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Research Article

Analysis of Bacterial Community Composition and Ecological Function during Soft Rot Process in Pitaya (*Hylocereus* spp.) Stems

Zhijun Peng, Jingqiang Guan, Dan Mou, Xiao Zou, Bin Wang, Jilin Jin, Xingwu Zhang, and Hui Luo

Institute of Fruit Science, Guizhou Academy of Agricultural Sciences, Guiyang 550006, China

Correspondence should be addressed to Hui Luo; luohui8732@163.com

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The soft rot in pitaya (*Hylocereus* spp.) stems seriously affected the harvest of pitaya fruits, but the dynamic change characteristics of bacterial community in pitaya stems during soft rot stages had not been revealed. In this study, we analyzed the bacterial community composition of different soft rot stages and visualized functional annotations of the core bacterial community in five soft rot periods via the Illumina high-throughput sequencing technology, MetaCoMET online network platform, and FAPROTAX database. The results showed that the dominant bacteria in healthy and diseased pitaya stems were Proteobacteria and Firmicutes. *Pseudomonas*, *Enterobacter*, *Sphingomonas*, and *Sphingobacterium* were the core bacteria microbiomes during the infection stages. Meanwhile, the ecological function analysis results showed that the *Enterobacter* and *Pseudomonas* bacteria may play an important role in causing soft rot of pitaya stems. Therefore, the results shown in this paper could provide a useful reference for the study on microecological mechanism of soft rot of pitaya stems.

1. Introduction

Pitaya (*Hylocereus* spp.) is often planted in tropical and subtropical regions and has high economic and nutritional value. Hainan Province in China began to grow pitaya in the late 1990s, followed by Fujian, Guangdong, Guangxi, Guizhou, Yunnan, and other southern provinces (regions) began to large-scale introduction. In recently years, Guizhou Province is one of the major pitaya producing areas in China, and pitaya in Luodian County has become a national geographic landmark product.

At present, pitaya yield is threatened by many diseases; among which, soft rot is one of the main diseases. Various studies showed that a variety of fungi could produce soft rot on pitaya fruits, for example, *Scytalidium dimidiatum*, *Fusarium* spp., *F. dimerum*, *Gilbertella persicaria*, and *F. oxysporum* [1–6]. If the pitaya stem is infected by *F. semitectum*, *F. oxysporum*, *F. moniliforme*, and other *Fusarium* fungi, it will show symptoms of tissue softening, ulceration, and depression at the stem ridge [2, 7]. *Bipolaris* was a plant pathogenic fungus with strong distribution in the world, which could cause soft rot in cacti plants, for example, fruit rot of pitaya and stem rot of cactus [8, 9]. In addition, Liang et al. showed that *B. cactivora* could harm the stem of pitaya under natural conditions, which was the first report on the harm of stem rot of this fungus at home and abroad [10].

However, there are few reports about the bacterial diseases of pitaya. Yuan et al. found that *Erwinia* sp. could make the nodes of pitaya stems infected with soft rot in western Guangdong Province [11]. Sang et al. also confirmed that the soft rot of pitaya stem was mainly caused by *Erwenella* [12]. Meanwhile, Masyahit et al. [13] first sampled the pitaya stems of soft rot from 11 sample areas in Malaysia and found that *Enterobacter cloacae* could cause soft rot and yellow-brown of pitaya stems. Lin et al. [14] isolated the endogenous bacteria in pitaya seedlings, and the identification results showed that *Enterobacter* bacteria induced typical soft rot symptoms in pitaya plants. In

Table 1: Statistics of sequencing date and alpha diversity index in different soft rot stages.

Samples	Raw reads	Effective reads	Numbers	Coverage (%)	Simpson index	Shannon index	Sobs index	Ace index	Chao1 index
CK	36580	36024	56	99.953	0.627	0.705	49	67.360	68.429
B-1	41511	40771	56	99.968	0.690	0.632	50	68.989	57.800
B-2	41016	40002	80	99.955	0.546	0.897	70	86.786	82.750
B-3	36948	35749	139	99.964	0.566	1.130	121	127.709	128.091
B-4	38898	36044	185	99.989	0.090	3.287	152	153.213	152.667

addition to *Erwenella* and *Enterobacter*, Zhang et al. [15] found that *Paenibacillus polymyxa* could also cause soft rot in the stem of pitaya. When plant stems are affected by soft rot pathogens, this type of disease will further spread and eventually affect the fruit yield [16]. However, the change characteristics of endogenous bacterial community during soft rot of pitaya stem are not clear.

In this study, we analyzed the bacterial community composition in the pitaya stem tissue in different soft rot stages by the Illumina high-throughput sequencing technology and annotated the core bacterial microbiome to provide theoretical guidance for the prediction of pitaya soft rot disease in different soft rot stages.

