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Diversity Analysis of Endophytes in Wheat Infected by Powdery Mildew

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Abstract

Common wheat (*Triticum aestivum* L.) is one of the most important food crops. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most serious diseases on wheat. In this study, the changes of endogenous bacteria in root, stem and leaf tissues of wheat infected and uninfected with powdery mildew were measured based on 16S rDNA. Integration, OTU cluster analysis, taxonomic analysis, diversity index, Shannon-Index curve, Rank-Abundance curve and PCoA analysis were carried out for each sample, and the roots, stems and leaves of different tissue parts were classified and summarized. The results showed that the infection of wheat powdery mildew had a certain effect on endophytic bacteria in stem tissue. There are also differences in the control and treatment of leaf tissue and root tissue. This indicated that endophytic bacteria were distributed differently in different parts of wheat.

Keywords

Common Wheat, Powdery Mildew, 16S rDNA, Endophytic Bacteria

1. Introduction

Common wheat (*Triticum aestivum* L.), one of the most important staple food crop, is widely planted in most temperate countries all over the world. Its annual global production reaches about 600 million tons and, thus, plays the most fundamental role in food security to human beings and economic activities [1]. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is one of the most serious diseases on wheat, especially in these wheat-growing regions with cool and humid climates, and critically threatens the wheat safety production worldwide [2]. In the last decade, the proper management measures can retrieve

about 1.5 million metric tons of yield losses, and the actual annual grain loss caused by *Bgt* was limited to 0.3 million metric tons [3]. Despite that, it is still urgent to develop alternative management approaches for durable and effective control of *Bgt* disease.

Endophytic bacteria are defined as those bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plants and that are not harmful to the host plants [4]. Many endophytic bacteria ubiquitously inhabiting in plant species have been isolated from a variety of plants [5]. And researchers have indicated that similar to plant growth-promoting rhizobacteria, endophytic bacteria can promote plant growth and suppress plant diseases probably due to their benefit contribution [6]. The portion of microbial diversity suggested by conventional cultivation techniques is less than 1% of the present bacterial species [7]. Therefore, to fully understand the bacterial community, recent years culture-independent molecular approaches based on 16S rDNA analysis strategies, including amplified ribosomal DNA restriction analysis, denaturing gradient gel electrophoresis, and terminal restriction fragment length polymorphism have been successfully developed and used in the analysis of bacterial community to a great variety of environments, for example, the soil ecosystems [8] [9], marine [10] [11], food [12], and human intestine [12].

The analysis based on 16S rDNA plays an important role in the classification [13] (Zou *et al*., 2023) and identification of micro-organisms and micro-ecological research. Although bacterial community analysis has been performed to multiple environment and variety species, it is still much unclear about the bacterial diversity on wheat, especially during disease development processes. There is no previous research has been done on endophytic bacteria in the context of powdery mildew-infected wheat plants. The objective of this study was to investigate the endogenous bacterial changes in roots, stems, and leaves tissues of powdery mildew infected and non-infected wheat plants. This will provide effective biological resources for the study of biological control of wheat powdery mildew.

2. Materials and Methods

2.1. Plant Growth and Sample Collection

Seedlings were grown in the chamber under controlled temperature (25˚C), humidity (90%), and light conditions (16 hours of light and 8 hours of darkness). When the third leaves were fully expanded, seedlings were inoculated with powdery mildew (race E20). Seven days later, infected and control tissue samples from seedling roots, stems and leaves were collected. Each sample has three biological replications. Each replication was mixed with the tissues from 5 individual plants. A total of 9 samples were used for further processing.

2.2. Surface Sterilization

The wheat roots, stems and leaves samples were cleaned with tap water for remove soil particles. For surface sterilization, wheat roots, stems and leaves tis-

sues were put in sterile falcon tubes and washed with sterilized water for 1 min, soaked in 1% sodium hypochlorite solution for 5 min, washed with 75% ethanol for 1 min, and then washed with sterilized water for five times. The surface sterilized tissues were then crushed in sterile mortar and suspended in sterile saline solution (2% NaCl). Suspensions were incubated for 2 h at 28˚C under shaking saline plated in duplicates onto Nutrient agar (NA). Peptone sucrose agar (PSA) was supplemented with 0.01% cycloheximide. Besides the washed wheat roots, stems and leaves samples, the last washed water was also spread onto different media plates for confirming the surface sterilization effect.

2.3. DNA Extraction

The genomic DNA of wheat roots, stems, and leaves in each group were extracted using E.Z.N.A.HP Plant DNA Kit (OMEGA Bio-Tek, USA, https://www.omegabiotek.com/). Total DNA concentration and purity were monitored on 1% agarose gels (Biowest Agarose, http://www.genehk.com).

2.4. Amplicon Generation and Illumina HiSeq Sequencing

The primers 335F (5'-CADACTCCTACGGGAGGC-3') and 769R (5'-ATCCTGTTTGMTMCCCVCRC-3'), which targeting the V3 and V4 hyper variable regions of bacterial 16S rDNA genes, were selected for bacterial taxa analysis [14]. Both forward and reverse primers were tagged with adapter, pad, and linker sequencing. The suitable system of PCR was 50 μL in which containing 0.2 μL Q5 High-Fidelity DNA Polymerase (NEB, USA), 10 μL High GC Enhancer (NEB, USA), 10 μL Buffer, 1 μL dNTP, 10 μM of forward and reverse primers, and 40 ng template DNA. The procedures were as follows: an initial denaturation at 95˚C for 5 min, each of 15 cycles at 95˚C for 1 min, 50˚C for 1 min, and 72˚C for 1 min, with a final extension at 72˚C for 7 min. Finally, the library was sequenced on an Illumina Hiseq2500 platform at Biomarker Technologies Co, LTD, Beijing, China.

Following amplification, 2 μL of PCR product was used to verify successful amplification by 2% agarose gel electrophoresis. The products of triplicate PCR reaction from each group were combined and the pooled mixtures were purified with Gel Extraction Kit (OMEGA Bio-Tek, USA, www.omegabiotek.com) and analyzed on an Agilent 2100 Bioanalyzer using High Sensitivity DNA Chips (Agilent Technologies, Germany) for size distribution. The sequencing libraries were generated using NEB Next Ultra DNA Library Prep Kit for Illumina (NEB, USA) following manufacture's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific, USA) and Agilent Bioanalyzer 2100 system (Agilent Technologies, Germany).

