Effect of *Aureobasidium Pullulans* S-2 on the Postharvest Microbiome of Tomato During Storage

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Research

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Abstract

Background: Biological control of fruit postharvest diseases by antagonistic microorganisms has been considered an effective alternative to chemical fungicides. The influence of microbial antagonists on fruit-associated microbiome will provide a new perspective for in-depth study of the antagonistic mechanism. In this study, the biocontrol efficacy of *A. pullulans* S-2 against postharvest diseases of tomatoes was investigated. Meanwhile, the fungal and bacterial microbiota on tomato surfaces were examined by high-throughput sequencing.

Results: *A. pullulans* S-2 can significantly inhibit the decay rate, maintain fruit firmness and reduce weight loss of tomatoes. In addition, the treatment group can maintain higher titratable acid, ascorbic acid and lycopene than the control group. After using *A. pullulans S-2*, more dramatic changes were observed in fungal diversity than bacterial in the microbiota. *Aureobasidium* was significantly enriched in the treatment group, while *Cladosporium*, *Mycosphaerella*, *Alternaria* and *Penicillium* were deficient compared with the control group. *Pantoea*, *Brevibacterium*, *Brachybacterium*, *Serratia*, *Glutamicibacter* and *Pseudomonas* also had significant differences between the two groups.

Conclusions: This study demonstrated that the application of *A. pullulans S-2* resulted in alterations in the bacterial and fungal community and that could inhibit pathogens and decrease fruit disease incidence. It provides new insights into the dynamics of the tomato's surface microbiome after microbial antagonist treatment.

1. Background

Tomato (*Solanum Lycopersicon* L.) is an important fruit that is widely grown globally, whose annual production is more than 180 million tons in 2019 (http://www.fao.org/faostat/en/#data/QC). However, during harvesting and transportation, the tomato will be easily damaged due to its fragile skin and soft flesh, the disease development results in a substantial economic loss [1]. The primary diseases of postharvest tomatoes are gray mold caused by *Botrytis cinerea* [2], black rot caused by *Alternaria alternata* [3] and so on.

Chemical fungicides are still the most common method to control postharvest decay. Still, the long-time dependence on chemical fungicides has resulted in fungicide residues, environmental hazards, and pathogen resistance. Recently, the use of microbial antagonists and their active compounds to control postharvest decay of fruits has been a research hotspot, and this eco-friendly method may replace chemical fungicides in the future [4].

The microbial antagonists are mostly isolated from the plant and soil, and their ability of rapid growth and colonization in wounds is the main criterion. Yeast and yeast-like fungi occupy a very important position in the research of microbial antagonists. Their biocontrol mechanisms mainly consist of competition for nutrients and space, induction of resistance mechanisms and secreting antimicrobial substances [5]. *Aureobasidium pullulans*, a yeast-like fungus, has been used in postharvest disease

prevention and control of several fruits such as avocado, citrus, peach, apple [6–8], It can antagonize several pathogens, such as *Penicillium spp.*, *Botrytis cinerea*, *Phytophthora cactorum* [9–11].

Previous research mainly focused on the aspects of microbial antagonists, pathogens and fruits to elucidate the mechanism. However, an important part, where the microorganisms act on the surface, is ignored [12, 13]. Microorganisms play an important role in the growth of plants, including beneficial, pathogenic and other microorganisms. After harvest, the microbial community dynamics could change due to the factors related to fruit physiology and other abiotic effects, including packaging, storage, and other postharvest treatments [14]. The changes of microorganisms on the fruit surface are closely related to fruit rot during storage [15]. It is generally believed that high microbial diversity and resilient microbiome structure are beneficial for fruits and vegetables. The occurrence of postharvest diseases of fruits is due to the proliferation of pathogenic fungi disturbing the microbial balance [16].

