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Bacterial community diversity on the surface of Chinese wolfberry fruit and its potential for biological control

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Abstract

The bacterial diversity on the surface of the Chinese Wolfberry (*Lycium barbarum*) affects the storage tolerance of the fruit. We analyzed the bacterial diversity on the surfaces of eight varieties of Chinese wolfberries using high-throughput sequencing before and after storage. A total of 737 OTUs, including 584 species, 423 genera, 243 families, 153 orders, and 62 classes, were identified by bioinformatic analysis of the OTUs at the 97% similarity level. There were no significant differences among fruit varieties just after picking (O period). The dominant genera during the O period were *Massilia* and *Pseudomonas*. However, after a 144-hour storage period (S period), there were significant changes in both the decay rate and surface bacterial compositions. The Z1 variety showed the highest rate of decay, and its surface microbiota was dominated by *Pantoea* (84.34%), while the variety with the lowest decay rate was O, dominated by both *Pantoea* (69.06%) and *Rosenbergiella* (16.99%).

Keywords: fresh fruit; storage tolerance; bacterial diversity; high throughput sequencing; decay rate.

Practical Application: The results of this article were mainly applied to the postharvest preservation and biological control of fresh Chinese wolfberry fruits.

1 Introduction

The Chinese wolfberry (*Lycium barbarum*) belongs to the family Solanaceae. Its dried fruit is used as a traditional Chinese medicine, and its main ingredient, lycium barbarum polysaccharide, has been found to have anti-anxiety, anti-oxidant, and neuroprotective effects on humans and rodents (Fakhfakh et al., 2020; KarakaŞ et al., 2020; Lian et al., 2020). Additional components, such as polyphenols and flavonoid minerals, have strong antioxidant activity and the ability to remove DPPH (Thiruvengadam et al., 2020), while zeaxanthin dipalmitate (ZD) has anti-fibrosis, anti-oxidant, anti-inflammatory, anti-apoptotic, and anti-tumor effects, as well as providing protection against chemical-induced and liver damage (Azami & Sun, 2019). Likewise, natural derivatives of ascorbic acid (AA-2βG) help to maintain health by regulating the intestinal flora (Dong et al., 2020), while methanol extract (LEM) provides protection from cisplatin-induced liver and kidney injury (Rjeibi et al., 2018).

The drying process can adversely affect the quality of the product, with a loss of both nutrients and active ingredients (Zhang et al., 2017). Berry species are considered the latest "super fruit" due to their unique taste and high nutritional and medicinal value, and their production has risen in northern and central Europe (Jatoi et al., 2017; Kafkaletou et al., 2017) as. However, the Chinese wolfberry has a high water content and delicate tissue and is thus easily affected by mechanical damage and microbial infection (Ban et al., 2015). As a result, after two to three days at room temperature, the fruit tends to change color and deteriorate on storage, adversely affecting the marketization of the Chinese wolfberry.

In addition to its poor storage tolerance, the Chinese wolfberry is vulnerable to pathogenic microorganisms, both in the field or after harvest. Likewise, surface pathogenic fungi affect such as *Fusarium*, *Alternaria*, *Penicillium*, *Rhizopus*, *Streptospore*, *Alternariaalternata*, and *Cladosporium* (Liu et al., 2017; Wang et al., 2018) lurk on the pericarp surface during or before harvest, invading from the wound to damage the fruit (Shen et al., 2018b; Zambounis et al., 2020). In addition, some yeasts and bacteria invade the fruit and can make the pulp sour (Allard et al., 2020). Thus, consumers are increasingly concerned about the safety and quality of fresh Chinese wolfberries. Previous research on the wolfberry has focused mainly on the isolation of pathogenic fungi on the fruit surface, however, the influence of microbial diversity and interactions on the quality and storage tolerance of the fresh fruit during storage has not been addressed to any extent. There are complex interactions among plants, fungi, and bacteria, which can potentially affect both the quality and storage tolerance of the fruit (Poosakkannu et al., 2017; Soldan et al., 2019). For example, bacteria on the fresh fruit surface may inhibit the development of pathogenic fungi, leading to anti-corrosion effects (Mamphogoro et al., 2020).

The most effective protection against decay is provided by the microbiota associated with the fruit, and these have been utilized as active components in existing commercial biocontrol products

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(Dukare et al., 2019; Almenar & Wilson, 2020). At present, most of the biological control products for the prevention of fruit rot are antagonists that have been isolated from the fruit surface and which are easy to cultivate (Dukare et al., 2019). It has been found that mixtures of antagonists are more effective than individual agents (Solanki et al., 2019; Zhimo et al., 2020). Therefore, the development of microbial colonies on the surfaces of Chinese wolfberry fruit may accelerate and/or delay the rate of decay. It is thus important to develop new biological control agents to delay the rate of decay of fresh fruit.

The diversity of bacterial communities may be assessed by culture-independent techniques that include analysis of the nuclear ribosomal small subunit (16S rRNA) gene fragments (Wang et al., 2017). Recently, high-throughput sequencing methods have been widely used for microbial diversity studies with the aim to better understand the role of diversity in biological control (Balmonte et al., 2019; Ritschard et al., 2018). Compared to traditional capillary sequencing methods, high-throughput sequencing produces more data on microbial diversity in a range of habitats, allowing sequence comparison of 16S, 18S, and internal transcribed spacer (ITS) that are commonly used to characterize fungal communities (Beckett, 2016). High-throughput sequencing has also been widely used to investigate microbial diversity in different fruit species, including tomato, grape, peach, and apple (Allard et al., 2020; Angeli et al., 2019; Ding et al., 2019). There is limited information on bacterial community composition associated with the surface of fresh fruit, which is crucial for screening micro-organisms that can antagonize pathogenic strains.