2. Materials and Methods

2.1. Sample Collection. In July 2019, the stems of pitaya (variety "Purple Red Dragon") in five different soft rot stages were sampled from Luodian County, Guizhou Province, China. According to the methods of Masyahit et al. [13], the soft rot disease grade of pitaya stem was divided into five grades, which were normal (CK), early soft rot grade (B-1), middle soft rot grade (B-2), middle and late soft rot grade (B-3), and late soft rot grade (B-4), respectively. Three stems were collected at each period. The samples were encapsulated in sterile ziplock bags, stored in ice bags, and transported to the laboratory for further processing. Tissue blocks with a size of 5 mm × 5 mm were cut at the junction of the disease with a sterile scalpel, 3 tissue blocks were cut from each stem, and 9 tissue blocks were mixed evenly in each period. According to the treatment method of Xu et al. [17], tissue blocks in each period were disinfected: first soaked in 75% alcohol for 40 s, then soaked in 5% sodium hypochlorite solution for 1 min, and finally washed with sterile water for 4 times. The dried tissue was encapsulated in a sterile ziplock bag and stored in a -80°C refrigerator.

2.2. DNA Extraction, PCR Amplification, and Sequencing. Under aseptic conditions, plant tissue blocks were ground into a fine powder with liquid nitrogen. Approximately, 50 mg tissue powder was taken at each onset stage, and total microbial genomic DNA was extracted according to the instructions in the E.Z.N.A.® SoiL DNA Kit (Omega Bio-Tek, Norcross, GA, USA). According to the method of Wei et al. [18], the V3-V4 region of bacterial 16S rDNA was amplified by ABI Gene Amp®9700 (ABI, CA, USA). Polymerase chain reaction (PCR) reaction system is as follows: 0.4 μL of TransStart Fastpfu DNA Polymerase (2.5 U·μ/L), 4 μL of Fastpfu Buffer, 2 μL of dNTPs (2.5 mol/L), 0.8 μL of forward and reverse primers of 338F (5 μmol/L) and 806R (5 μmol/L), 0.2 μL of BSA (0.8 μg·μ/L), 10 μL of DNA template (1 ng/μL), and 20 μL of ddH_2O. PCR reaction procedure is as follows: predenaturation at 95°C for 3 min; denaturation at 98°C for 30 s, 55°C for 30 s, 72°C for 45 s, a total of 30 cycles; extended at 72°C for 10 min [19, 20]. PCR products were detected by 1% agarose gel electrophoresis and sequenced using the Illumina Miseq platform.

2.3. Data Analysis. In order to obtain effective Tags data, the sequence of each sample was splicing and quality optimization (Tags interception, filtering, and chimeric removal) by referring to the processing method of Robert [21]. The UPARSE software was used to cluster the 97% nonrepeating sequences (excluding single sequences) into operational taxonomic units (OTUs). Mothur method was used to compare OTUs with species in SSU rRNA database. Finally, α diversity analysis was performed on the obtained data.

The species abundance of each sample was counted at the taxonomic level of phylum and genus, and the bacterial communities of samples in each period were analyzed by using the Origin Pro 2018C software. The distribution differences of OTUs in different soft decay stages were analyzed by Venn diagram. At the genus level, the species annotation and abundance of each sample were analyzed. The top 26 groups with relative abundance were selected, and the changes of dominant bacterial groups in samples at different soft rot stages were analyzed by heat map.

Core microbiome is a key component of Holobionts, which is of great significance for the study of symbiotic and pathogenic microorganisms [22]. OTUs were uploaded to the MetaCoMET platform (http://probes.pw.usda.gov/MetaCoMETT). The membership definition method was used to obtain the core bacterial microbiomes of different soft decay stages.

FAPROTAX is a kind of database collected for culturable bacteria and is often used to predict the ecological function of the microbiome. The database has included more than 4600 culturable bacteria, including more than 80 functional groups and 7600 functional annotations, which can be applied to predict the ecological function of culturable bacteria [23]. Through the FAPROTAX database, the OTU function of the core bacterial microbiome in different soft decay stages was predicted.

