Effects of sodium hypochlorite cleaning on microbial diversity and phenotype of fresh-cut onions based on high-throughput sequencing

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Abstract: Fresh onion was used as raw material, sodium hypochlorite cleaning was used as the treatment group, and water cleaning was used as the control group. Stored at 4°C, high-throughput sequencing technology was used to compare the microbial succession and phenotype prediction of fresh-cut onion at the 1st, 3rd and 5th day, and to explore the effects of sodium hypochlorite cleaning on the microflora structure and phenotype of fresh-cut onion during storage. The results showed that at the level of family and genus, sodium hypochlorite cleaning had a significant inhibitory effect on Enterobacteriaceae, Enterobacter, Pantoea and Kosakonia. Compared with the control group, the relative abundance of facultative anaerobic, gram-negative and potential pathogenic phenotypes of bacteria in fresh-cut onion after sodium hypochlorite cleaning decreased, while the relative abundance of aerobic, anaerobic and gram-positive phenotypes increased. After cleaning with sodium hypochlorite, it could to some extent reduce the contamination of pathogenic bacteria in fresh-cut onion, thereby improving the quality and safety of fresh-cut onion.

1. Introduction

Onion is a vegetable belonging to Allium cepa L. in the liliaceae family, known as the "Queen of vegetables"[1] due to its high nutritional content. Fresh-cut onion, due to cutting and processing, caused their juice to overflow, providing nutrients for the growth and reproduction of microorganisms, thereby accelerating their decay rate and reducing the quality of freshly cut onions. Cleaning could remove the excess juice on the surface of fresh-cut onion and delay its decay and spoilage. Therefore, cleaning was a crucial link in the processing, storage, and preservation of fresh-cut onion[2].

At present, the cleaning techniques of fresh-cut fruits and vegetables mainly include physical cleaning techniques such as ultrasound[3], chemical cleaning techniques using chemical reagents such as chlorine dioxide[4], and biological cleaning techniques such as bacteriophages[5]. The research results of MaribelAbadias et al.[6] indicated that sodium hypochlorite could effectively inhibit and kill pathogenic microorganisms on the surface of fresh-cut fruits and vegetables. However, there have been no reports of phenotypic changes caused by changes in bacterial community structure after cleaning with sodium hypochlorite.

Taking fresh-cut onion as the research object, high-throughput sequencing technology was used to explore the microbial community succession of fresh-cut onion after cleaning with sodium hypochlorite. BugBase prediction was performed on the phenotype, and differences in microbial community structure were analyzed. Phenotypes were compared to clarify the impact of sodium hypochlorite cleaning on the microbial community structure and phenotype of fresh-cut onion during storage, providing a theoretical basis for the pre-processing industry of fresh-cut onion.

2. Materials and methods

2.1. Materials

Onion, a red skin variety.

2.2. Pretreatment of Onions

Onions cleaned with 100 mg/L sodium hypochlorite were used as the treatment group, while onions cleaned with deionized water were used as the blank control group, PE film was packaged, about 100 g per package, and stored at 4°C. As show in table 1.

Table.1 Corresponding table of sample number

Treatment method	Sequencing area	Sample number
Day 1 Control group (CK)	16s:V3+V4 (bacteria)	W1
Day 1 Control group (CK)	16s:V3+V4 (bacteria)	W3

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Day 1 Control group (CK)	16s:V3+V4 (bacteria)	W5
Day 1 Treatment group (CLSN)	16s:V3+V4 (bacteria)	Y1
Day 1 Treatment group (CLSN)	16s:V3+V4 (bacteria)	Y3
Day 1 Treatment group (CLSN)	16s:V3+V4 (bacteria)	Y5

2.3. Construction and diversity analysis of 16S rDNA library

PCR amplification of microbial DNA was performed using 16S V4 region primers. The products were purified, quantified and homogenized to form a sequencing library. The established library was first subjected to library quality inspection, and the qualified library was sequenced by Illumina Hiseq2500.

2.4. Data processing

Use R software (Version 2.15.3) draw dilution curve. Rank abundance curves, and species accumulation curves, conduct inter group difference analysis of Alpha diversity index, and PCA graphs were drawn.

3. Results and analysis

3.1. OTUs Analysis

From Fig.1, it could be seen that there were a total of 63 OTUs in the control group, 63 OTUs in the treatment group. There were 59 OTUs in the control group on the first and third days, and 60 OTUs on the fifth day, this indicated that there was not much change in the diversity of microorganisms in the control group during storage. On the first day, there were 57 OTUs in the treatment group, 61 OTUs on the third day, and 60 OTUs on the fifth day, indicating that fresh-cut onions treated with sodium hypochlorite would have new bacterial communities on the third and fifth days. Compared with the control group, this indicates that sodium hypochlorite still had the effect of inactivating bacterial communities when just treated.