2.5. Combination and Data Preprocessing

The forward and reverse sequences were merged by overlapping paired-end reads using FLASH (V1.2.11, http://ccb.jhu.edu/software/FLASH/) [15]. All se-

quence reads with the same tag were assigned to the same sample according to the unique barcodes (raw tags). The raw tags were further strictly filtered by previous methods (clean tags) [16], and the quality of clean tags were detected by Qiime (V2.1.0, http://qiime.org/index.html) [17]. The low quality tags were removed. The tags with chimera were detected and removed using UCHIME Algorithm (V4.2, http://www.drive5.com/usearch/manual/) [18]. The effective sequences were then clustered into operational taxonomic units (OTUs) at 97% sequence similarity using the UPARSE-OTU and UPARSE-OTUref algorithms of UPARSE software package (Uparse v7.0.1001, http://drive5.com/uparse/). The indices of alpha diversity were calculated based on the above algorithms [19]. Finally, the RDP (Ribosomal Database Project) Classifier (V2.2, https://sourceforge.net/directory/windows/) was used to assign representative sequence to the microbial taxa [20]. Sequence data have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number SRP056016 (16S).

2.6. Statistical Analysis

Cluster analysis was preceded with principal component analysis (PCA) using the QIIME (V1.8.0) software package [21]. QIIME calculates both weighted and unweighted unifrac distances, which are phylogenetic measures of beta diversity [22]. Phylogenetic relations among different microbial taxa were further displayed by KRONA [23]. Alpha diversity indices of Chao1, ACE, Shannon, Simpson, and coverage were calculated to reflect the diversity and richness of the endophytic community in different samples [24].

3. Result

3.1. Overview of 16S rDNA Gene Sequencing Data

To fully understand the bacterial community, we performed 16S rDNA sequencing to wheat roots, stems, and leaves tissues under *Bgt* (*Blumeria graminis* f. sp. *tritici*) infection and un-infection conditions (**Table 1**, samples designated as PM-Root, CK-Root, PM-StemPM-Stem, CK-Stem, PM-Leaf, and CK-Leaf, respectively). Totally, we obtained 1,424,920 sequencing reads and, among them, 1,317,962 reads were raw tags. After splicing the original data, quality filtering the spliced sequences, and removing the chimeras, finally we obtained 1,121,671 high quality tag sequences, with a range from 150,591 to 217,010 sequences per sample. The average length of effective bacterial sequences was 437 bp (**Table 1**). Overall, at a similar level of 97%, these sequence reads were classified into 1279 Operational Taxonomic Units (OTUs), with a range from 422 to 943 OTUs per sample (**Table 1**). Generally speaking, these OTUs were divided into 20 Phylum, 58 Classes, 91 Orders, 165 Families, 277 Genus and 197 Species. And wheat samples were dominated by those OTUs taxonomy into *Proteobacteria* (60%), *Bacteroidetes* (20%), *Acidobacteria* (16%), *Firmicutes* (13%), and *Actinobacteria* (10%). Rarefaction curves, showing the increasing number of detected OTUs

with the increasing number of obtained sequences per sample, were obtained for the three tissues upon *Bgt* infected and uninfected conditions, as shown in Figure 1.

3.2. Diversity

Further analysis revealed that, in leaves, we detected the most OTUs (1701), and close number of OTUs were detected in powdery mildew infected and uninfected leaves (854 and 847 respectively). The detected number of OTUs in root samples was 1406, with 726 and 680 OTUs in infected and uninfected roots respectively. OTUs in the stem samples was the lowest (1365) and obvious difference of OTUs number was observed between infected and uninfected powdery

Table 1. The characteristics of effective tags from samples of wheat sprouts, stems, and roots.

Sample	PE Reads	Raw Tags	Effective Tags	Average length (bp)
CK-Root	239,137	229,394	217,010	442
CK-Stem	231,891	210,209	186,232	443
CK-Leaf	239,920	216,458	151,005	422
PM-Root	239,618	229,477	207,497	443
PM-Stem	234,557	216,697	195,485	448
PM-Leaf	239,797	215,727	150,591	422
Total	1,424,920	1,317,962	1,107,820	2620

CK represents uninfected wheat powdery mildew, PM represents infected wheat powdery mildew.

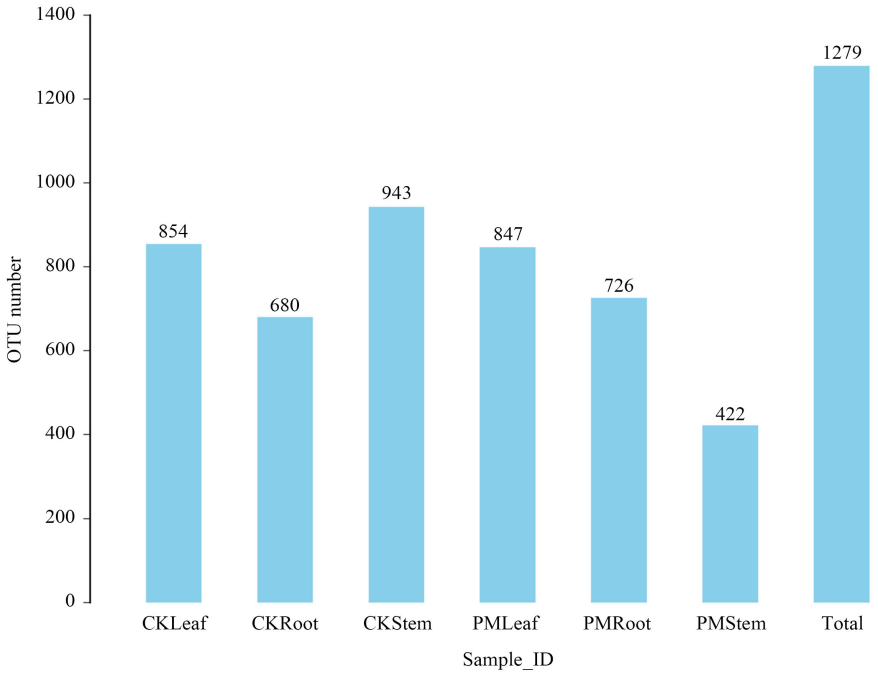


Figure 1. OTU quantity and distribution of different samples.

mildew (422 and 943 respectively). Comparingly, it seemed the disease happening had different effect on the distribution of OTUs in root and stem tissues (Figure 1). The overlap and unique OTUs between infected and uninfected samples of three tissues were further analyzed. The results showed that 122 OTUs were overlapped by the six treatments (Figure 2). Meanwhile, 15, 7, 0, 0, 5, and 6 unique OTUs were detected in PM-Root, PM-StemPM-Stem, PM-Leaf, CK-Root, CK-Stem, and CK-Leaf samples, suggesting that the OTUs diversity are some differences among samples.

At the *Phylum* level, the heat map was drawn according to the species annotation and abundance information (Figure 3). It showed that samples from same tissues were clustered together, suggesting that OTUs have some tissue specific manners. However, as can be seen from Figure 3, the abundance was fair difference between infected and uninfected samples, suggesting the impact of disease happening on OTUs community.