As the storage times of the Chinese wolfberry differ between different strains, it is likely that the bacterial colony compositions may vary accordingly. In this study, we analyzed the bacterial diversity on the surfaces of eight varieties of Chinese wolfberry during the picking and storage periods using high-throughput sequencing. Our specific objectives included 1) determination of the numbers and proportions of different strains on the fresh fruit using quantitative PCR; 2) clarification of the changes of bacterial composition during the storage of the eight varieties; 3) analysis of the differences in the surface microbial populations in the eight varieties; 4) examination of the relationship between the surface microorganisms and the decay rate during storage; and 5) investigation of the bacterial groups that may reduce the loss after harvest. The answers to these questions may lay a foundation for the development of a new strain of Chinese wolfberry and the identification of a biological control agent for preventing the decay of the fresh fruit.

2 Materials and methods

2.1 Sampling

The Chinese wolfberry (*Lycium barbarum*) strains were selected from the National Germplasm Resource Nursery of China (38°38" N and 106°9"13" E) (Table 1). The fresh fruit from the eight varieties were collected from four-year-old trees. The fruit were picked in mid-July 2020 between 09:00 and 11:00 wearing disposable sterile gloves. The fresh fruit were then placed in a sterile box, with each variety consisting of six boxes of fresh fruit and 100 wolfberries per box. Three boxes of fruit were selected for each variety and the surface microbes were analyzed immediately (O period). The remaining three boxes of fresh fruit were stored at 6 °C and 42% humidity for 144 h, and the decay rate and surface microbes were analyzed over this period (S period).

Table 1. Investigation on the decay rate of fresh *L. barbarum* L. from eight varieties.

Category	O	X	BQ	JS	N	Z4	YL	Z1
144 h (%) decay rate	20	22	26	32	34	38	40	58

The letters in the table represent the names of different variety.

2.2 Surface microorganisms

We first wiped the surfaces of 30 fresh Chinese wolfberries three to five times with cotton swabs soaked in aseptic deionized water to collect the surface microorganisms. The sampling swabs were collected and stored at −40 °C for less than two weeks followed by the extraction of microbial DNA. The total DNA of the microorganisms was extracted using the MP DNA extraction kit (Omega Bio-Tek, Norcross, GA, USA), according to the manufacturer's instructions. The quality of the extracted DNA was assessed by 1% agarose gel electrophoresis, while the concentration and purity of the DNA were determined using a NanoDrop2000 spectrophotometer (Thermo Fisher, Waltham, MA, USA).

2.3 Quantitative Polymerase Chain Reaction (qPCR)

We used the following reaction system: Ex Taq buffer, 10×, μL, 5 dNTP Mix, 2.5 mM, μL Ex Taq, 4 5U, 0.5 μL, Primer F, 10P, μL, 1 Primer R, 10P, μL, 1 Template, μL, 1 ddH2O 12.5 μL, Total, 25 μL. Primer sequences consists of 338F (5'-ACTCCTACTACGGGAGGCAG-3') and 806R (5'-GGGACTACHVGGTWTCTAA T-3'), while reaction conditions were set to 95 °C for 3 min × 1 cycle; 95 °C for 3 min × for 30 sec, 60 °C for 3 min × for 30 sec, 72 °C for 3 min × for 40 sec × 35 cycles.

2.4 Diversity of PCR amplification

We used the 338F (5′-ACTCCTACGGGAGGCAG-3′) and 806R (5′-GGACTACHVGGTW TCTAAT-3′) primers for PCR amplification of the variable V3-V4 regions of the 16S genes. The amplification procedure consisted of 95 °C pre-denaturing for 3 min, 27 cycles (95 °C denaturation for 30 sec, and 55 °C for 30 sec, and annealing at 72 °C for 30 sec), followed by standing at 72 °C for 10 min. Finally, 10 ng of DNA were amplified at 4 °C using an ABI GeneAmp®9700 instrument and 5 × TransStart FastPfu buffer 4 μL, 2.5 mM dNTPs 2 μL PCR reaction system, followed by the upstream primer (5 μM), 0.8 μL, downstream primer (5 μM) 0.8 μL, and TransStart FastPfu DNA polymerase 0.4 μL.

2.5 Illumina Miseq sequencing

The PCR products of the same sample were mixed and recovered using Axygen 2% agarose gel electrophoresis (Biosciences, Union City, CA, USA), and fluorometry measurements (Quantus™Fluorometer, Promega, Madison, WI, USA) followed by detection and quantification of the recovery products. Library construction was performed using the NEXTFLEX Rapid DNA-Seq Kit (PerkinElmer, Waltham, MA, USA) that includes a four-step procedure: (1) linking; (2) magnetic bead screening to remove joint self-connected fragments; (3) library template enrichment using PCR amplification; (4) magnetic bead recovery. We used the Illumina Miseq PE300 platform for sequencing (Shanghai Meiji Biopharmaceutical Technology Co., Ltd., China), and all the raw data were uploaded to the NCBI SRA database (serial number: SRP***).