In recent years, several reports were demonstrating changes in microbial communities on fruit surfaces following different physical or chemical treatments. For example, the combination of ozone treatment and polyethylene packaging [17], hot water [18], waxing [19]. These researches have shown that a deeper understanding of changes taking place on fruit surfaces after harvest could provide new ideas for applying post-harvest control methods. However, there is limited information on the effect of microbial antagonists on microbial communities on the surface.

In this study, we screened a strain, *A. pullulans* S-2, from healthy tomatoes and used it on tomatoes to observe whether it could reduce the incidence of diseases during storage and examine its influence on the quality of tomatoes. The changes in the bacterial and fungal communities on tomato fruit surface were also investigated using amplicon sequencing of the 16S and ITS conserved regions in the rDNA.

2. Methods

2.1 Tomato

The tomato fruits of the cultivar ‘Provence’ (introduced from the Netherlands) were harvested at the red-ripe stage from Hanya Organic Farm (32°11'N, 119°27'E), Zhenjiang City, Jiangsu Province, China. After harvest, fruits were packed in foam boxes and transported to our laboratory within one hour time. Medium size tomatoes with similar maturity indices were selected for experiments.

2.2 Biocontrol tests on tomato fruit

A. pullulans S-2 was cultivated in nutrient yeast dextrose broth (NYDB: 8 g nutrient broth, 5 g yeast extract and 10 g dextrose in 1 L of distilled water) at 180 rpm, 28°C for 24 h (two consecutive culturing). Then, the yeast was centrifuged at 5000 × *g* for 5 min, and washed with sterile water twice, finally suspended in sterile physiological saline (0.85% NaCl). The concentration of cells was adjusted to 1×10^8 cells/mL using a binocular microscope and a hemocytometer.

The tomatoes were randomly divided into two groups. The first group of tomatoes was dipped in *A. pullulans* S-2 suspension of 1×10^8 cells/mL for 1 min designated as the treatment group. The control group was dipped in sterile physiological saline. Treated tomatoes were then kept in a plastic box (48 × 38 × 15 mm) to dry. After drying, the boxes were wrapped with plastic film to maintain high humidity, and incubated at 10℃ for 8 d and then transferred to shelf life for 4 d at 20℃. Decay incidence and severity, and fruit quality indices (firmness, weight loss rate, titratable acid, ascorbic acid and lycopene level) were determined.

2.2.1 Firmness

TA-XT2i Texture Analyser (Stable Micro Systems, UK) was used to test the firmness of tomatoes [20]. TA settings were: P5; the test speed was 1 mm/s, the pre-test and after-test speed were 5 mm/s, and displacement was 5 mm. Each tomato was tested at three sites in the equatorial area, and finally, the maximum force (N) was recorded.

2.2.2 Weight loss

The weight of each tomato during storage was recorded, and the percentage of fruit weight after storage relative to the initial fruit weight was calculated.

2.2.3 Titratable acid

Ten grams of tissues from the sarcocarp of 6 tomatoes were ground into a homogenate with 20 mL distilled water. After 1 h, the homogenate was centrifuged at 7000 × *g* for 10 min to get the supernatant. The supernatant was transferred into a 100 mL volumetric flask and used as the final extracting solution. 1% phenolphthalein (indicator) was dripped into 10 mL extracting solution and titrated against 0.1 M NaOH [20].

2.2.4 Ascorbic acid

Twenty grams of tissues from the sarcocarp of 6 tomatoes were crushed with 20 g/L oxalic acid solution and diluted to 100 mL. After which, it was centrifuged at 4°C, 7000 × *g* for 10 min, and the filtrate was collected. Ten milliliters of the filtrate were titrated with 2,6-dichlorophenol indophenol dye [21] and the volume of the titrant was recorded. Ten milliliters of 20 g/L oxalic acid solution were used as a blank.

2.2.5 Lycopene

Lycopene can be extracted with petroleum ether solvent without light [22]. Ten grams of tomatoes were homogenized, then 25 mL water and petroleum ether were added respectively into the homogenate. After 30 min, the supernatant was extracted and 25 mL petroleum ether was added to the homogenate again for extraction. The two supernatants were mixed and the absorbances at 472 nm were recorded.