3.3. *Bgh* Impacts the Bacterial Community

Consist with the highest Chao1 index and ACE index (992.2143 and 972.3016 respectively), the most OTUs were detected in CK Stem sample (943), indicating that the endophytic bacteria community in leaf was more abundant and diversity. However, there are a minimum of 422 OTU clusters of endophytic bacteria in PM-Stem. From the perspective of community richness index (Chao1 index and ACE index), the Simpson index and Shannon index of the sample are 0.0641 and 3.3244, respectively (Figure 4).

This may be due to the changes in endophytic bacterial species affected by wheat powdery mildew, thereby regulating the migration of its internal mechanisms. Among the number of O T Uin the leaf tissue samples, the number of OTU infecting wheat powdery mildew was more than the number of OTU in the endophytic bacteria clustering of uninfected wheat powdery mildew. In brief, our results suggested that the pathogens that infect wheat powdery mildew and uninfected wheat powdery mildew have the highest bacterial abundance and uniformity.

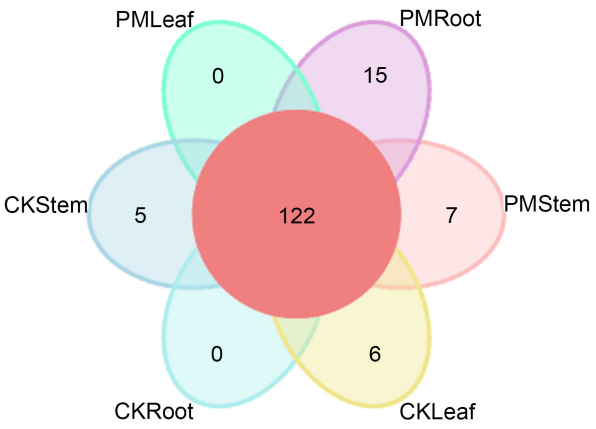


Figure 2. OTUs overlapping petal map of endophytic bacterial communities in different tissue parts.

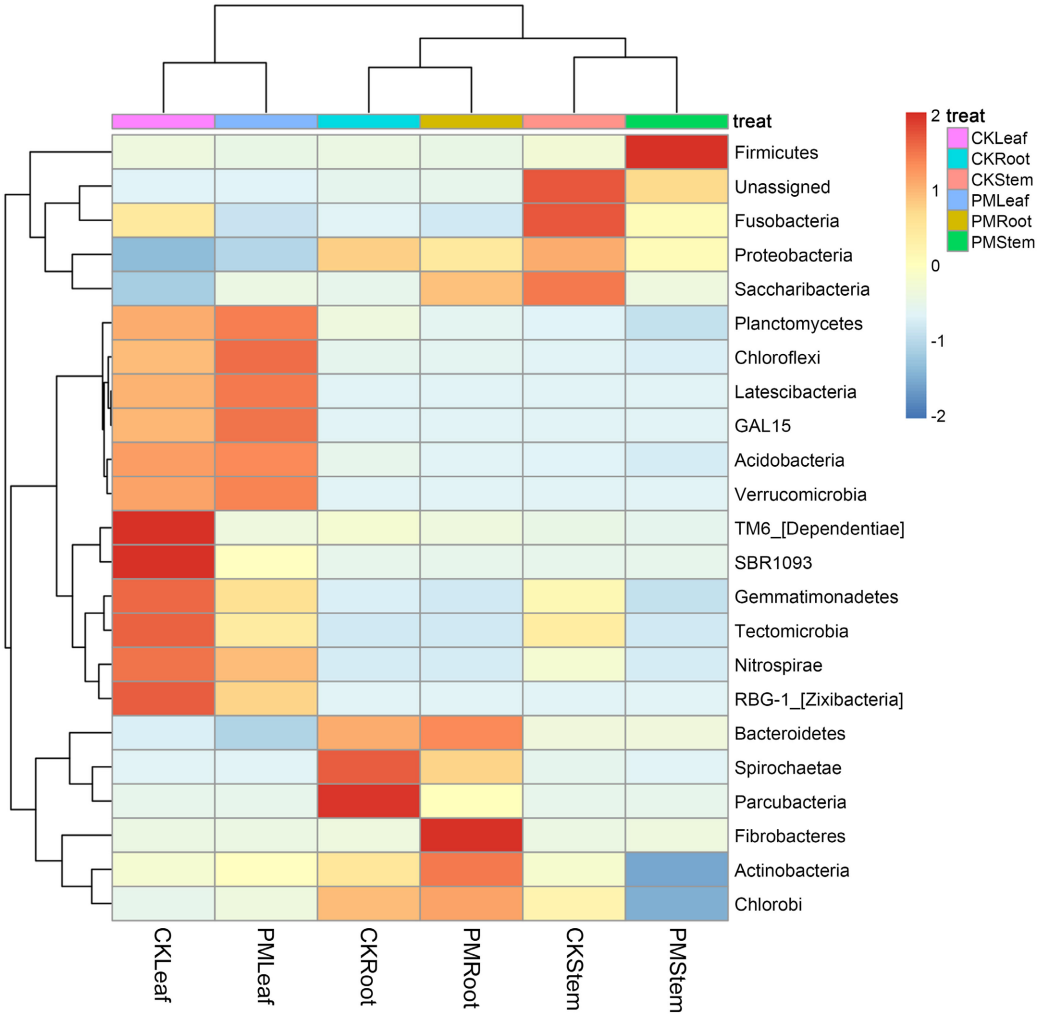


Figure 3. Abundance and clustering analysis at Phylum level of endophytic bacterial in different tissue samples.

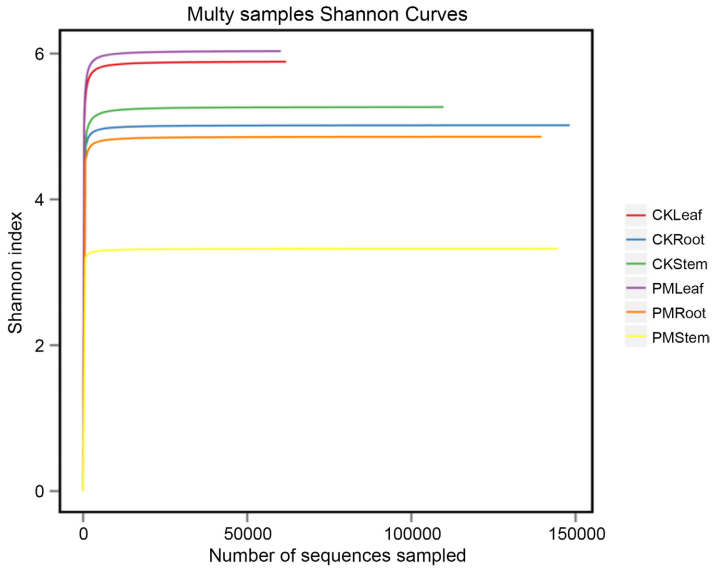


Figure 4. Shannon-Index curves of curves of different infestation sites.