2.6 Bioinformatics analysis

Bioinformatics analysis was performed using Trimmomatic software original sequencing sequences for quality control, while FLASH software was used for splicing:

(1) The read bases were filtered with a tail mass below 20, following by setting up a window of 50 bp with an average mass value in the window of less than 20. We then filtered the reads below 50 bp after quality control to remove reads consisting of N bases.

(2) According to the overlap relationship between the PE reads, the paired reads were merged into one sequence, with minimum overlap lengths of 10 bp.

(3) The maximum allowable mismatch ratio of the spliced sequence in the overlap region was 0.2.

(4) The samples were differentiated based on barcodes and the primers at both ends of the sequence with an allowable mismatch of 0 and a maximum primer mismatch of 2.0.

We used UPARSE software version 7.1 (Edgar, 2013) to carry out 97% similarity assessment, followed by clustering of the OTUs and elimination of the chimeras *(8)* We then used RDP classifier (Wang et al., 2007) to classify 97% of the OTU representative sequences at similar levels using the Silva database (Release132, Yarza et al., 2014) by setting the comparison threshold to 70. Analysis of species composition was performed in R with the "Qiime" package used to calculate the beta diversity distance matrix.

2.7 Rotting statistics

Count the number of rotting fruits in Chinese wolfberries after storage for 144 h and calculate the rotting rate. Rot rate (%) = (number of rotten fruits/total fruits) %.

3 Results and discussion

3.1 Investigation of the decay rate of fresh Chinese wolfberries after storage

Table 1 shows the decay rate of the eight varieties, including 'O', 'X', 'BQ', 'JS', 'N', 'Z4', 'YL', and 'Z1', after 144 h of storage. The decay rate varied significantly among the varieties, with the Z1 variety showing the highest decay rate at 58% and the variety O showing the lowest decay rate at 20%.

3.2 Quantitative PCR of total microorganisms fluorescence on Chinese wolfberry surfaces

The total microbial biomass on the eight wolfberry varieties were much less at the start of storage than after 144 h of storage (Figure 1). The variety JS had the largest difference between the fresh and stored fruit, with a difference of 3.96e + 09 copies/g, while the variety Z4 had the smallest difference, with a difference of 4.89e + 07 copies/g. After 144 h of storage, the variety X had the least number of bacteria on the surface (7.2046 e + 07 copies/g), which was lower than the variety JS with the largest bacterial diversity by 3.91e + 09 copies/g. There appeared to be no overall

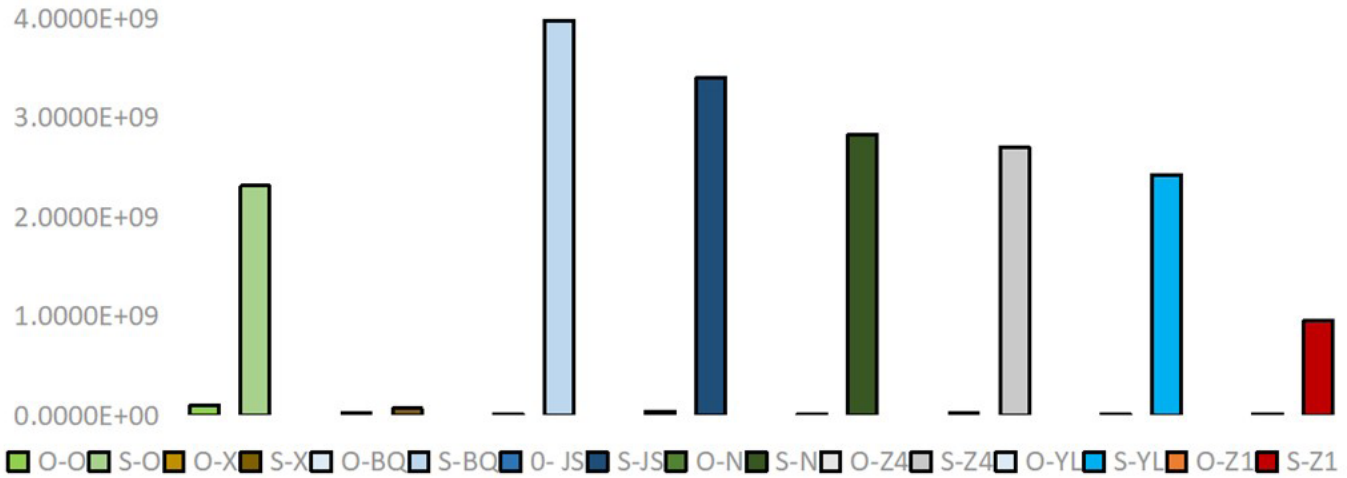

Figure 1. Quantitative PCR of microbial fluorescence on the surfaces of eight varieties of fresh Chinese wolfberries. The abscissa indicates the fresh fruit of the eight varieties in the two periods, and the ordinate represents the copy number per unit mass, in units of copies/g.

relationship between the bacterial numbers and the rate of decay; on the contrary, the number of bacteria in other varieties decreased while the decay rate increased, except for the variety O and the variety X, indicating that relative to the number of bacteria, the decay rate of the fruit was strongly influenced by the species of bacteria on the surface.