3. Results

3.1. Sequence Data. As shown in Table 1, a total of 178,745 valid sequences and 516 OUTs were obtained, with an

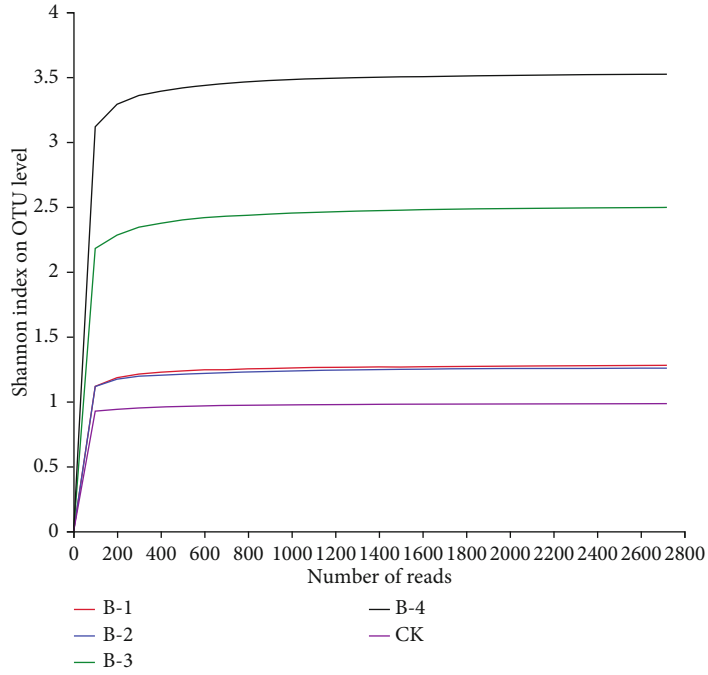

Figure 1: The Shannon-Wiener curves in different soft rot stages.

average of 103 OUTs per sample. Sequencing results showed that the coverage rate of each sample was above 99%, which could accurately reflect the real situation of the bacterial community of the sample [24]. According to the sobs index differences shown in Table 1, bacterial abundance in different onset periods was B-4 > B-3 > B-2 > B-1 > CK, indicating that the abundance and diversity of bacteria in stem tissue of pitaya changed with the progression of disease degree. Meanwhile, based on Simpson and Shannon indexes in Table 1, Simpson index was the lowest (0.090), and Shannon index was the highest (3.42) in B-4 stage (late onset), indicating that the bacterial community gradually tended to diversify during the soft rot of pitaya stem tissue. In addition, Shannon-Wiener index can reflect the relationship between species diversity and sequencing amount [25, 26]. As can be seen from Figure 1, Shannon-Wiener index curve becomes flatter with the increase of sample sequence number, indicating that the data depth of this experiment can fully reflect the microbial information in the sequenced samples. Analysis of OTUs of samples in 5 periods (Figure 2) revealed that 2, 4, 5, 20, and 34 endemic OTUs (Figure 2) were shared by the five samples of CK, B-1, B-2, B-3, and B-4, respectively.

3.2. Composition of Bacterial Community in Different Soft Rot Stages. As shown in Figure 3, except Proteobacteria and Firmicutes, Proteobacteria and Firmicutes have always been at the level of dominant phyla, while bacteria at other phyla levels have undergone great changes. From CK to B-4, Proteobacteria accounted for more than 70%, which was the dominant flora in the whole pathological process (>1%). Firmicutes were the dominant bacteria next to Proteobacteria; the abundance of Firmicutes was more than 2% in all periods. From B-2 to B-4, the abundance of Actinobacteria and Bacteroidetes showed an increasing trend and gradually established a dominant position in B-3 and B-4. Verrucomicrobia belonged to the dominant group in B-1 and B-2 stages, but with the development of disease (B-3 to B-4 stage), it gradually changed to non-dominant group (<1%).

As shown in Figure 4, a total of 116 genera of bacteria were detected in five samples. In the CK group, *Pseudomonas* was the main dominant groups accounting for 93.75%. From B-1 to B-4, *Pseudomonas*, *Enterobacter*, *Sphingomonas*, and *Sphingobacterium* were the dominant genera accounting for more than 2%. The bacterial community structure became more complex, and the species diversity became richer when the soft rot disease of pitaya stem was aggravated.

3.3. Dynamic Analysis of Bacterial Community in Different Soft Rot Stages. As shown in Figure 5, in the CK group, *Pseudomonas* of Bacteroidetes and *Enterococcus* of Firmicutes were obviously dominant. From B-1 to B-2, the abundance of *Pseudomonas*, *Enterobacter*, *Sphingosphinx*, and *Sphingosphinx* showed an increasing trend. With the soft rot grade rising to B-3 stage, *Sphingosphinx* of Bacteroides and *Enterobacter* of Firmicutes were the dominant genera. In B-4 stage, *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, *Rhizobium*, *Devosia*, *Kineococcus*, *Enterobacter*, and *Aureimonas* had obvious dominant characteristics.

3.4. Core Bacterial Group and Functional Analysis during Soft Decay Period. MetaCoMET analysis of data sets from B-1 to B-4, as shown in Figure 6, showed that there were five core bacterial microbiomes at the genus level, namely,