2.3 DNA extraction and amplicon sequencing

Microorganisms on the tomato surface were isolated at 0, 4, 8, and 12 d during storage. The tomatoes were washed with a sterile PBS solution (pH 7.2), and the washed-out solution was collected. Then the solution was filtered through a microporous membrane (diameter 50 mm, pore diameter 0.22 μm) by vacuum filtration [13].

The total DNA was extracted by a CTAB (Cetyl trimethyl ammonium bromide) method [23], and the quantity and concentration of the total DNA were detected using a Thermo NanoDrop ONE (Thermo Fisher Scientific, USA). The 16S and ITS amplicons were obtained by using different primers. The universal primers were the 16S V3-V4 region (341F: 5'-CCTACGGGNGGCWGCAG-3'; 806R: 5'-GGACTACHVGGGTATCTAAT − 3') and ITS2 region of ITS (ITS3_KYO2: 5'-GATGAAGAACGYAGYRAA-3'; ITS4: 5'-TCCTCCGCTTATTGATATGC-3') [24, 25]. The parameters for PCR reaction were set as: 94℃ for 2 min, followed by 30 cycles of denaturation at 98℃ for 10s, 62–66℃ for 30s, and extension at 68℃ for 30s and a final extension at 68℃ for 5 min. The PCR amplicons were extracted from 2% agarose gel electrophoresis and purified using the Axyprep DNA Gel extraction kit (Axygen Biosciences, USA). Then, the purified products were used to construct a library, and sequenced on an Illumina MiSeq platform according to the standard protocols at Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China).

2.4 Sequence data analysis

To obtain more clean tags, the adaptor sequences, low-quality sequences, and poly N-containing reads were filtered by QIIME. Then, the effective tags were clustered into operational taxonomic units (OTUs) with ≥ 97% similarity using the UPARSE pipeline. The representative sequence (the highest abundant tag sequence in each OTU) was classified into organisms by a naive Bayesian model using an RDP classifier based on the SILVA database [26] and ITS2 database [27], with the confidence threshold value of 0.8.

Alpha diversity indexes were calculated by Welch's t-test and Wilcoxon rank test in R project Vegan package. Considering the presence or absence of species and changes in species abundance, the principal component analysis diagram of β diversity was obtained based on the Weighted UniFrac algorithm. An online platform of OmicShare tools was used to analyze the sequence data (http://www.omicshare.com/tools).

2.5 Statistical analysis

Data were analyzed by Statistical Program SPSS (PC) ver. II. X (SPSS Inc. Chicago, Illinois, USA), and $P < 0.05$ was considered to be statistically significant. All experiments were performed in three technical replicates and three biological replicates.

3. Results

3.1 The effect of *A. pullulans* S-2 on disease development and fruit quality

Tomatoes in the control group began to rot on the 8th day, with a rot rate of 5.56%, and the rot rate increased to 59.46% on the 12 d (Fig. 1A). While the tomatoes in the *A. pullulans* S-2 treatment group did not rot on 8 d, the rot rate on the 12 d was 35.56%, which was significantly lower than the control group. Although the tomatoes were separated from the plant, they also undergo various physiological activities, and the weight loss rate gradually increases. At the end of storage, the weight loss rate of tomatoes in the control group reached 1.79% and that in the treatment group was less than 1% (Fig. 1B). The firmness of tomatoes changes due to the loss of moisture. The decrease rate of firmness increased (Fig. 1C), but the firmness of the treatment group was always higher than that of the control group. At the same time, the titratable acid (Fig. 1D), ascorbic acid (Fig. 1E), and lycopene (Fig. 1F) of the treatment group were significantly higher than those of the control group during the entire storage period.