3.3 Bacterial diversity

The dilution curve of three replicates of bacterial samples from the surfaces of Chinese wolfberries from the eight varieties before and after storage.

We obtained a total of 2 367 573 high-quality 16S reads from 48 samples. Of the total 16S reads, 1 039 984 16S reads (43 332 sequences per sample) were associated with the fresh fruit samples, while 1 327 589 16S reads (55 316 sequences per sample) were from the stored samples. Based on the high-throughput sequencing of OTUs with 97% similarities, we obtained a total of 737 OTUs, including 584 species, 423 genera, 243 families, 153 orders, 62 classes, and 28 phyla. Application of the dilution curve to the analysis of bacterial α diversity showed that when 5000 reads are reached, the curve tends to be flat. This indicates that the amount of sequencing data is reasonable and includes the vast majority of species.

Figure 2 is a more intuitive representation of the unique and overlapping OUT compositions in the eight varieties at the O and S periods. At the O timepoint, the highest number of OTUs was seen in the variety O (OO), with 235 OTUs, while the lowest was in the variety X (OX) with only 63 OTUs. There was an overlap in 29 OTUs among the eight varieties at timepoint O. The variety YL contained the most unique OTUs, 94 in all, while the variety BQ was the least unique, with only 3 unique OTUs. In the S period, 28 OTUs overlapped among the eight varieties. Among different varieties, the variety N and the variety S had 41 and 40 unique OTUs, respectively, while the variety Z4 and the variety YL had only 3 unique OTUs each. Compared to the O period, there was a slight increase in the number of unique OTUs in the N, JS, BQ, and X varieties, while the number of unique OTUs in the Z4, YL, Z1, and O varieties decreased significantly.

A total of 774 bacterial genera were detected on the surfaces of fresh Chinese wolfberries. Of these, 457 genera were found on the fresh fruit surfaces before storage (O period) and 317 genera after 144 h storage (S period), with an overlap of 214 genera between the two periods. In other words, there were 243 endemic species in the O period, while 103 endemic species were present during the S period.

The bacterial genera identified on the surfaces of the eight varieties of Chinese wolfberry before and after storage are shown in Figure 3. The most abundant genus was *Pantoea* (32.28%) followed by *Massilia* (21.23%), *Pseudomonas* (10.38%), *Rosenbergiella* (6.3%), and *Curtobacterium* (6.29%). Over the eight varieties before storage, the most dominant genus was *Massilia*, with an abundance ranging between 15.98% and 77.20%, followed by *Pseudomonas* with an abundance ranging between 9.63% and 46.10%, and *Pantoea* with an abundance of 2.75%-25.62%. The strain compositions among the eight varieties were slightly different, with unique individual genera observed in particular strains. For example, the abundance of *Paenibacillus* was 18.8% in OJS and was less than 0.5% in the other varieties. The abundance of *Rosenbergiella* in OYL reached 12.57%, in contrast to less than 0.6% in the other varieties, while the abundance of *Saccharibacillus* in OZ1 and OZ4 reached 7.88% and 3.36%, respectively.

Our results based on fluorescence quantitative PCR and colony histograms demonstrated that samples in S period had more diverse and abundant bacterial communities than samples in the O period. Therefore, it is possible that fruit in long-term storage has a higher bacterial diversity, consistent with the results of fungal studies during apple refrigeration (Shen et al., 2018a).

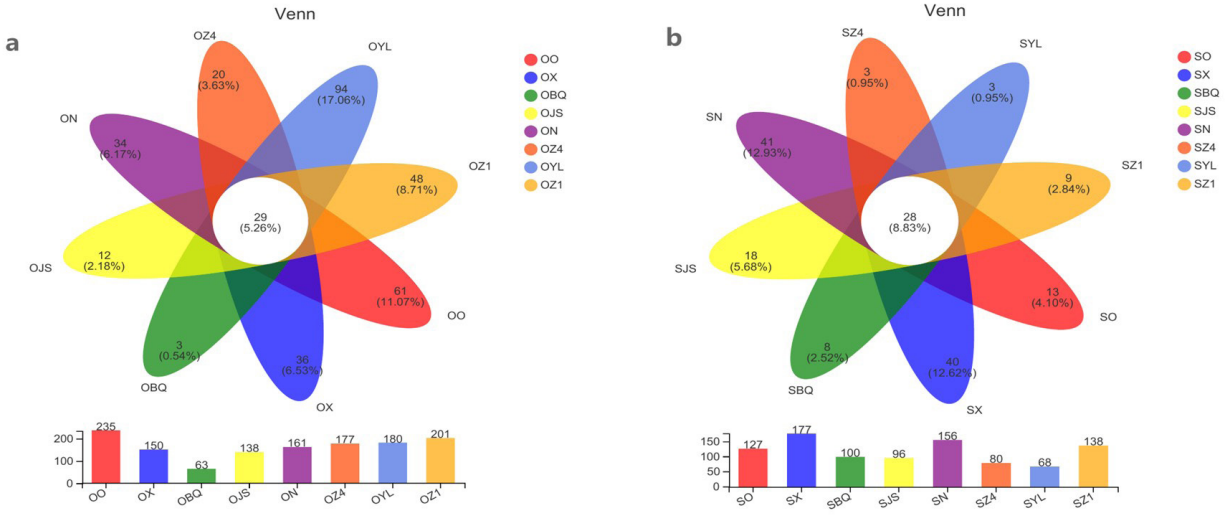

Figure 2. The number of OTU (Operational Taxonomic Unit) is in the O period (A) and S period (B) for the eight varieties. Differently colored petals represent the unique characteristics of different strains, while the center circle of the petals represents the common OTUs of the eight varieties sampled at the same time.