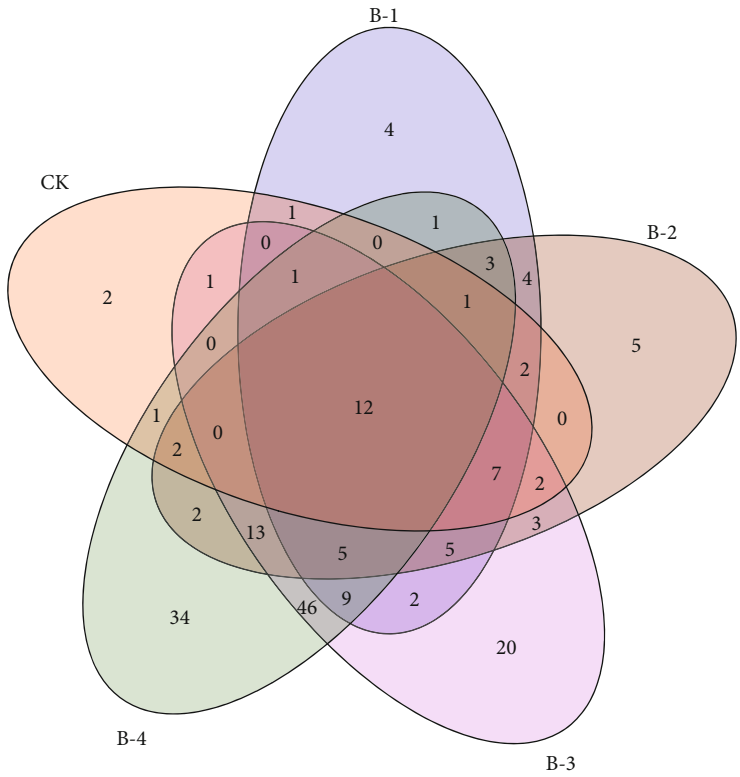

Figure 2: Analysis of OTUs from different soft rot process with Venn diagram.

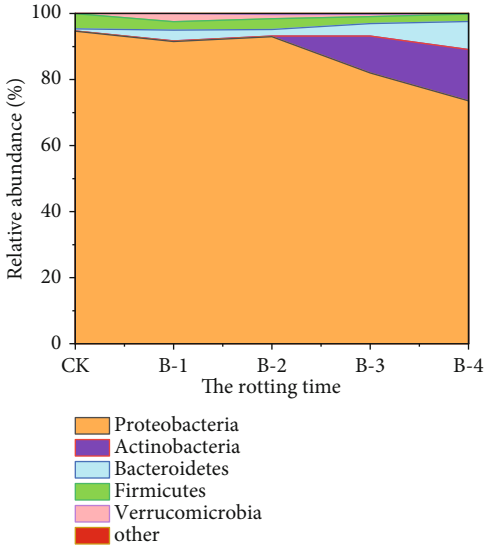

Figure 3: The stacked area graph of bacteria on phylum level.

Pseudomonas, *Sphingomonas*, *Enterobacter*, and *Sphingobacterium*. Meanwhile, Figure 7 showed that the main functional groups of the core bacterial groups in the different soft rot periods were chemoheterotrophy, aerobic chemoheterotrophy, animal parasites or symbiotics symbionts, and plant pathogens, among which chemoheterotrophs and oxidative functional groups dominated. This suggested that microbes needed to break down organic matter in the stem tissue to obtain a supply of nutrients. In the whole soft rot process, Pseudomonas played an important role in chemoheterotrophy, heterotrophy, animal parasitism, and symbiosis. It is noteworthy that, combined with the change process of species abundance, the change of species abundance of Enterobacter was synchronized with the change of plant pathogenic functional groups. With the increase of species abundance, the role of Enterobacter in plant pathogenic functional groups became more and more prominent. Therefore, the Enterobacter sequenced in this study may be a class of important plant pathogens, with certain functional potential for the occurrence of soft rot diseases.

4. Discussion

Microbial invasion is often the main cause of fruit and vegetable quality degradation. In natural ecosystems, the quality of fruit and vegetable crops is affected by soft rot pathogens from planting, harvesting to storage. Bruises, cuts, and insect bites of plants can increase the advantages of microbial colonization [27]. At present, there are few reports on the change of bacterial community in pitaya stem tissue during soft rot. Therefore, exploring and revealing the relationship between bacteria and stem soft rot can provide important reference value for the maintenance of pitaya fruit quality and disease prediction.

The occurrence of soft rot in pitaya stem changed the composition and distribution of bacterial community in stem tissue. Alpha diversity analysis showed that bacterial