Due to the large difference in the community structure of endophytic bacteria between different parts of the sample, when the Rank-Abundance curve was drawn, the roots, stems and leaves of the infected and uninfected pathogens were drawn according to the classification criteria. The Rank-Abundance curve of endophytic bacterial diversity, in which the CK-Stem sample curve is the most gradual, and the curve has the largest span on the X-axis, indicating that the endophytic bacteria have the highest abundance and the endophytic bacteria distribution is the most. Evenly. The PM-Stem sample curve has the narrowest span and the steepest trend, which indicates that the endophytic bacteria in the stem tissue is the lowest after infestation of wheat powdery mildew. The CK-Leaf and PM-Leaf tissue samples were relatively flat, and the span on the X-axis was relatively close. The CK-Root and PM-Root were also close, indicating that the leaf tissue samples had less difference in richness (Figure 5, Table 2).

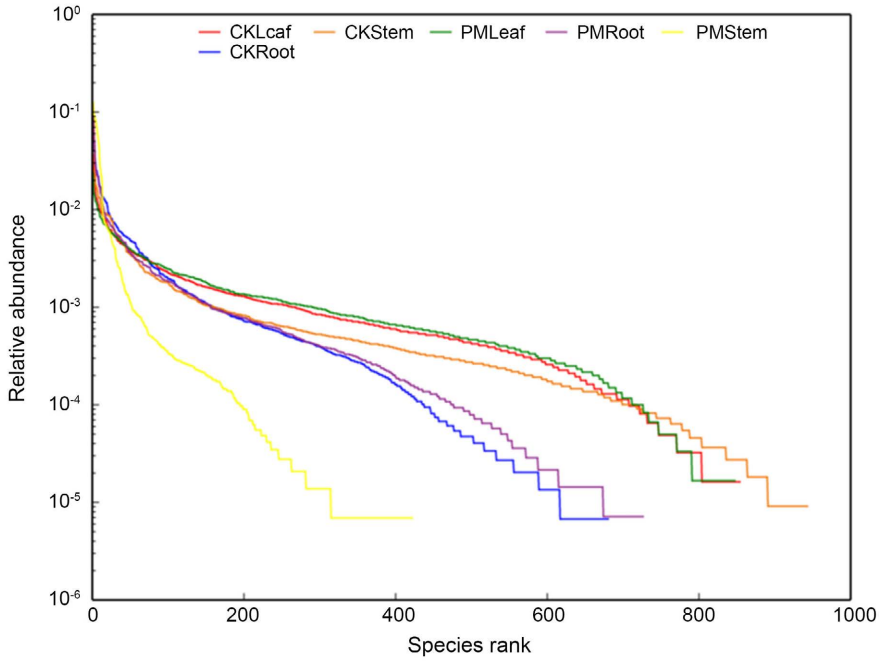


Figure 5. Rank-Abundance curves of different infestation sites.

Table 2. Alpha diversity index statistics.

Sample ID	OTU	ACE	Chao1	Simpson	Shannon	Coverage
CK-Leaf	854	885.467	891.5	0.0059	5.8876	0.9992
PM-Leaf	847	890.1842	923.0	0.0041	6.0327	0.9991
CK-Root	680	723.0841	749.5172	0.0149	5.0169	0.9996
PM-Root	726	763.5968	748.9667	0.0266	4.8592	0.9996
CK-Stem	943	972.3016	992.2143	0.0202	5.2653	0.9995
PM-Stem	422	659.0074	591.9412	0.0641	3.3244	0.9993

3.4. Beta Diversity Analysis

Beta diversity analysis was performed using QIIME software to compare the similarity of species diversity in different samples. Beta diversity analysis mainly uses binary jaccard, brays curtis, weighted unifrac (bacteria limited), unweighted unifrac (limited bacteria) and other four algorithms to calculate the distance between samples to obtain the beta value between the samples. Principal coordinates analysis (PCoA [25]), is a similar dimensionality reduction method to PCA, which can be used to classify multiple samples and reveal the diversity of species diversity among the samples.

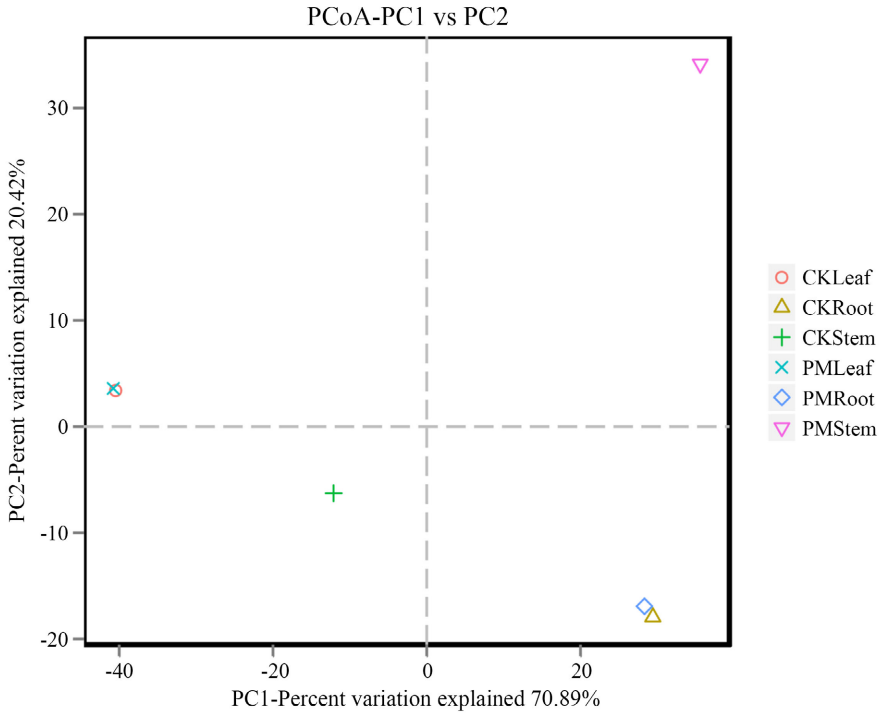
The PCoA map was drawn based on the endophytic OTUs of different tissues to reflect the endophytic Beat diversity of all samples. As a result, it was found that the stem (CK-StemCK-Stem, PM-Stem) sample was on the PC1 (70.89%) axis in **Figure 6(a)** and the PC1 (50.86%) axis in **Figure 6(b)**, and the PC1 (66.41%) axis in **Figure 6(c)** and **Figure 6(d)**. PC1 (61.73%) was well separated from the other two sets of samples on the axis, while the other two samples were basically inseparable. The number of reads of CK-StemCK-Stem and PM-Stem is quite different, while the difference between CK-Leaf and PM-Leaf, CK-Root and PM-Root is small (**Table 1**), so it is inevitable for stems. For roots and leaves, the difference between difference and similarity is a lower level, CK-Root and PM-Root are separated on the PC1 (61.73%) axis in **Figure 6(d)**, but the distance is closer. This indicates that wheat powdery mildew has an effect on the distribution of endophytic bacteria in roots, stems and leaves of wheat, and has a great influence on stem tissue parts.