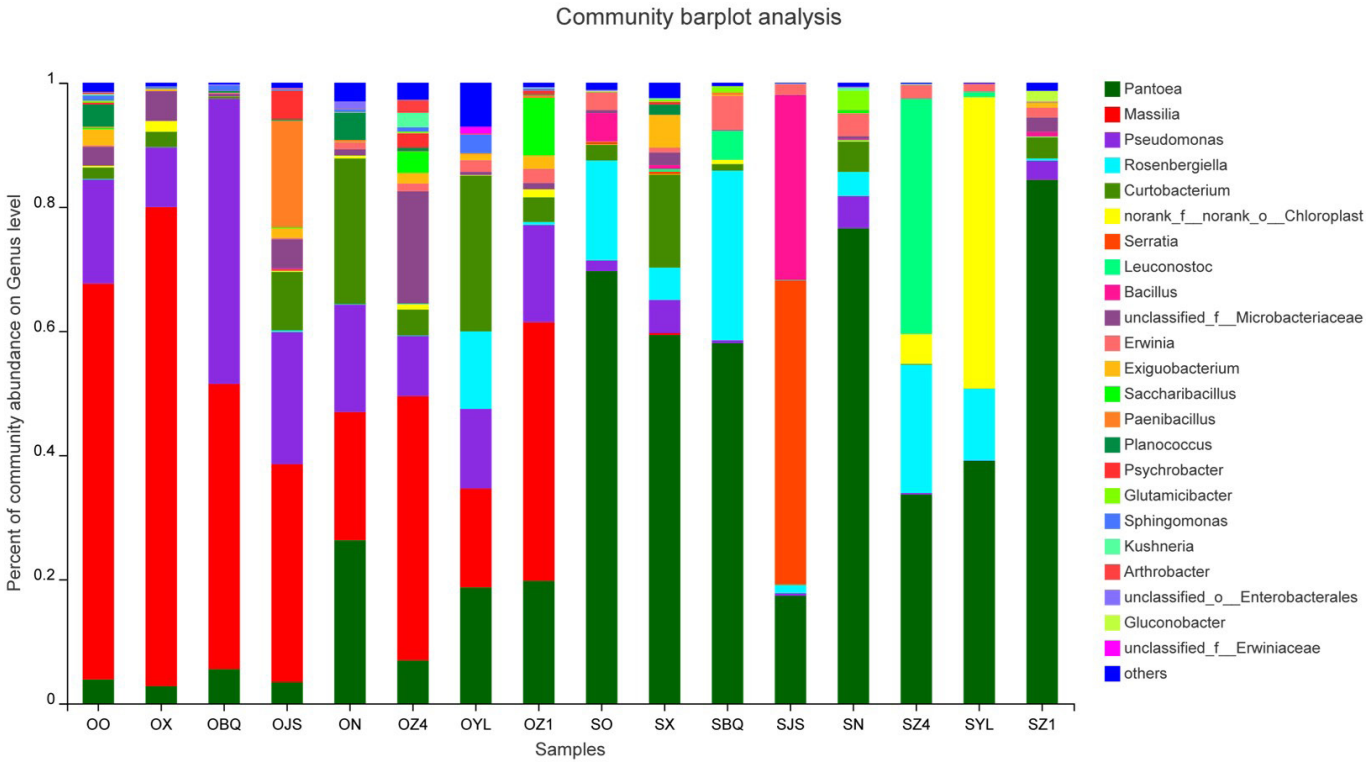

Figure 3. Bacterial colony distribution on the surfaces of fresh Chinese wolfberries showing the top 23 genera and the rest included as "others".

After storage, the species composition of the bacteria varied greatly over the eight varieties, with the most obvious change being the shift in genus composition from *Massilia* to *Pantoea*. Compared to the O timepoint, the proportion of *Pantoea* in all varieties at the S timepoint had increased significantly from 18.22% to 84.34%, while *Massilia* had decreased to almost none. *Pantoea* is generally a chemotrophic bacterium with both metabolic and fermentation characteristics, and its endophytic bacterial strains can alleviate the damage caused by oxidative stress to plants (Li et al., 2016). *Massilia* is widely distributed in both soil and air (Baek et al., 2022). The *Massilia* on the wolfberry surfaces decreased to zero in the S period likely due to storage conditions and/or environmental changes that inhibit *Massilia*.

Rosenbergiella was the most abundant genus in the S period, with an average abundance of 5.45%, although the abundance varied greatly among the varieties ranging between 0.38% and 26.84%. The different varieties also showed their own unique bacterial growth. For example, apart from the increase in *Pantoea* and decline in *Massilia* in the variety O, *Rosenbergiella* showed a change from 0.08% in the O period to 16.99% in the S period, while the high levels of *Paenibacillus* (18.8%) and *Psychrobacter* (5.02%) in OJS almost disappeared in SJS. In SJS, *Serratia* had the highest abundance (46.02%), followed by *Bacillus* (31.76%). Likewise, *Leuconostoc* (37.89%) increased significantly in SZ4 besides *Rosenbergiella. Serratia* showed the highest abundance in SJS (46.02%), followed by *Bacillus* (31.76%).