3.2 Sequencing statistics

The microbial populations from 24 tomato samples were assessed by DNA amplicon sequencing of the bacterial 16S rRNA gene and fungal ITS region. A total of 2,846,637 raw tags were detected from the 16S data, and finally, 5409 OTUs were obtained at 97% identity after filtering low-quality, mitochondria and chloroplast sequences. These OTUs were annotated to 35 phylum, 84 classes, 165 orders, 276 families, 616 genus, and 299 species (Additional file1). Meanwhile, the ITS dataset contained 2,945,259 raw tags, and finally, 473 OTUs were obtained. These OTUs were annotated to 99.15%, 97.25%, 95.98%, 80.55%, 70.4%, 30.66% of phylum, class, order, family, genus, species levels, respectively (Additional file1).

3.3 Alpha and beta diversity on tomato surfaces

The Ace index was used to reflect the alpha diversity, and the species abundance and changes in the two groups during the entire storage period were observed by the box diagram. From Fig. 2A, the Ace index of the treatment group was significantly lower than the control group due to the intervention of *A. pullulans* S-2 on 0, 4, 8 d. However, as the storage time prolonged, various pathogenic fungi in the control group became active, disrupting the balance of tomato surface species, resulting in a decrease in the species diversity of the control group and a decrease in the Ace index. At the same time, due to the activity of pathogenic fungi in the late storage period, the surface diversity of the treatment group increased, and the value of Ace index became larger.

According to the alpha diversity of the bacterium (Fig. 2B), the Ace index of the control group was significantly greater than that of the treatment group ($P < 0.05$) on 0, 4 d. However, there is no significant difference in the Ace index between the two groups ($P > 0.05$), which indicates that the usage of *A. pullulans* S-2 caused a decrease in the species abundance of bacteria in the early stage, but the species diversity increased with the extension of storage time in the later stage.

For beta diversity, from the PCoA chart of the fungi (Fig. 3A), the explanation degree of PCo1 was 90.47%, and that of PCo2 was 8.95%. The sum of the two was greater than 50%, and which makes the explanation degree enough. From the whole point of view, the control group is far away from the treatment group during the entire storage period, which means that the flora structure between the two

groups is not similar. At the same time, within 0–8 d, the structure of the respective flora of the two groups was close and similar. On the 12 d, due to the decay, the flora of the treatment group and control group altered, which was far away from the point in the previous 8 d, and the flora structure was different.

According to the PCoA diagram of bacterium (Fig. 3B), the explanation degree of PCo1 was 48.16%, and that of PCo2 was 33.84%. The explanation degree of the two was relatively similar, and the sum was greater than 50%. Analysis from PCo1, PY4 is far away from the other groups, and the other groups are close. Focusing on PCo2, PCK0, PY0; PCK8, PY8; PCK12 and PY12 are very close. From the above results, we witnessed that the structures of the bacterial colony between the two groups did not differ significantly during the entire storage period.

3.4 The influence on fungal community

According to the analysis of fungal phylum (Fig. 4A), the *Ascomycota* accounted for more than 99%, and the *Basidiomycota* constituted less than 1%. Then focus on the fungal genus category (Fig. 4B). *Aureobasidium*, *Cladosporium*, *Mycosphaerella*, *Alternaria*, and *Penicillium* were important genera during storage.

From Fig. 5A, in the treatment group, the change curve of *Aureobasidium* was stable within 8 d, and the proportion was always higher than 90%, then decreased to 79.11% on 12 d. The trend of *Aureobasidium* was maintained at a low level for 8 d, and increased slightly in the later storage period of the control group. Throughout the storage process, the *Aureobasidium* of the treatment group was significantly higher than the control group.

The proportions of *Cladosporium* in the two groups were stable for the first 8 d (Fig. 5B); the control group was always higher than 60%, and in the treatment group, it was less than 5%. In the last four days, the control group rapidly dropped to 29.4%, and the treatment group increased to 12.92%. *Cladosporium* of the control group was always more than the treatment group during the whole storage period.