3.5. Significant Differences between Groups

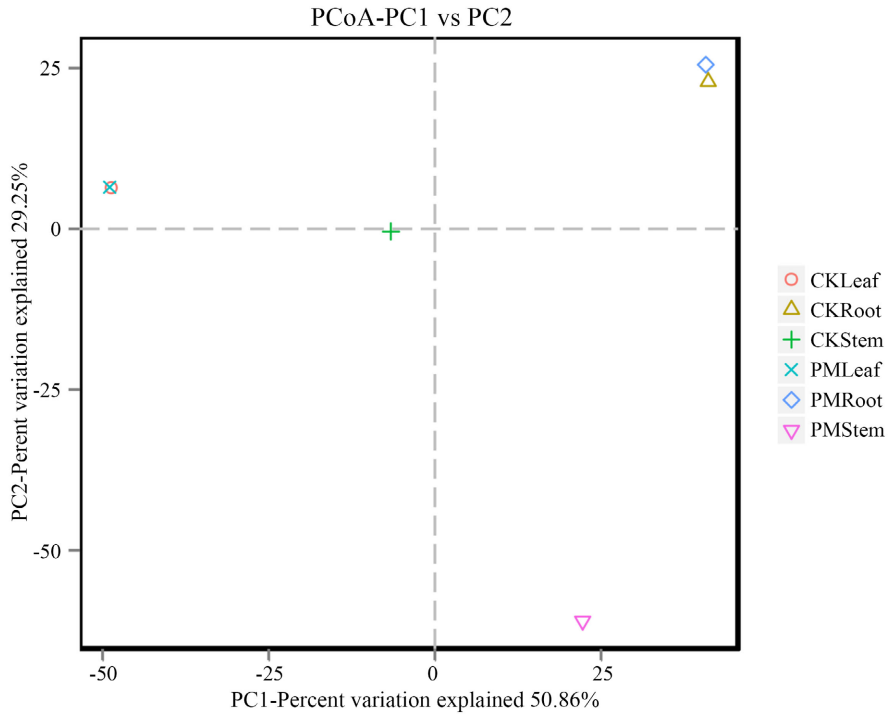
The significant difference between groups was mainly used to find Biomarker with statistical differences between different groups. According to the set Biomarker screening criteria (LDA score > 4), the eligible Biomarker was found and displayed as an icon, LEFSe (Line Discriminant Analysis (LDA) Effect Size [26]) is able to find statistically significant Biomarker between different groups (**Figure 7**, **Figure 8**). In the uninfected wheat powdery mildew mycorrhizal was enriched with *Devosia*, *Cytophagia*, *Cytophagaceae*, *Cytophafales*, *Streptomyces* and *Streptomycetales* (6 groups in totsl), the stems were enriched with *Proteobacterio*, *Burkhoideriales* and *Comamonadaceae* (3 group in total), the leaves enriched with *Blastocatellales*, *Nitrospirae*, *Desulfurellaceae* and *Latescibacteria* (4 groups in total), the fine lineages had an LDA value of 4 or higher in above groups (**Figure 5**). In infected wheat powdery mildew, the leaves were enriched with Acidobacteria, Chloroflexi, Gemmatimonadetes, Rhodospirillales, Nitrospirales, Sphingomonadales, Acidimicrobiales and Xanthomonadales, the roots were enriched with Myxococcales, Actinobacteria, Streptomycetac, Xanthomonadales, Hyphomicrobiaceae, etc. The stems were enriched with Gammaproteobacteria, Pseudomonas, Enterobacteriales, Staphylococcaceae, Brucellaceae, Ochrobactrum, Candidatus, etc. (**Figure 6**).

4. Discussion

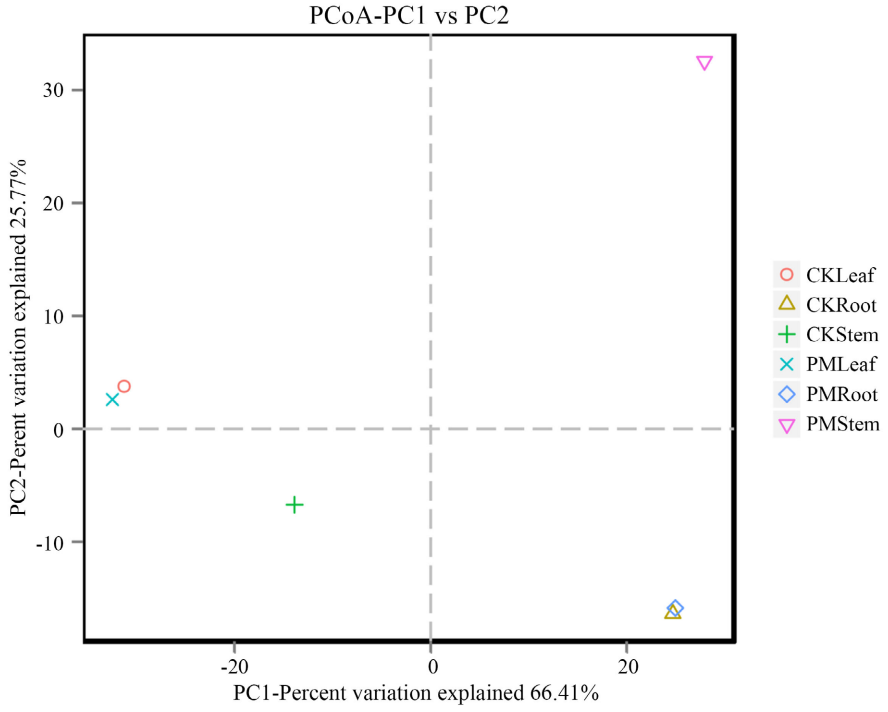
16S rDNA has long been used in microbial identification, but 16S rDNA analysis using high-throughput sequencing first appeared in 2006 [27]. Illumina MiSeq second-generation sequencing technology has been applied to the study of