Bacillus was barely present in any of the eight varieties in the O period. During the S period, its abundance was highest in the JS variety with a decay rate of 32% (middle position among the eight varieties), while in all other varieties it was less than 5%. *Bacillus* is a potential pathogen that promotes decay. Previous studies have shown increased levels of *Bacillus* during the fermentation of pepper, and/or the storage and decay of straw mushrooms and walnuts (Wang et al., 2019; Xu et al., 2021; Zhang & Wang, 2017). Although the *Bacillus subtilis*, *Bacillus amylolyticus,* and *Bacillus cereus* species are the best producers of antifungal volatile organic compounds (VOCs), *Bacillus* species also inhibit fungal growth (Chaves-López et al., 2015) . Some species from *Bacillus* have obvious antagonistic effects on postharvest pathogenic fungi (Chaves-López et al., 2015; Calvo et al., 2017) and can be used in the development of biological control agents (Kafkaletou et al., 2017; Lastochkina et al., 2019; Wang et al., 2018). Further study is needed to better understand the effects of *Bacillus* on fresh Chinese wolfberries.

Principal coordinate analysis (PCoA) was used to examine the relationship between samples using the Curtis distance for calculating beta diversity (Figure 4). The beta diversity according to the Curtis distance showed that the bacterial community changed significantly between the O and S periods (Figure 5a). Analysis of different groups using Anoism showed that there was no significant difference at the species level of bacteria on the wolfberry surfaces in the O period, but there was a significant difference in the S period. In the S period, there was a high abundance of *Pantoea* and the differences among the varieties were marked, while both the abundance and the difference among varieties of *Curtobacterium* were significant. There were significant differences in *Rosenbergiella*, *Bacillus,* and *norank_f__norank_o_chloroplast* abundance among the varieties (Figure 5b). Comparing the O period to the S period,

the abundances of eight species differed significantly ($P \leq 0.001$) including *Pantoea*, *Massilia*, *Pseudomonas*, *Rosenbergiella*, *Leuconostoc, unclassified_f__Microbacteriaceae, Bacillus,* and *Sphingomonas*. The abundance of *Serratia* was also significantly different ($0.001 < P \leq 0.01$) between the O and S periods, as was that of *Exiguobacterium* ($0.01 < P \leq 0.05$) (Figure 5c).

3.4 Comparison of bacterial colonies and decay rates in fresh Chinese wolfberries

Analysis of the relationship between the bacterial genera and the decay rate of the fruit (Table 2) showed no significant correlation between individual bacterial genera and decay rate. For some genera such as *Rosenbergiella*, the correlation coefficient was relatively high, but still not significant. This indicates that the decay rate was not directly related to the abundance of a single genus but may be more related to the composition of the bacterial community. The correlation analysis also showed that there was a significant positive correlation among *Pseudomonas*, *Curtobacterium*, *Serratia,* and *Bacillus* abundance. Additionally, the positive and negative correlations of the surface microorganisms suggested the presence of competitive or symbiotic relationships between microorganisms.

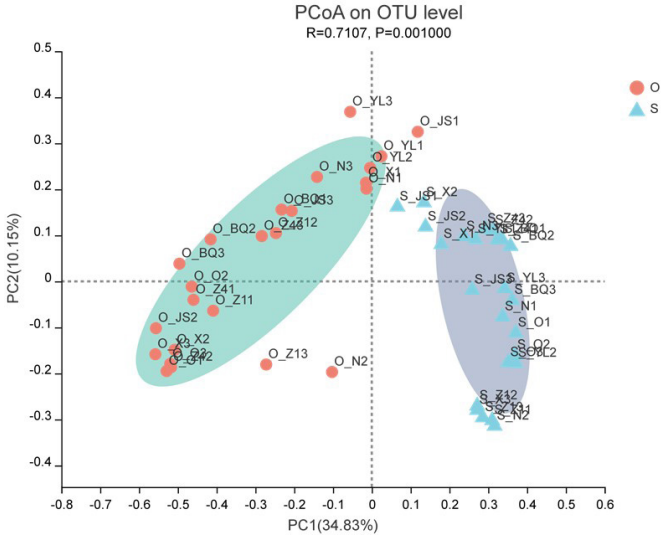

Figure 4. The Bray Curtis distance between the O and S fresh fruit samples of the Chinese wolfberry based on principal coordinate analysis (PCo).

3.5 Functional analysis

Figure 6 shows the different functions of different strains including amino acids, mineral transport, and metabolism, and the system of energy production and microbial gene function related to metabolism (Figure 6).