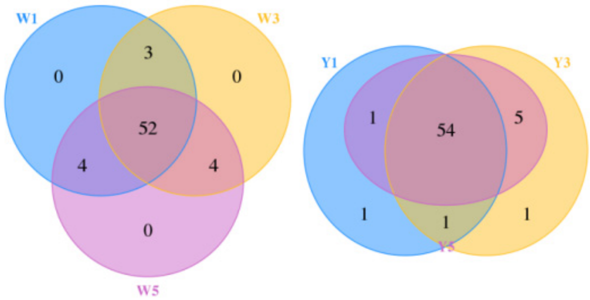


Fig.1 OTUs analysis of each group

3.2. Alpha (Alpha diversity) diversity analysis

As could be seen from Table 2, Simpson index value of Y1 was higher than W1, and Shannon index value of Y1 was lower than W1, indicating that bacterial diversity in Y1 was lower than W1, indicating that the microbial diversity of fresh-cut onion was reduced after cleaning with sodium hypochlorite, so sodium hypochlorite had bactericidal effects.

Table.2 Statistics of Alpha diversity index

Sample name	OTU	ACE	Chao1	Simpson	Shannon	Coverage/%
W1	59	59.76	59.25	0.1645	2.0539	1.00
W3	59	62.25	63.20	0.2742	1.6694	0.99
W5	60	61.57	63.33	0.1846	1.9294	0.99
Y1	57	57.00	57.00	0.3058	1.6920	1.00
Y3	61	61.49	61.25	0.5340	1.1161	1.00
Y5	60	61.03	61.00	0.4203	1.3952	1.00

3.3. Analysis of microbial diversity

3.3.1. The level of family

From Fig.2, the top ten microflora in the control group and the treatment group were: Lactobacillaceae, Moraxellaceae, Pseudomonadaceae, Acetobacteraceae, Streptococcaceae, Mitochondria, uncultured_bacterium, Allium_ampeloprasum_leek, Enterobacteriaceae, Leuconostocaceae. Compared with control group, the treatment group showed a significant increase in the relative abundance of Leuconostocaceae, uncultured_bacterium and Allium_ampeloprasum_leek in fresh-cut onion during storage after cleaning and disinfection with sodium hypochlorite, while the relative abundance of Enterobacteriaceae decreased significantly, indicating that sodium hypochlorite cleaning had a significant inhibitory effect on Enterobacteriaceae.

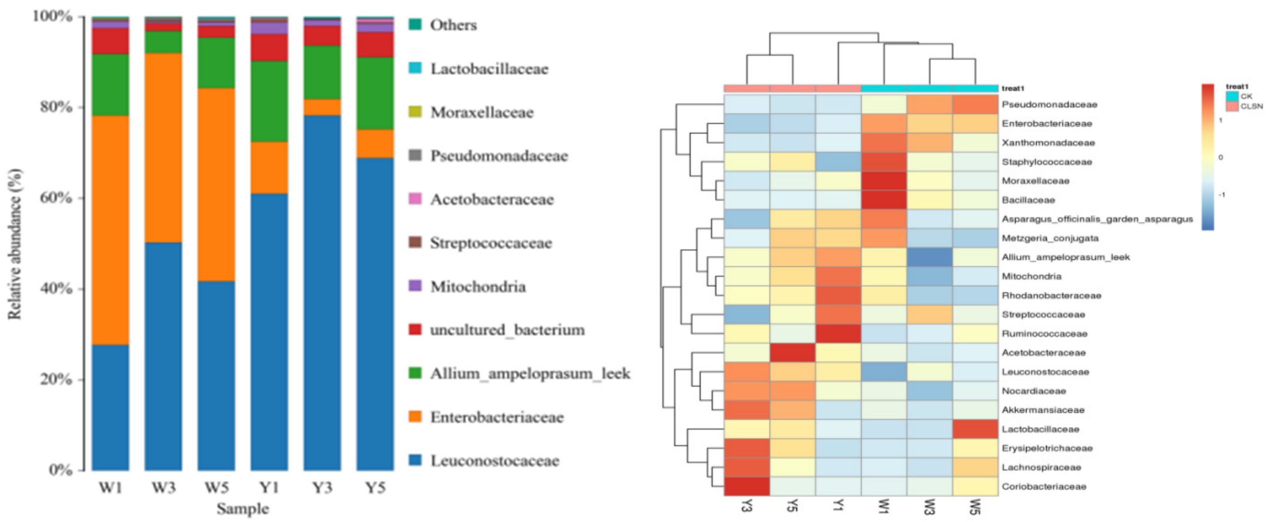


Fig.2 Distribution of bacterial community structure at the family level and cluster heatmap of abundance in samples

3.3.2. The level of genus

From Fig.3, it could be seen that during storage, the top ten bacteria genera with relative abundance in the treatment group and the control group were: Raoultella, Pectobacterium, Orontium_aquaticum, Kosakonia, uncultured_bacterium, Pantoea, Leuconostoc, Allium_ampeloprasum_leek, Enterobacter, Weissella. Compared with the control group, the relative abundation of Weissella and Allium_ampeloprasum_leek increased significantly in the treatment group, while the relative abundance of Enterobacter, Leuconostoc, Pantoea and Kosakonia decreased significantly. Enterobacter, Pantoea and Kosakonia all belong to the family of Enterobacteriaceae, indicating that the growth of Enterobacteriaceae could be effectively inhibited after cleaning with sodium hypochlorite.