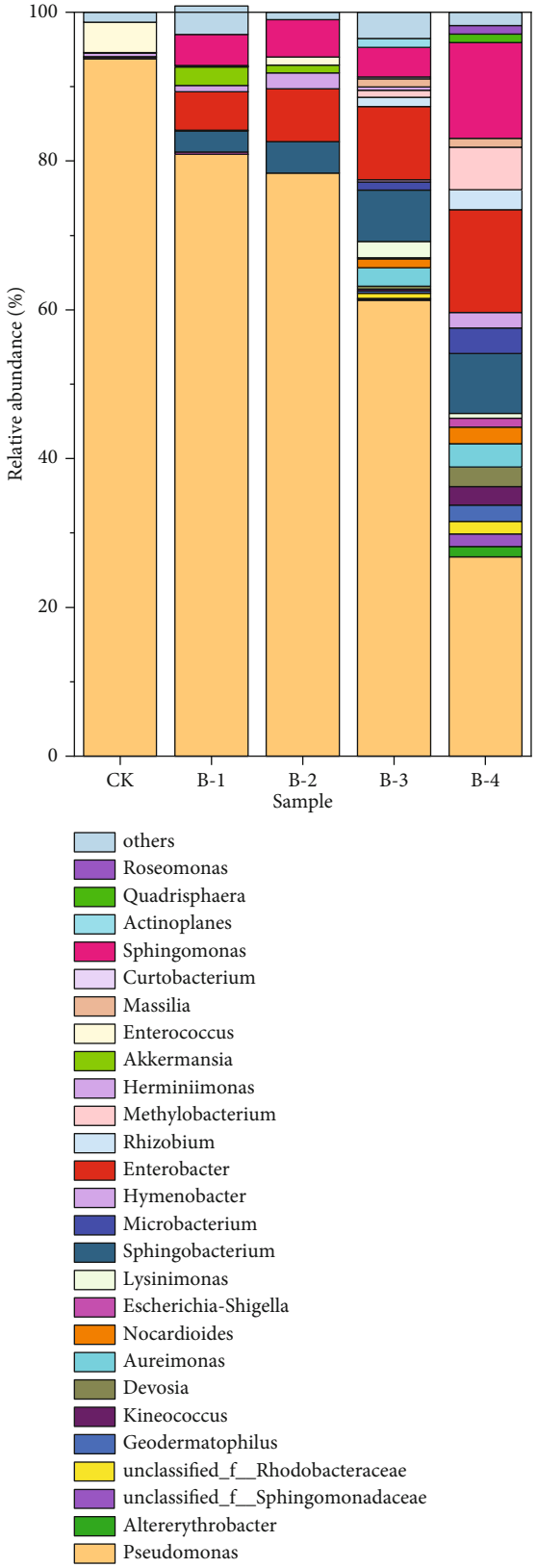

Figure 4: Histogram of sample's relative abundance on genus level.

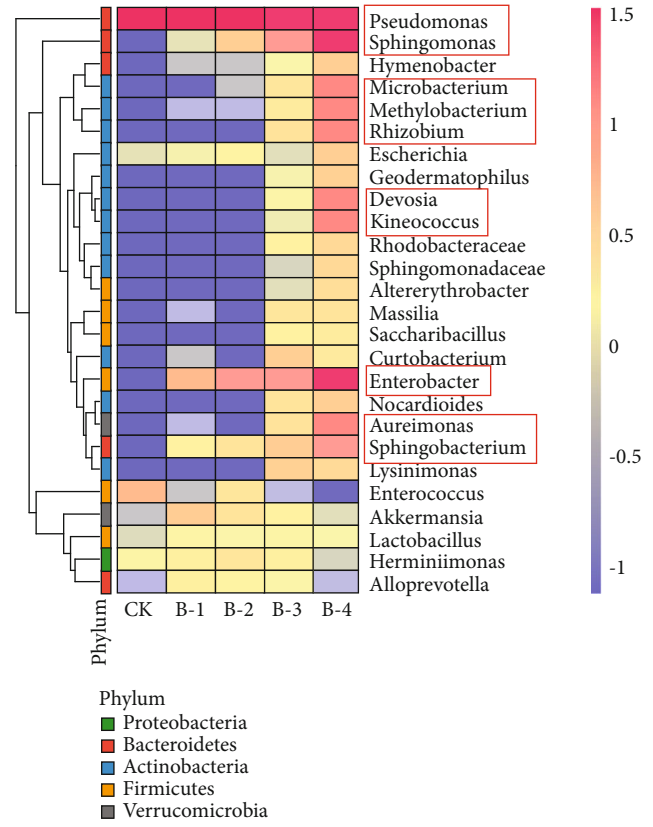

Figure 5: Heat map of the species abundance during different soft rot stages.

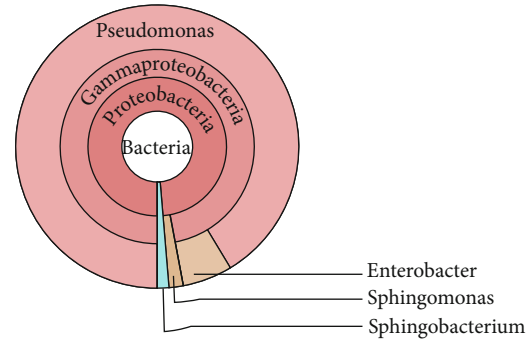

Figure 6: Core bacterial microbiota compositions during different soft rot stages.

community richness and diversity in stem tissues gradually increased with the deepening of the degree of soft rot disease. Welington et al. [28] believed that the healthy plants could only be colonized by a few dominant microorganisms, while the diseased plants without obvious diseases could recruit more community richness. This study also found a similar phenomenon: *Pseudomonas* and *Enterococcus* were the dominant bacteria in the stem tissue of the healthy pitaya. In the early stage of soft rot, there were not only Pseudomonas bacteria but also abundant *Enterobacter* and *Sphingomonas*. At the end of soft rot, the abundance and diversity of bacteria in stem tissue reached the maximum.