Controlling postharvest decay is possible under low temperature when combined with chemical fungicides. However, the long-term use of chemical agents can limit their effectiveness due to the emergence of drug-resistant strains with negative impacts on human health and the environment. Therefore, the biological control of postharvest diseases (BCPD) has become an effective alternative to the use of chemical fungicides (Early, 2019; Taoukis & Giannakourou, 2018). The use of antagonistic microorganisms represents an important biological control method. Globally, many microbial-based biological control products have been developed for commercial use with the potential to prevent post-harvest diseases (Bazioli et al., 2019; Wang et al., 2021; Zhimo et al., 2020). Antagonists can be applied directly to the fruit wound using a variety of methods including spraying, which can significantly reduce fruit decay. For example,

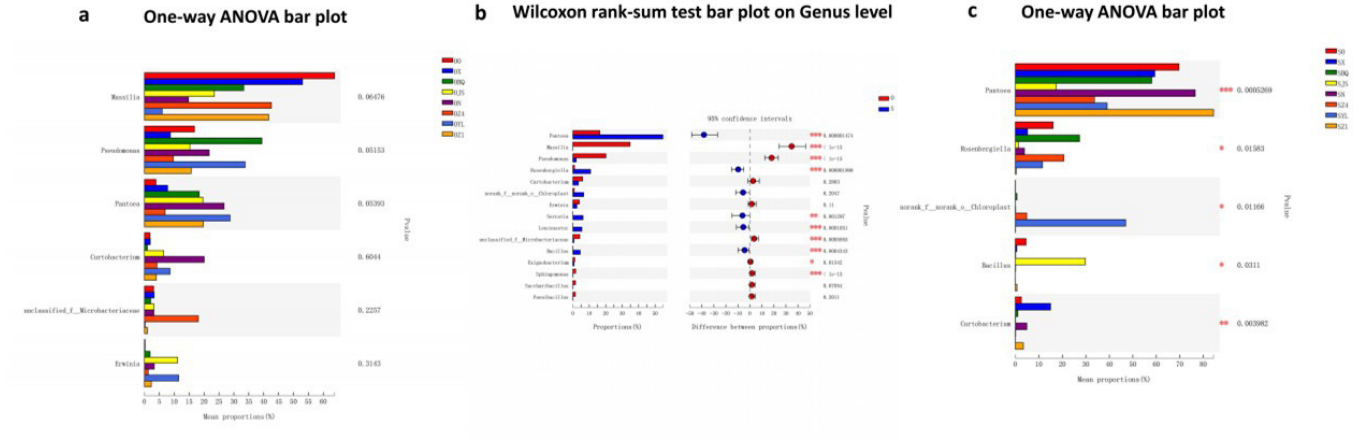

Figure 5. (a) Bacterial genera differing significantly between the O and S periods on the surfaces of Chinese wolfberries. The Y-axis represents the genus and the X-axis represents the average relative abundance among the species. Columns of different colors represent different groups; labels on the right-hand side are P-values. (b) Significance levels of bacterial abundance on the surfaces of the eight varieties of Chinese wolfberry in the S period (B). The Y-axis represents the genus and the X-axis represents the average relative abundance in different species belonging to that genus. Columns of different colors represent different groups and the labels on the right-hand side are P-values. (c) Significance levels of bacterial abundance on the surfaces of the eight varieties of Chinese wolfberry in the O period. The Y-axis represents the genus and the X-axis represents the average relative abundances of species within the genus. Columns of different colors represent groups and the labels on the right hand side are P-values.

Table 2. Correlations between bacterial abundance and rate of decay in eight varieties of Chinese wolfberries in the S period.

	Erwinis	*Bacillus*	*norank_f__ norank_o__ Chloroplast*	*Curtobacterium*	*Rosenbergiella*	*Pseudomonas*	*Pantoea*	*Serratia*	*Leuconostoc*
Bacillus	-0.180								
norank_f__ norank_o__ Chloroplast	-0.314	-0.195							
Curtobacterium	-0.336	-0.260	-0.300						
Rosenbergiella	0.595	-0.380	0.080	-0.342					
Pseudomonas	-0.143	-0.300	-0.404	0.846**	-0.531				
Pantoea	0.259	-0.614	-0.315	0.369	-0.134	0.640			
Serratia	-0.187	0.993**	-0.167	-0.256	-0.395	-0.297	-0.650		
Leuconostoc	0.019	-0.202	-0.044	-0.286	0.494	-0.371	-0.378	-0.172	
Decay rate	-0.290	-0.112	0.221	-0.322	-0.406	-0.086	0.171	-0.066	0.111

