Study on microbial diversity of fresh-cut potatoes in different storage periods

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Abstract. In order to extend the shelf life of fresh-cut potatoes, maintain their edible quality, and clarify the flora succession rules in fresh-cut potatoes during storage (4 °C), this experiment uses PE film natural packaging, and high-throughput sequencing was used to study the On day 0, day 7, and day 14, the microbial diversity was high. The results showed that the dominant bacteria in the samples stored on days 0, 7, and 14 were mainly Pseudomonas, Pantoea, Erwinia, Bucher Buchnera. There is basically no difference in the diversity of microorganisms during different storage periods, but the abundance changes.

1. Introduction
Fresh-cut fruits and vegetables have become a new trend in domestic consumption and have great market demand and market potential. In China, fresh-cut fruits and vegetables have become a new direction to extend the vegetable industry chain[1]. The sales counters of fast food restaurants, supermarkets and department stores at home and abroad have become more and more important [2-4]. Although its pretreatment provides convenience for subsequent processing, and saves a lot of time and waste disposal costs, it results in exposure of potato tissue to oxygen, which easily results in enzymatic browning, microbial proliferation, accumulation of harmful substances and nutrition Material loss[5]. The microorganisms on vegetables come from the following aspects. First, vegetable tissues are susceptible to soil bacteria infection due to their low acid content, such as Pseudomonas, Xanthomonas, and Erwinia [6-7]. The second is the pollution of fresh vegetables before processing, that is, the microorganisms that exist and remain after cleaning, such as Vibrio cholera on cabbage [8], which is affected by its technology and cultivation environment and microbial growth characteristics; The composition and pH show that the contaminated microorganisms in fresh-cut vegetables are mainly molds, bacteria, and yeasts, among which there are many bacteria and molds. Common bacteria are Erwinia, Pseudomonas, Xanthomonas, Corynebacterium, Paenibacillus, Clostridium.

This experiment is different from previous studies. By studying the microbial diversity of fresh-cut potato flakes at different storage times, obtaining microbial flora information at different storage times, measuring sensory and enzyme activities and other indicators at different storage times, and...
analyzing them Correlation with different microbial flora, exploring the effect of different microbial flora on the quality of fresh-cut potatoes, providing useful information for further research on fresh-keeping of fresh-cut potatoes.

2. Materials and Reagents

2.1. Materials
Dutch Potato 15 were provided by Beijing Yunong High Quality Cultivation of Agricultural Products Company.

3. Experimental Methods

3.1. Collection of fresh-cut potato microorganisms
Fresh-cut potato chip samples during the storage period of 0, 7 and 14 days were rinsed with sterile PBS buffer, and 5 mL sterilized centrifuge tubes were collected and marked. A fresh-cut potato sample on day 0 is denoted as A1, a fresh-cut potato sample on day 7 is denoted as B1, and a fresh-cut potato sample on day 14 is denoted as C1.

3.2. Extraction of microbial DNA from fresh-cut potato samples
For three groups of fresh-cut potato chip samples, DNA was extracted using the Tiangen kit. For specific methods, see the kit instructions. The quality of the extracted DNA samples was tested using a 1.5% agarose gel, and the qualified samples were stored on dry ice and sent to Beijing Nuohe Zhiyuan Bioinformation Technology Co., Ltd. for sequencing analysis.

3.3. 16S rDNA library construction and diversity analysis
16S V4 region primers (515F: 5'-GTGCCAGCMGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify microbial DNA. The Illumina Hiseq platform was used to analyze the amplified sequencing results. After cutting the Barcode and primer sequences, the reads of each sample were spliced using FLASH, and then the chimera sequence was removed to obtain the final valid data. Uparse v7.0 1001 was used to cluster all valid data of all samples. By default, the sequences were clustered into OTU with 97% consistency. Annotate the representative sequences of OTUs, use Mothur method and SILVA's SSUrRNA database to perform species annotation analysis (set threshold is 0.8 1.0), and obtain taxonomic information at each classification level: kingdom, phylum, class, order, famiy, Genus, and species count the community composition of each sample. The PyNAST software was used to perform rapid multi-sequence alignment with the "Core Set" data information in the GreenGene database to obtain the systematic relationship of all OTUs representative sequences. Finally, the sample data is used as a standard to uniformize the sample data. Use Qime software to calculate Observed-species, Shannon index, Goods-coverage, Alpha [9].