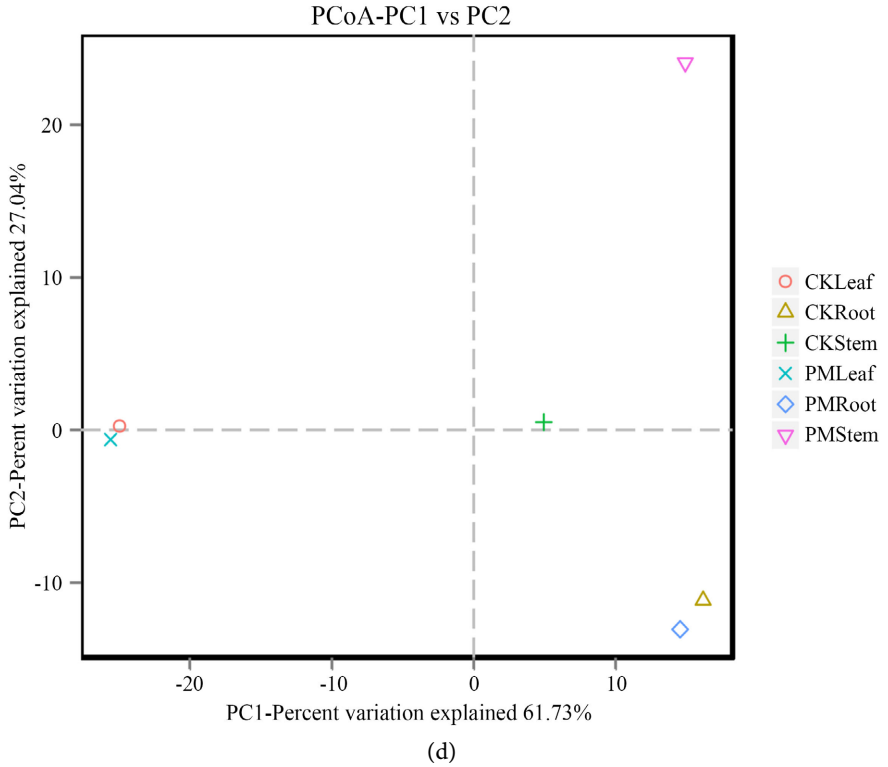
(a)



(b)



(c)



(d)

Figure 6. Principal coordinates analysis, The points represent each sample; different colors represent different groups; red represents wheat leaves, green represents wheat roots, blue represents wheat stems The coordinate represents the first principal component; the percentage represents the contribution of the first principal component to the sample difference; the ordinate represents the second principal component and the percentage represents the second principal component of the sample difference contribution value.

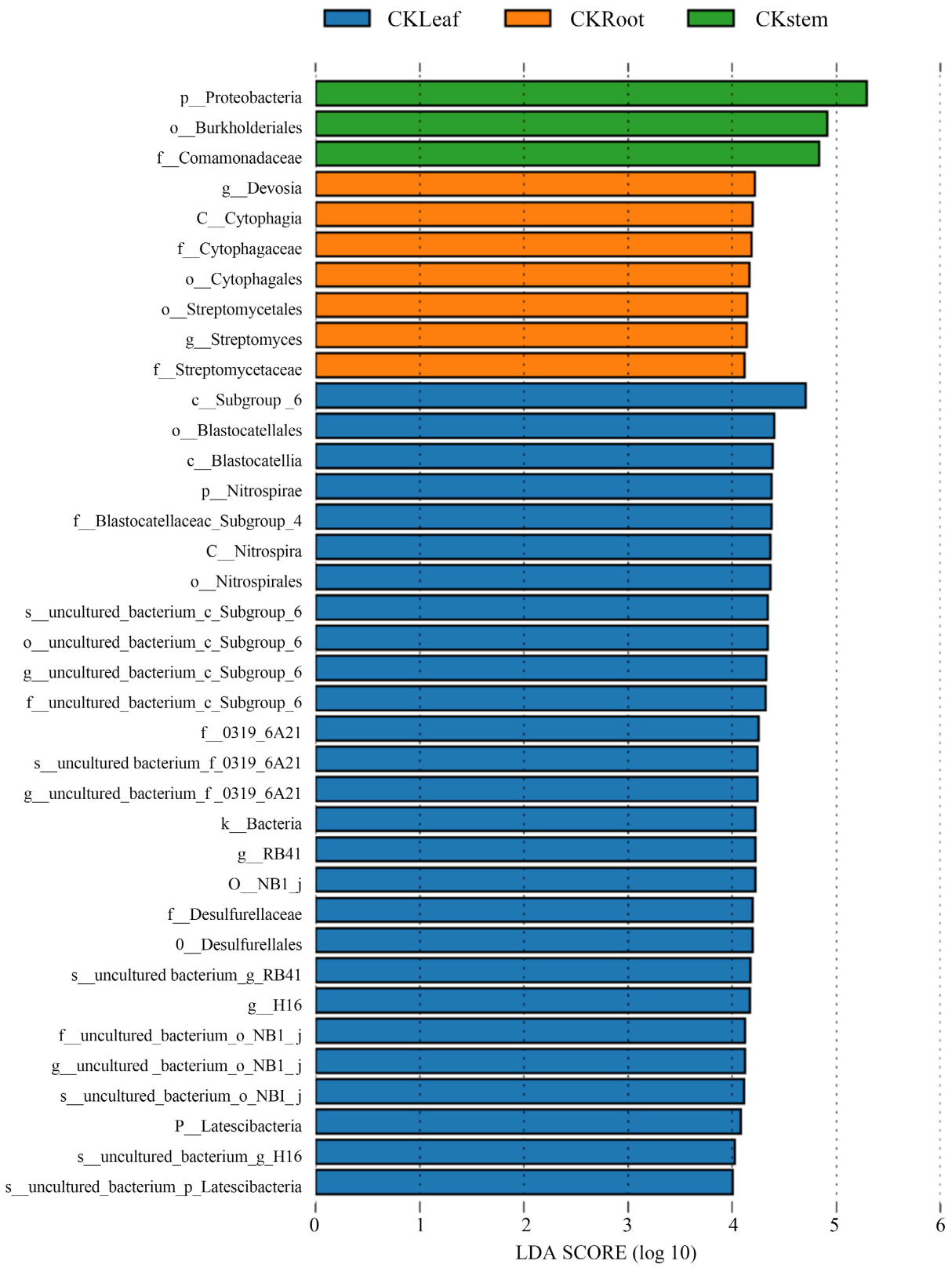


Figure 7. LDA distribution histogram, The species with LDA Score that is not infected with the powdery mildew of wheat is greater than the set value (set by default at 4.0). The length of the histogram represents the size of the difference species (ie LDA Score), and the different colors represent different grouped species.