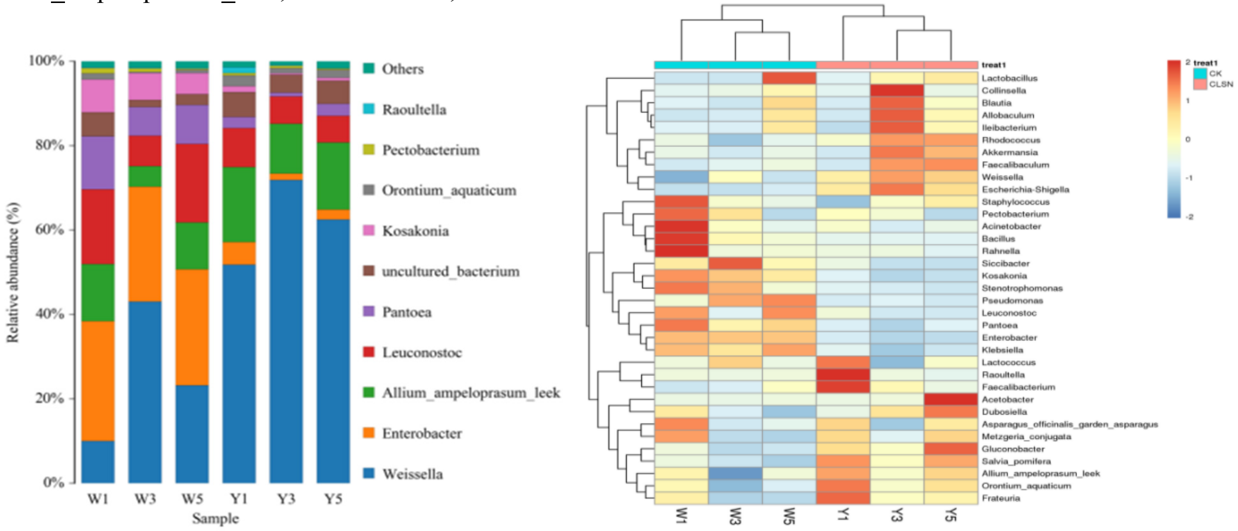


Fig.3 Distribution of bacterial community structure at the genus level and cluster heatmap of abundance in samples

3.4. Analysis of significant differences between groups

From Fig.4, the treatment group had higher relative abundance at the family level during storage, including Nocardiaceae and Leuconostocaceae. At the genus level, the relative abundance of Rbodococcus and Weissella was higher. During storage, the control group had a relatively high abundance of Enterobacteriaceae at the family level, while at the genus level, the relative abundance of Enterobacteriaceae, Klebsiella, Kosakonia, Pantoea, Siccibacter, and Pseudomonas genera was higher. Comparing the treatment group and the control group, it was found that during storage, the treatment group cleaned with sodium hypochlorite had almost no levels of Enterobacteriaceae, Enterobacter at the family to genus level, indicating that sodium hypochlorite had a significant inhibitory effect on Enterobacteriaceae.

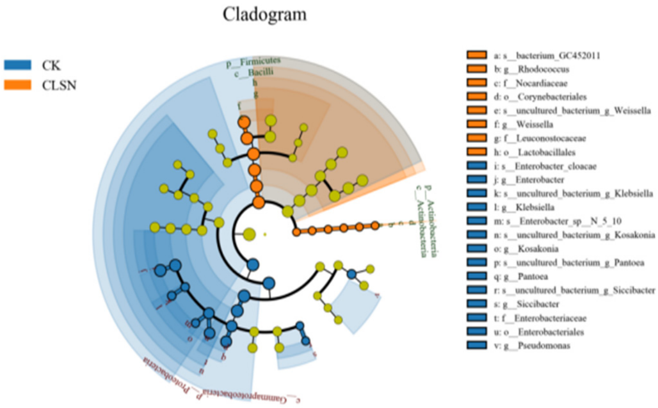
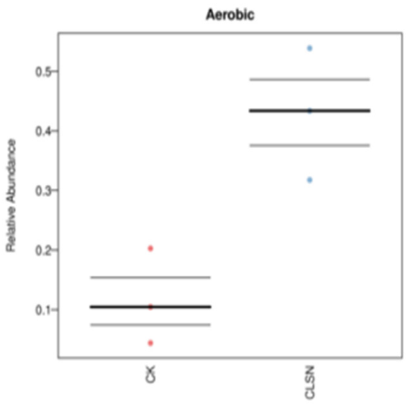