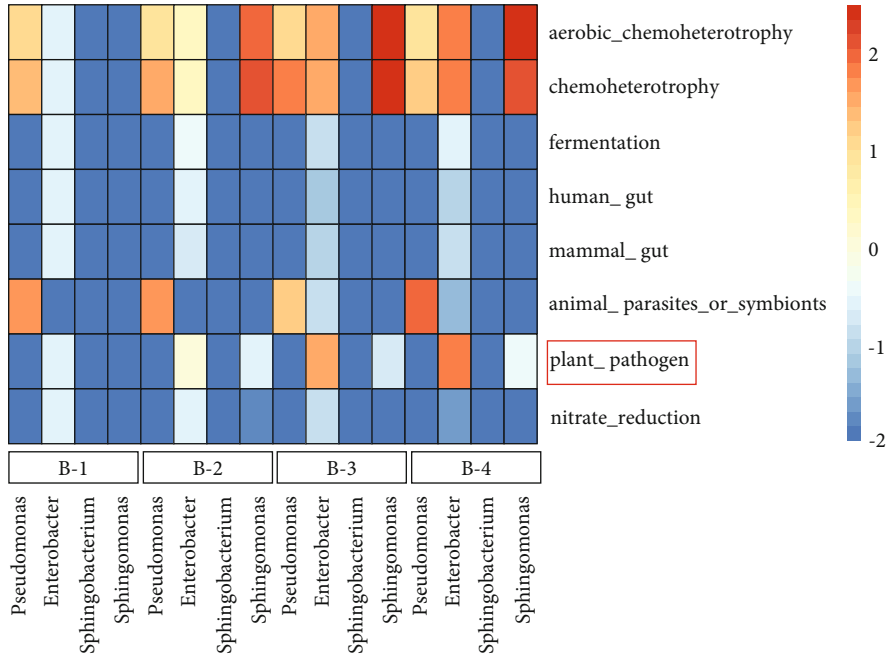

Figure 7: Changes of the functional groups of the core bacterial microbiota at different soft rot stages.

The distribution differences of OTUs in different soft rot stages indicated that there was a transient succession process in the bacterial community in the stem tissue of pitaya at different soft rot stages: the bacterial community structure was most complex when the bacterial community in the stem tissue succeeded to the end of soft rot. It can be seen that plants are a complex microecosystem, in which bacterial communities occupy a certain ecological niche and constantly compete with each other for nutrients and water in host tissues [29].

MetaCoMET analysis and FAPROTAX functional analysis showed that the functional groups of the core bacterial groups in the five soft decay stages were mainly dominated by chemoheterotrophic and oxidation-requiring functional groups. These results suggested that microorganisms need to decompose organic matter in the stem tissue to obtain a supply of nutrients. It should be noted that *Pseudomonas* and *Enterobacter* may play an important role in the occurrence of pitaya stem soft rot disease by analyzing the changes of bacterial community composition and the functional changes of core bacterial group. In healthy pitaya stem tissue, *Pseudomonas* was the dominant bacterium with the highest species abundance, accounting for 93.75%. However, with the aggravation of soft rot, the species abundance of *Pseudomonas* decreased gradually and reached the lowest value (26.8%) at the end of soft rot. Studies had showed that after inoculation with *Pseudomonas*, the fruit quality of grape and cotton can be well maintained and improved [30]. Based on this research significance, Pseudomonas bacteria can be used as an important indicator for the prediction of soft rot in pitaya in subsequent studies.

In the early stage of soft rot disease, *Enterobacter* began to colonize in the stem tissue, and its species abundance increased to the maximum (13.79%) in the late stage of soft rot disease. FAPROTAX functional analysis showed that *Enterobacter* played an increasingly important role in plant pathogenic functional groups with the increase of species abundance. *Enterobacter* was a common human pathogen in Enterobacteriaceae, which could often activate pectinase regulation pathway and cause the occurrence of plant soft rot. At present, *Enterobacter cloacae*, *Enterobacter nimipressuralis*, and *Enterobacter pyrinus* could cause soft rot in plants [14]. In recent years, studies on the harm of *Enterobacter* bacteria to fruits, vegetables, and other plants had been reported. Masyahit et al. [13] first found that *Enterobacter cloacae* could cause bacterial soft rot in stem segments of pitaya fruit and causes the disease in 36% of pitaya fruit. Lin et al. [14] first discovered *Enterobacter* bacteria in pitaya stems from Taiwan, China. According to morphological characteristics, molecular identification, pathogenicity determination, and other methods, the bacteria causing the soft rot of pitaya stem was *Enterobacter* group. Oniha and Egwari [16] isolated *Enterobacter* spp. bacteria that could cause soft rot from the fruit and stem of *Carica papaya* L. In addition to the harmful effects of *Enterobacter* bacteria on fruits, researchers had observed the symptoms of decay in cucumbers, carrots, cabbage, and onions inoculated with *Enterobacter* spp. [31]. The *Enterobacter* bacteria obtained in this test may play an important role in the occurrence of soft rot of pitaya stem, but whether it has the above soft rot pathogenic ability is not clear and needs to be isolated and identified. It is worth discussing that studying the change characteristics of bacterial community in plants can not only provide help for predicting plant diseases but also reveal the role of dominant microorganisms in orchard ecosystem.