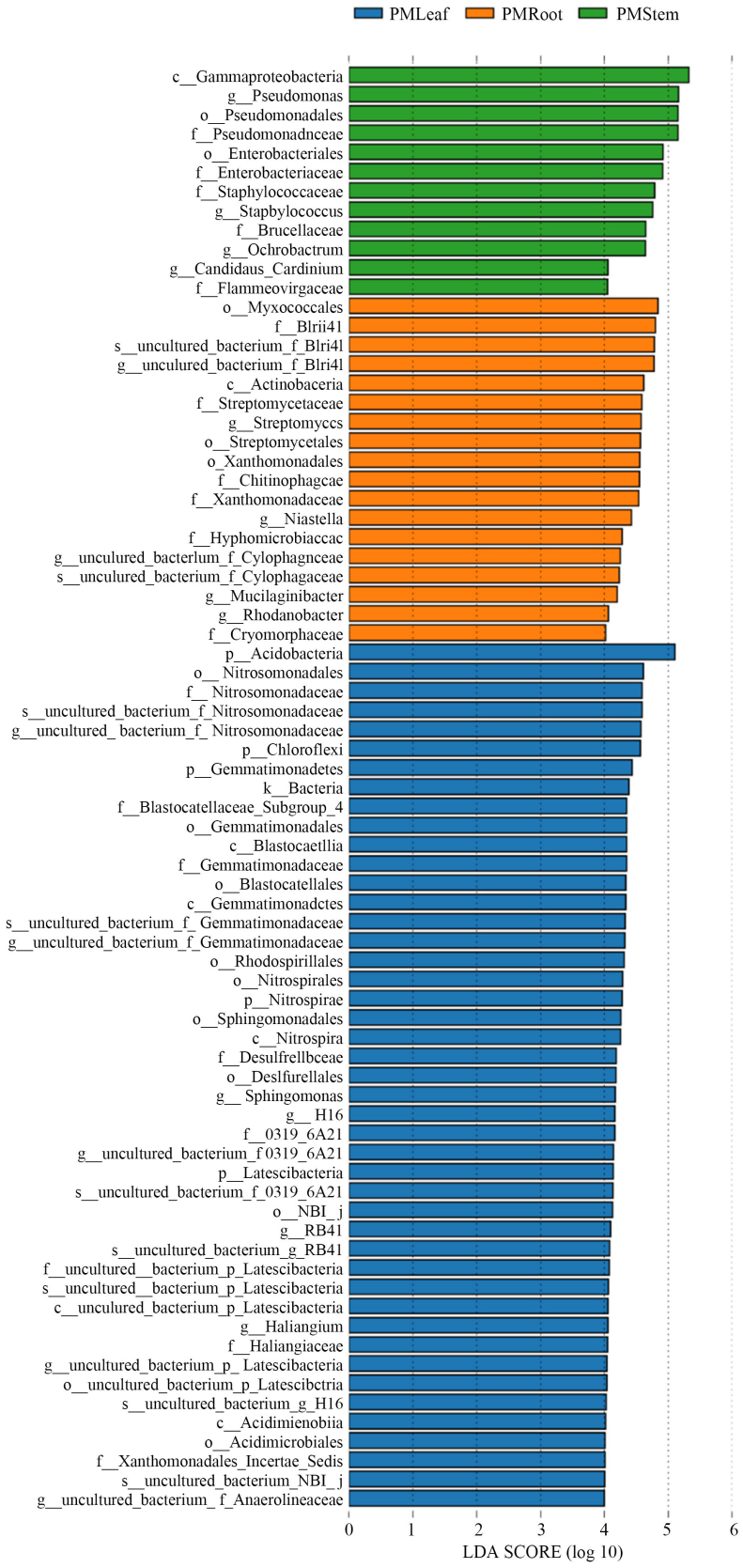


Figure 8. Indicator microbial groups within the three types of wheat powdery mildew with LDA values higher than 4.

endophytic bacteria in plants. It overcomes the shortcomings of low fluxes in traditional molecular biology methods, and analyzes microbial community structure at the genome level. Many anaerobic endogenous microorganisms have not been isolated and cultivated technical bottlenecks [8]. Six high-throughput sequencing techniques were used to sequence 16SrRNAs from three different parts of the roots, stems and leaves of infected and uninfected wheat powdery mildew, and the endomes of different parts of infected and uninfected wheat powdery mildew. Bacterial diversity was analyzed. The quality control data showed that the dilution curves were stable, indicating that the total DNA extraction quality of each sample was qualified and the sequencing depth was reasonable. It could reflect the community structure of endophytes well.

After obtaining valid sequencing data, the data was obtained by the software. Integration, analysis of OTU clustering, taxonomic analysis, diversity index, Shannon-Index curve, Rank-Abundance curve and PCoA analysis of each sample were carried out to classify and summarize the roots, stems and leaves of different tissue parts. Among them, the difference between CK-StemCK-Stem and PM-StemPM-Stem OTUs is the largest, indicating that the endophytic bacteria in stem tissue is the highest, and the infection of wheat powdery mildew has certain influence on endophytic bacteria in stem tissue. CK-Leaf and PM-Leaf, CK-Root and PM-Root have smaller gaps in OTUs, and PM-Leaf has fewer OTUs than CK-Leaf. Among them, there are Gemmatimonadetes, Acidobacteria and Nitrospirae in CK-Stem and not in PM-StemPM-Stem. There are also differences in control and treatment between leaf tissue and root tissue. This indicates that the endophytic distributions in different parts of wheat are different, and they also differ in the level of phylum classification, and are more likely to change by self-regulation.

Although a large number of tags were designed for each sample, rarefaction curves of OTUs were far from the plateau, which indicated that there were more undetermined tags either from real rare species or artificial sequences produced by PCR and sequencing mistakes. Obtaining 26 endophytic bacteria using conventional separation methods, it belongs to Bacillus cereus, Bacillus polymyxa, Bacillus megaterium, etc., but the main researches in this study are Proteobacteria, Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria, Gemmatimonadetes, Chloroflexi, Nitrospirae, and Latescibacteria. The species and quantity of endophytes in plants are closely related to plant species, growth environment, climatic conditions, sampling time and location, and separation [28] (Spiering, Lane *et al.*, 2005).

In this study, R39 wheat varieties, wheat seedlings, short growing period, single collection area, single species, may be part of the reason for the composition of endophytic bacteria. In addition, since the total DNA in the microorganisms in the plant separation is directly extracted, in order to prevent pollution, the treatment of the plant tissue after sterilization time and concentration may cause damage to the plant tissue and affect the extraction quality.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., Pretty, J., Robinson, S., Thomas, S.M. and Toulmin, C. (2010) Food Security: The Challenge of Feeding 9 Billion People. *Science* (*New York*, *N.Y.*), **327**, 812-818. https://doi.org/10.1126/science.1185383

[2] Green, A.J., Berger, G., Griffey, C.A., Pitman, R., Thomason, W. and Balota, M. (2014) Genetic Resistance to and Effect of Leaf Rust and Powdery Mildew on Yield and Its Components in 50 Soft Red Winter Wheat Cultivars. *Crop Protection*, **64**, 177-186. https://doi.org/10.1016/j.cropro.2014.06.023

[3] Liu, W., Liu, Z., Huang, C., Lu, M., Liu, J. and Yang, Q. (2016) Statistics and Analysis of Crop Yield Losses Caused by Main Diseases and Insect Pests in Recent 10 Years. *Chinese Plant Protection*, **42**, 1-9.