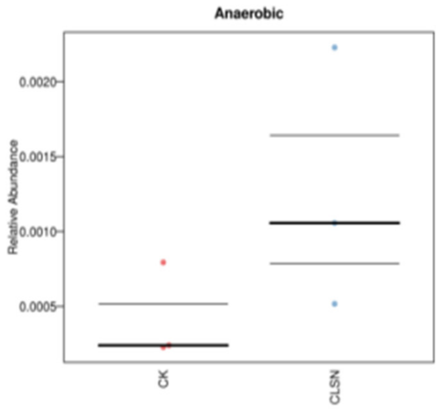
Fig.4 Evolutionary branching diagram of LEfSe analysis

3.5. BugBase function prediction

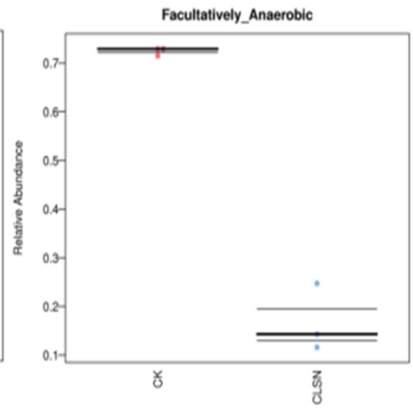
From Fig.5, it could be seen that fresh cut onions treated with sodium hypochlorite had a higher relative abundance of aerobic, anaerobic, and gram positive microorganisms during storage. The treatment group showed significant inhibitory effects on gram negative bacteria such as Enterobacteriales, Enterobacteriaceae, Enterobacteriaceae, and Pantobacteria. In Fig.e, the relative abundance of gram negative bacterial types in the treatment group cleaned with sodium hypochlorite was lower, which is consistent with the above results. From Fig.f, it could be seen that the relative abundance of potential pathogenic microorganisms in the treatment group cleaned with sodium hypochlorite was lower, indicating that sodium hypochlorite cleaning could effectively inhibit the growth of pathogenic bacteria and better protect the safety of fresh cut onions during storage.



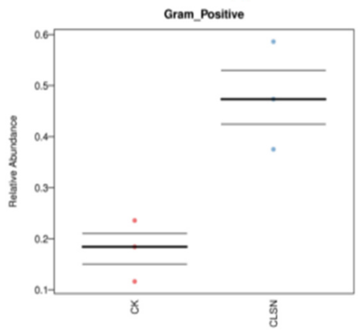
a-aerobic type



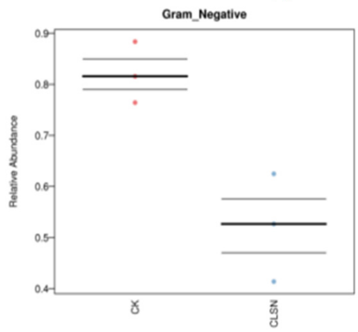
b-anaerobic type



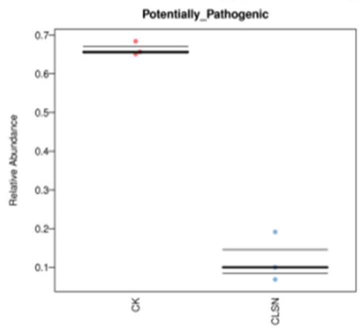
c-facultatively_anaerobic type



d-gram_positive type



e-gram_negative type



f-potentially_pathogenic type

Fig.5 Phenotype prediction chart

4. Conclusion

In this paper, the results showed that the diversity of microorganisms on the surface of fresh-cut onion decreased after sodium hypochlorite cleaning. High-throughput sequencing technology was used to investigate the effects of sodium hypochlorite on the microflora structure of fresh-cut onion. After sodium hypochlorite cleaning, the abundance of Enterobacteriaceae, Enterobacter, Pantoea and Kosakonia on the surface of fresh-cut onion decreased. Alejandro Tomas-Callejas et al.[7] showed that sodium hypochlorite had an inhibitory effect on Escherichia coil O157:H7, which was consistent with the results in this paper. It was found that bacteria in fresh-cut onion cleaned by sodium hypochlorite tended to aerobic, anaerobic and gram-positive, and the relative abundance of potential pathogenic types was significantly reduced, indicating that sodium hypochlorite cleaning could to some extent reduce the risk of contamination of fresh-cut onion by pathogenic bacteria. Therefore, it is necessary to pay attention to the cleaning of pre-processed onion, so as to improve its quality and safety.

References

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