**Significant at the P < 0.01 level (two-tailed).

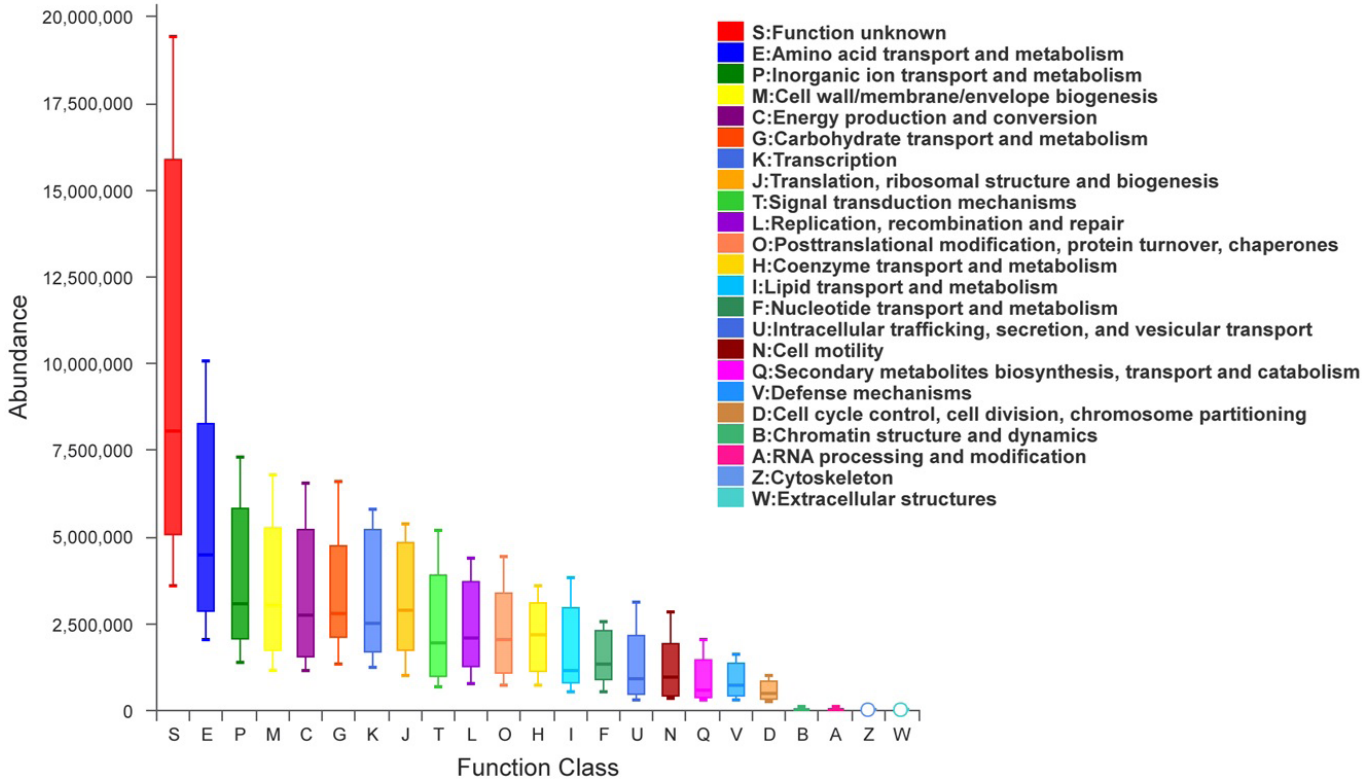

Figure 6. COG(Cluster of Orthologous Groups) function classification statistics box (box length represents abundance at bacterial OTU levels).

Pseudomonas aeruginosa UPMP3 can inhibit rotting of the base stem in oil palm seedlings and has been developed as a biological control agent using antifungal derivatives including phenazine (Parvin et al., 2020). Likewise, the isolation of antagonistic bacteria in tomatoes inhibits the growth of post-harvest *B. cinerae* (Shi & Sun, 2017) while QBA5 has great potential to reduce the damage caused by tomato gray mold (Gao et al., 2018). Similarly, peach fruit treated with JK-14 *Bacillus subtilis* reduced the average incidence of *A. tenuis* and *B. cinerea* by 81.99% and 71.34%, respectively, and reduced the lesion diameters caused by the two species by 82.80% and 73.57%, respectively (Zhang et al., 2019). Many bacterial biocontrol agents have been registered and used to control post-harvest diseases (Early, 2019; Liu et al., 2018). For example, the pioneer biocontrol products Bio-Save 110 (*Pseudomonas aeruginosa*) and Aspire (*Pseudomonas aeruginosa* I-182) were registered with the and have been successfully used to control the post-harvest decay of grapes, citrus, and pears (Taoukis & Giannakourou, 2018; Liu et al., 2018).

At present, the research on the storage of fresh Chinese wolfberries has mainly focused on the isolation of pathogenic fungi. Fungal isolation, however, is limited by the culture conditions and is unable to fully reflect the microbial diversity

on the surfaces of the Chinese wolfberry. In contrast, high-throughput sequencing is widely and successfully used for the study of microbial diversity on fruit surfaces and is not limited by culture conditions. Our findings will assist in the control of post-harvest pathogens on fresh Chinese wolfberries, as well as providing directions for the development of safe and environmentally friendly biological preservatives, and a basis for the genetic improvement of the Chinese wolfberry.

4 Conclusion

We analyzed the bacterial diversity on the surfaces of eight varieties of Chinese wolfberries using high-throughput sequencing before and after storage. A total of 737 OTUs, including 584 species, 423 genera, 243 families, 153 orders, and 62 classes, were identified by bioinformatic analysis of the OTUs at the 97% similarity level. There were no significant differences among fruit varieties just after picking (O period). The dominant genera during the O period were *Massilia* and *Pseudomonas*. However, after a 144-hour storage period (S period), there were significant changes in both the decay rate and surface bacterial compositions. The total amount of bacteria on the surface of fruit at S period was much greater than that at O period, and the composition of bacterial colony among varieties at S period was significantly different. The most obvious change from O period to S period is the *Pantoea* increase and decrease of *Massilia*. The Z1 variety showed the highest rate of decay, and its surface microbiota was dominated by *Pantoea* (84.34%), while the variety with the lowest decay rate was O, dominated by both *Pantoea* (69.06%) and *Rosenbergiella* (16.99%).

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