5. Conclusion

In conclusion, the dominant bacterial genera with the stem samples of healthy pitaya were *Pseudomonas* and *Enterococcus*. From the initial infection stage to the late infection stage, the dominant bacteria in pitaya stems were *Pseudomonas*, *Enterobacter*, *Sphingomonas*, and *Sphingobacterium*. At the terminal stage, the bacterial genera with dominance in soft rot pitaya stems were *Pseudomonas*, *Sphingomonas*, *Sphingobacterium*, *Microbacterium*, *Methylobacterium*, *Rhizobium*, *Devosia*, *Kineococcus*, *Enterobacter*, and *Aureimonas*, of which *Pseudomonas*, *Enterobacter*, *Sphingomonas*, and *Sphingobacterium* were the core microbiota of bacteria during the stages of infection. Meanwhile, the functional groups of these core microbiomes were chemoheterotrophy, aerobic chemoheterotrophy, animal parasites or symbionts, and plant pathogen. The *Enterobacter* and *Pseudomonas* bacteria may play an important role in causing soft rot disease of pitaya stems.

Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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References

[1] M. Li, M. J. Hu, Z. Y. Gao, D. R. Xue, D. P. Yang, and B. Yang, "Identification and biological characteristics of a pathogen causing fruit rot of *Hylocereus undatus* (Haw.) Britt. et. Rose," *Chinese Journal of Tropical Crop*, vol. 33, no. 11, pp. 2044–2048, 2012.

[2] T.-f. Ma, B. Yang, Y. Yu et al., "Market disease pathogens detection of imported fruits in Shanghai," *Agricultural Sciences in China*, vol. 8, no. 9, pp. 1087–1096, 2009.

[3] Z. Yingying, G. Zhaoyin, L. Min, C. Liang, and H. Meijiao, "Identification and biological characteristics of dragon fruit (Hylocereus undatus Britt) Fusarium rot pathogen," *Chinese Journal of Tropical Crop*, vol. 37, no. 1, pp. 164–171, 2016.

[4] L. W. Guo, Y. X. Wu, H. X. He, Z. C. Mao, P. B. He, and Y. Q. He, "A new fruit rot disease in *Hylocereus costaricensis* in Yunnan Province of China," *Journal of Fruit Science*, vol. 31, no. 1, pp. 111–114, 2014.

[5] F. Zheng, G. Xu, F. Qiu, F. Q. Zheng, L. Zheng, and C. P. Xie, "Identification and biological characteristics of the pathogenic fungus causing the soft rot of Hylocereus costaricensis in Hainan, China," *Plant Protection*, vol. 45, no. 4, pp. 137–142, 2019.

[6] Z. J. Cui, Y. W. Wang, Y. Yu, and L. Xu, "Pathogens analysis of soft rot disease of imported pitaya in Shanghai," *Microbiology China*, vol. 38, no. 10, pp. 1499–1506, 2011.

[7] W. Zheng, Y. Q. Cai, and L. Y. Dai, "Research progress on main diseases and insect pests of pitaya," *Guizhou Agricultural Sciences*, vol. 6, pp. 139–142, 2007.

[8] S. Taba, N. Miyahira, K. Nasu, T. Takushi, and Z. Moromizato, "Fruit rot of strawberry pear (pitaya) caused by Bipolaris cactivora," *Journal of General Plant Pathology*, vol. 73, no. 5, pp. 374–376, 2007.

[9] Z. L. Wang and Z. Z. Lin, "Fruit rot of pitaya and stem rot of cacti in Taiwan," *Plant Pathology Bulletin*, vol. 14, no. 4, pp. 269–274, 2005.

[10] Q. L. Liang, J. Wei, X. Y. Li, and L. Q. Wang, "Identification on causal agent of dragonfruit stem rot and indoor determination of fungicide toxicity," *South China Fruits*, vol. 40, no. 1, pp. 9–12, 2011.

[11] C. L. Yuan, W. F. Zhang, and H. X. Yuan, "Preliminary study on disease investigation and control measures of pitaya in Yuexi area," *South China Fruit*, vol. 33, no. 2, pp. 49-50, 2004.

[12] W. J. Sang, D. F. Wang, Q. Wei, R. Yang, G. Q. Fan, and Y. L. Jin, "Preliminary identification of pitaya disease in Guizhou province," *Journal of Mountain Agriculture and Biology*, vol. 26, no. 3, pp. 267–270, 2007.