4. Results and discussion

4.1. Analysis of Bacterial 16S rRNA Gene Side Sequence Results
At the similarity level of 97%, the number of OTUs of each sample is obtained. The Venn diagram can be used to show the number of common and unique OTUs between the samples (number 2 to 5), which intuitively shows the overlap of OTUs between samples. A total of 54 OUTs were obtained from microbial samples of fresh-cut potatoes during storage, of which A1, B1, and C1 samples contained 35, 39, and 33 OTUs. Goods coverage is the proportion of the entire genome sequenced to assess the integrity of the sample. The coverage rate of all samples in this study is 100%, which indicates that the test results have good coverage. The diversity analysis of a single sample can reflect the richness and diversity of the microbial community in the sample. Shannon is used to estimate one
of the microbial diversity indexes in the sample. A larger value indicates a higher community diversity. B1 in this study The highest Shannon index of the sample was 1.8192, indicating that the fresh-cut potato had rich bacterial diversity on the seventh day of storage. The lowest Shannon index of the A1 sample was 0.7, and the lowest index of the C1 sample was 1.5196. The results showed that the bacterial abundance was highest on the 7th day during storage of fresh-cut potatoes (Table 1).

| Sample ID | OTU | ACE   | Chao1  | Simpson | Shannon | Coverage |
|-----------|-----|-------|--------|---------|---------|----------|
| A1        | 35  | 36.3259 | 35.75  | 0.6512  | 0.7     | 1.0      |
| B1        | 39  | 39.0    | 39.0   | 0.2797  | 1.8192  | 1.0      |
| C1        | 33  | 34.3867 | 34.0   | 0.3371  | 1.5196  | 1.0      |

4.2. Analysis of bacterial diversity
Statistical analysis of relative abundance of species at the gate level according to the results of species annotation. It can be seen from Fig. 1 that the samples on day 0, day 7, and day 14 include: Proteobacteria, Cyanobacteria, Cyanobacteria, Actinobacteria, Bacteroidetes. In the day 0 sample, Cyanobacteria was the dominant flora with 79.26%, followed by Proteobacteria (20.52%). In the day 7 sample, the relative abundance of Proteobacteria (51.11%) and Cyanobacteria (45.02%) was higher.Secondly, Cyanobacteria (3.8%). Compared with the sample on the 14th day, the relative abundance of Proteobacteria (51.11%) was basically unchanged, and Cyanobacteria (48.26%) increased slightly. The species differences in the samples on day 0, day 7, and day 14 were not significant, but the relative abundances were different, and the dominant bacteria groups on day 7 and day 14 were not significantly different.
f Enterobacteriaceae (27.65%), Buchnera (4.67%), Pectobacterium (4.18%), Nicotiana otophra (3.77%), Pantoea (3.37%). The samples on the 14th day mainly contained Lachnoelostridium 5 (47.92%) and Pectobacterium (34.49%), followed by Erwinia (5.35%) and did not contain Uncultured bacterium. The bacterial diversity of the three groups of samples was not significantly different.

4.3. Core microorganism colony analysis
At the similarity level of 97%, the number of OTUs of each sample is obtained, and the coincidence of OTUs between samples is visually displayed by drawing a Wayne diagram. Combine the species represented by OTU to find common microorganisms in different environments. The OTU-Venn of each sample drawn according to different groups is as follows: different samples are represented by different colors, and the number of overlapping parts between different color graphics is the number of OTUs shared between the two samples. As can be seen from Figure 3, there are 35 OTUs of fresh-cut potato chips on day 0, including Nicotiana otophora (79%), Uncultured bacterium (17.6%), Buchnera (3.1%), and 39 OTUs on day 7, including Lactococcus (44.85%), Uncultured bacterium Enterobacteriaceae (27.65%), Buchnera (4.67%), there were 33 OTUs of fresh-cut potato chips on the 14th day, and the dominant genus mainly included Lachnoelostridium (47.92%) and Pectobacterium (34.49%), Followed by Erwinia (5.35%). Sixteen of these OTUs were common during storage. During the storage of fresh-cut potato chips, several fungi are common, and they are mainly: Erwinia, Nicotiana otophora, Buchnera, Pantoea, Pseudomonas.

Fig.2 Distribution of generic level bacterial population structure in samples
5. Conclusions
This experiment uses a high-throughput sequencing analysis method to analyze the microbial diversity of fresh-cut potatoes at different storage times to understand the dominant flora of fresh-cut potatoes at different storage times. The results show that: at the level of the door, the main flora is Proteobacteria, Firmicutes; at the level of the class, the dominant flora is Bacteroidia, Actinobacteria, Alphaproteobacteria, Bacilli, Clostridia, Oxyphotobacteria, Gammaproteobacteria; at the level of genus, abundance The top 10 are: Pseudomonas, Pantoea, Erwinia, Buchnera, Uncultured bacterium, Uncultured bacterium Enterobacteriaceae, Pectobacterium, Lactococcus, Lachnoelosridium, Nicotiana otoplora and the main microorganisms on fresh cut fruits and vegetables are Pseudomonas and Erwinia. Basically consistent with the experiment.

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