004279	1900.530122	1908.023256	0.9978

Fig. 3: Non-metric multidimensional scaling analysis

observed that soil diversity indexes were higher in late growth stage, as compared with those at early ones, which indicates that soybean growth stage is significantly influenced by the bacterial community structure in rhizosphere soil. This study provides a theoretical basis for understanding the alterations and diversity in bacterial community structures in rhizosphere.

Acknowledgments

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