[4] Hallmann, J., Quadt-Hallmann, A., Rodríguez-Kábana, R. and Kloepper, J.W. (1998) Interactions between *Meloidogyne incognita* and Endophytic Bacteria in Cotton and Cucumber. *Soil Biology and Biochemistry*, **30**, 925-937. https://doi.org/10.1016/S0038-0717(97)00183-1

[5] Mastretta, C., Barac, T., Vangronsveld, J., Newman, L., Taghavi, S. and Lelie, D. (2006) Endophytic Bacteria and Their Potential Application to Improve the Phytoremediation of Contaminated Environments. *Biotechnology and Genetic Engineering Reviews*, **23**, 175-188. https://doi.org/10.1080/02648725.2006.10648084

[6] Feng, Y., Shen, D. and Song, W. (2006) Rice Endophyte *Pantoea agglomerans* YS19 Promotes Host Plant Growth and Affects Allocations of Host Photosynthates. *Journal of Applied Microbiology*, **100**, 938-945. https://doi.org/10.1111/j.1365-2672.2006.02843.x

[7] Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic Identification and *in Situ* Detection of Individual Microbial Cells without Cultivation. *Microbiological Reviews*, **59**, 143-169. https://doi.org/10.1128/mr.59.1.143-169.1995

[8] Tholozan, J.L., Cappelier, J.M., Tissier, J.P., Delattre, G. and Federighi, M. (1999) Physiological Characterization of Viable-but-Nonculturable *Campylobacter jejuni* Cells. *Applied and Environmental Microbiology*, **65**, 1110-1116. https://doi.org/10.1128/AEM.65.3.1110-1116.1999

[9] Dunbar, J., Takala, S., Barns, S.M., Davis, J.A. and Kuske, C.R. (1999) Levels of Bacterial Community Diversity in Four Arid Soils Compared by Cultivation and 16S rRNA Gene Cloning. *Applied and Environmental Microbiology*, **65**, 1662-1669. https://doi.org/10.1128/AEM.65.4.1662-1669.1999

[10] Cottrell, M.T. and Kirchman, D.L. (2000) Community Composition of Marine Bacterioplankton Determined by 16S rRNA Gene Clone Libraries and Fluorescence *in Situ* Hybridization. *Applied and Environmental Microbiology*, **66**, 5116-5122. https://doi.org/10.1128/AEM.66.12.5116-5122.2000

[11] Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H. and Berg, G. (2001) Bulk and Rhizosphere Soil Bacterial Communities Studied by Denaturing Gradient Gel Electrophoresis: Plant-Dependent Enrichment and Seasonal Shifts Revealed. *Applied and Environmental Microbiology*, **67**, 4742-4751. https://doi.org/10.1128/AEM.67.10.4742-4751.2001

[12] Cocolin, L., Rantsiou, K., Iacumin, L., Cantoni, C. and Comi, G. (2002) Direct Identification in Food Samples of Listeria spp. and *Listeria monocytogenes* by Molecular Methods. *Applied and Environmental Microbiology*, **68**, 6273-6282. https://doi.org/10.1128/AEM.68.12.6273-6282.2002

[13] Zou, X.Y., Liu, J., Dong, Z.P., Ma, J.F., Shi, A.L., Bai, H. and Li, Z.Y. (2022) The Composition and Difference Analysis of Endophytic Bacterial Community in Different Resistant/Susceptible Foxtail Millet Varieties. *Acta Agriculturae Boreali-Sinica*, **37**, 300-308.

[14] Walters, W.A., Berg-Lyons, D., Huntley, J., *et al.* (2012) Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms. *The ISME Journal*, **6**, 1621-1624. https://doi.org/10.1038/ismej.2012.8

[15] Magoc, T. and Salzberg, S.L. (2011) FLASH: Fast Length Adjustment of Short Reads to Improve Genome Assemblies. *Bioinformatics*, **27**, 2957-2963. https://doi.org/10.1093/bioinformatics/btr507

[16] Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A. and Caporaso, J.G. (2013) Quality-Filtering Vastly Improves Diversity Estimates from Illumina Amplicon Sequencing. *Nature Methods*, **10**, 57-59. https://doi.org/10.1038/nmeth.2276

[17] Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., *et al.* (2012) Ultra-High-Throughput Microbial Community Analysis on the Illumina HiSeq and MiSeq Platforms. *The ISME Journal*, **6**, 1621-1624. https://doi.org/10.1038/ismej.2012.8

[18] Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C. and Knight, R. (2011) UCHIME Improves Sensitivity and Speed of Chimera Detection. *Bioinformatics*, **27**, 2194-2200. https://doi.org/10.1093/bioinformatics/btr381

[19] Edgar, R.C. (2013) UPARSE: Highly Accurate OTU Sequences from Microbial Amplicon Reads. *Nature Methods*, **10**, 996-998. https://doi.org/10.1038/nmeth.2604

[20] Wang, Q., Garrity, G.M., Tiedje, J.M. and Cole, J.R. (2007) Naive Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology*, **73**, 5261-5267. https://doi.org/10.1128/AEM.00062-07

[21] Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., *et al.* (2010) QIIME Allows Analysis of High-Throughput Community Sequencing Data. *Nature Methods*, **7**, 335-336. https://doi.org/10.1038/nmeth.f.303

[22] Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Duenas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M. and Larsson, K.-H. (2013) Towards a Unified Paradigm for Sequence-Based Identification of Fungi. *Molecular Ecology*, **22**, 5271-5277. https://doi.org/10.1111/mec.12481

[23] Ondov, B.D., Bergman, N.H. and Phillippy, A.M. (2011) Interactive Metagenomic Visualization in a Web Browser. *BMC Bioinformatics*, **12**, Article No. 385. https://doi.org/10.1186/1471-2105-12-385

[24] Kemp, P.F. and Aller, J.Y. (2004) Bacterial Diversity in Aquatic and Other Environments: What 16S rDNA Libraries Can Tell Us. *FEMS Microbiology Ecology*, **47**, 161-177. https://doi.org/10.1016/S0168-6496(03)00257-5

[25] Sakaki, T., Takeshima, T., Tominaga, M., Hashimoto, H. and Kawaguchi, S. (1994) Recurrence of ICA-PCoA Aneurysms after Neck Clipping. *Journal of Neurosurgery*, **80**, 58-63. https://doi.org/10.3171/jns.1994.80.1.0058

[26] Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S. and Huttenhower, C. (2011) Metagenomic Biomarker Discovery and Explanation. *Genome Biology*, **12**, R60. https://doi.org/10.1186/gb-2011-12-6-r60

[27] Sogin, M.L., Morrison, H.G., Huber, J.A., Mark Welch, D., Huse, S.M., Neal, P.R., Arrieta, J.M. and Herndl, G.J. (2006) Microbial Diversity in the Deep Sea and the Underexplored "Rare Biosphere". *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 12115-12120. https://doi.org/10.1073/pnas.0605127103

[28] Spiering, M.J., Lane, G.A., Christensen, M.J. and Schmid, J. (2005) Distribution of the Fungal Endophyte *Neotyphodium lolii* Is Not a Major Determinant of the Distribution of Fungal Alkaloids in *Lolium perenne* Plants. *Phytochemistry*, **66**, 195-202. https://doi.org/10.1016/j.phytochem.2004.11.021