The trend curve of *Mycosphaerella* (Fig. 5C) and *Alternaria* (Fig. 5D) in the control group were increased initially and then decreased. In contrast, *Penicillium* (Fig. 5E) was decreased initially and then increased. The proportions of these three genera in the treatment group were very low, even *Alternaria* and *Penicillium* were always less than 1%. The contents of these three genera in the control group were significantly higher than the treatment group during the entire storage period.

3.5 The influence on bacterial community

From the perspective of bacterial phylum taxonomy (Fig. 6A), *Proteobacteria*, *Actinobacteria* and *Firmicutes* were the most important phyla during the entire storage, accounting for 72%, 19% and 2%, respectively. Among them, the dynamic trend of *Proteobacteria* (Fig. 7A) was increased and then decreased during the 12 d of storage. The treatment of *A. pullulans* S-2 advanced the decline time point from 8 d to 4 d, and the content of the treatment group in the late storage period was significantly lower than the control group.

During the 12 d storage, *Actinobacteria* (Fig. 7B) was first declined and then increased. The increasing time point was advanced from 8 d to 4 d in the treatment group, and the proportion of *Actinobacteria* was greater than the control group during the later storage period. The *Firmicutes* (Fig. 7C) trend was always decreasing, and *Firmicutes* in the control group were always higher than the treatment group.

From the bacterial genus category (Fig. 6B), the six genera, namely *Pantoea*, *Brevibacterium*, *Brachybacterium*, *Serratia*, *Glutamicibacter*, and *Pseudomonas* were showed their importance. The panoramic view of the situation (Fig. 8) showed that the tendency of *Pantoea* and *Pseudomonas* was raised at the beginning and declined later. But, *Brevibacterium*, *Brachybacterium*, and *Glutamicibacter* were showed the opposite trend. The proportion of *Serratia* was stable during 0–8 d, and rapidly increased in 8–12 d.

After using *A. pullulans* S-2, the proliferation rate of *Pantoea* (Fig. 8A) was slowed down, and the decay rate became faster; the decay rate of *Brevibacterium* (Fig. 8C) and *Brachybacterium* (Fig. 8F) became faster in the early stage of storage, and the proliferation rate was slower in the later stage.

The proportion of *Serratia* (Fig. 8D) and *Glutamicibacte*r (Fig. 8E) was increased in the late storage period, and they were the dominant bacterium in the treatment group. *Pseudomonas* (Fig. 8B) was the dominant bacteria in the early stage of the treatment group. Still, due to the rapid growth in the early stage, the total amount of *Pseudomonas* had been decreasing since the fourth day, but it was still significantly higher than the control group in the end.

4. Discussion

According to previous research, antagonistic yeast may replace chemical fungicides, prevent the postharvest diseases of fruits and vegetables, and significantly maintain the good qualities of fruits and vegetables. In this study (Fig. 1), the application of *A. pullulans* S-2 significantly reduced the incidence of tomato postharvest diseases, also maintained fruit moisture, firmness, TA, ascorbic acid and lycopene. Our results were highly comparable with the previous study [28].

The influence of *A. pullulans* S-2 on the change of microbial community structure on tomato surface is the key point of our study. The high-throughput sequencing technology was used to identify the microbiome of tomato surface during storage. From the analysis of ACE index (Fig. 2), we found that the application of *A. pullulans* S-2 had a more significant impact on fungal diversity. The rapid colonization of *A. pullulans* S-2 and inhibition of other pathogenic fungi in the early stage of storage resulted in the decrease of fungal diversity, so the diversity of the treatment group was less than the control group. At the later stage of storage, the tomato fruit began to rot, the abundance of pathogenic microorganisms and the microbial diversity of the treatment group was increased. The previous study has shown that rotting could significantly reduce fungal diversity [15], so the microbial diversity of the control group decreased due to the severe rot (Fig. 2A).