[13] M. Masyahit, K. Sijam, Y. Awang, and M. Ghazali, "First report on bacterial soft rot disease on dragon druit (*Hylocereus* spp.) caused by Enterobacter cloacae in Peninsular Malaysia," *International Journal of Agriculture and Biology*, vol. 11, no. 6, pp. 1560–8530, 2009.

[14] W. Z. Lin, R. F. Liao, X. H. Chen et al., "Isolation and identification of the pathogen causing soft rot in *Hylocereus undatus*," *Acta Phytopathologica Sinica*, vol. 45, no. 2, pp. 220–224, 2015.

[15] R. Y. Zhang, X. S. Zhao, Q. Z. Tan, and C. H. Zhu, "First report of bacterial stem rot disease caused by *Paenibacillus polymyxa* on *Hylocereus undulatus* in China," *Plant Disease*, vol. 101, no. 6, p. 1031, 2017.

[16] M. Oniha and L. Egwari, "Fruit, leaf and stem diseases of *Carica papaya* L," *Journal of Agriculture and Food*, vol. 3, no. 1, article 398-4.7, 2015.

[17] L. Xu, X. W. Li, and K. N. Teng, "Detection and control of postharvest pathogenic fungi in fruits and vegetables," *Food Science*, vol. 7, pp. 155–158, 2003.

[18] D. Wei, Q. H. Wu, Y. P. Liu et al., "Microbial community diversity and its characteristics in Magnolia Officinalis Cortex "sweating" process based on high-throughput sequencing," *Zhongguo Zhong Yao Za Zhi*, vol. 44, no. 24, pp. 5405–5412, 2019.

[19] J. Walter, G. W. Tannock, A. Tilsala-Timisjarvi et al., "Detection and identification of gastrointestinal Lactobacillus species by using denaturing gradient gel electrophoresis and species-specific PCR primers," *Applied and Environmental Microbiology*, vol. 66, no. 1, pp. 297–303, 2000.

[20] Y. Youngseob, L. Changsoo, and K. Jaai, "Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction," *Biotechnology and Bioengineering*, vol. 89, no. 6, pp. 670–679, 2005.

[21] C. E. Robert, "UPARSE: highly accurate OTU sequences from microbial amplicon reads," *Nature Methods*, vol. 10, no. 10, pp. 996–998, 2013.

[22] C. B. Dong, Z. Y. Zhang, Y. F. Han, and Z. Q. Liang, "Research and application prospects of core microbiome," *Mycosystema*, vol. 38, no. 1, pp. 1–10, 2019.

[23] S. Louca, L. W. Parfrey, and M. Doebeli, "Decoupling function and taxonomy in the global ocean microbiome," *Science*, vol. 353, no. 6305, pp. 1272–1277, 2016.

[24] C. C. Liu, S. B. Feng, Q. Wu et al., "Flavor-related microbiota and their flavor metabolism during highland barley baijiu fermentation," *Microbiology China*, vol. 47, no. 1, pp. 151–161, 2020.

[25] W. H. Mao, S. L. Wu, and X. Zhang, "Establish and application of the high throughput sequencing method for soil microbial 16S rDNA using Ion Torrent PGM," *Acta Agriculturae Zhejiangensis*, vol. 27, no. 12, pp. 2165–2170, 2015.

[26] U. Kõljalg, R. H. Nilsson, K. Abarenkov et al., "Towards a unified paradigm for sequence-based identification of fungi," *Molecular Ecology*, vol. 22, no. 21, pp. 5271–5277, 2013.

[27] A. O. Charkowski, "Decaying signals: will understanding bacterial–plant communications lead to control of soft rot?," *Current Opinion in Biotechnology*, vol. 20, no. 2, pp. 178–184, 2009.

[28] W. L. Araújo, J. Marcon, W. Maccheroni Jr., J. D. Van Elsas, J. W. Van Vuurde, and J. L. Azevedo, "Diversity of endophytic bacterial populations and their interaction with Xylella fastidiosa in citrus plants," *Applied and Environmental Microbiology*, vol. 68, no. 10, pp. 4906–4914, 2002.

[29] M. M. Wei, X. C. Liu, Y. H. He et al., "Biochar inoculated with *Pseudomonas putida* improves grape (*Vitis vinifera* L.) fruit quality and alters bacterial diversity," *Rhizosphere*, vol. 16, pp. 100261–100269, 2020.

[30] L. X. Yao, Z. S. Wu, Y. Y. Zheng, I. Kaleem, and C. Li, "Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton," *European Journal of Soil Biology*, vol. 46, no. 1, pp. 49–54, 2010.

[31] B. C. Adebayo-Tayo, N. N. Odu, C. U. Esen, and I. O. Okonko, "Microorganisms associated with spoilage of stored vegetables in Uyo Metropolis, Akwa Ibom State, Nigeria," *Nature and Science*, vol. 10, no. 3, pp. 23–32, 2012.