Then, we analyzed the effect of *A. pullulans* S-2 on the microbial community structure of tomato surface from the perspective of colony composition. From the perspective of fungi (Fig. 4B), after *A. pullulans* S-2 treatment, the five genera namely *Aureobasidium*, *Cladosporium*, *Mycosphaerella*, *Alternaria*, and *Penicillium* were changed significantly in the control and treatment groups. In the treatment group, only *Aureobasidium* was significantly higher than the control group, and the other four genera were lower compared to the control group (Fig. 5). *Aureobasidium* is a ubiquitous strain on the surface of tomatoes, and it also occupies a certain proportion in the naturally grown group (CK).

Cladosporium, *Mycosphaerella*, *Alternaria*, *Penicillium* are several common pathogens. Studies have shown that *Cladosporium* mainly exists in the leaves of various plants and can cause tomato leaf mold disease [29, 30]. *Mycosphaerella* is the primary source of melon and banana leaf spot [31, 32], but it doesn't seem to cause postharvest diseases. *Alternaria* and *Penicillium* can infect various fruits and vegetables, leading to black spot and blue mold of postharvest tomato [33–35].

It was observed that *A. pullulans* S-2, showed an outstanding ability to survive and colonize, and compete with pathogenic fungi such as *Cladosporium*, *Mycosphaerella*, *Alternaria*, *Penicillium*, for nutrition and space, thereby inhibiting the growth of pathogenic fungi and achieving the effect of disease control. Our results are consistent with the previous report that *A. pullulans* can compete for nutrition and space to prevent pathogenic fungi [36].

Stating from the bacterial genus (Fig. 8), the main genera found in our study was reported to be beneficial to plants on the surface. Among them, *Pantoea* is quite special. It is widespread in plants, and most of them are screened out from diseased plants. Therefore, it is generally believed that bacteria belonging to this genus were pathogenic in the early stage. Among them, the notorious pathogens, including *Pantoea stewartia* subsp. *Stewartii* and *Pantoea ananatis*, were reported to infect corn and rice [37]. However, with the deepening of research, it was found that some species in the genus have nitrogen fixation and are considered to plant growth-promoting bacteria. They play a role in controlling *Botrytis cinerea*, which is very complex and fascinating [38–40].

Most species in the genus *Brevibacterium* have been proven to promote plant growth, among which *Brevibacterium casei* MH8a can enhance the ability of white mustard seeds to absorb metals, and *Brevibacterium linens* in Compost teas have a specific control effect on tomato diseases [41, 42]. In order to enhance the beneficial effects of *Brevibacterium* strains, it has been used in combination with other strains. For example, the synthetic community of *Brevibacterium frigoritolerans* HRS1 and the other three bacteria has a stronger immune effect against tomato bacterial wilt [43]. *Brevibacterium halotolerans* and *Trichoderma harzianum* have a synergistic interaction in improving the growth and yield of peppermint [44]. There are reports that the bacteria in the genus *Brachybacterium* also can promote plant growth [45].

Studies proved that *Serratia*, *Glutamicibacter*, and *Pseudomonas* had been recognized as plant growth-promoting bacteria. *Serratia proteamaculans* can effectively control tomato early blight and promote plant growth [46]. A salt-tolerant PGPR strain *Glutamicibacter* sp. YD01 can stimulate plant growth and development and alleviate the adverse effects on plants under salt stress conditions [47]. *Pseudomonas*

was often isolated from the rhizosphere of plants, and some of them have been reported as rhizosphere bacteria that promote plant growth [48, 49].

The effect of *A. pullulans* S-2 on bacteria is ‘simple and clear’ on the fungal community and mainly affects the growth and decay rate of many bacterial species. The proportion of *Pantoea*, *Brevibacterium*, and *Brachybacterium* in the treatment group was significantly lower than that of the control group, and the three genera *Serratia*, *Glutamicibacter*, and *Pseudomonas* were significantly higher compared to the control group. It can be understood as the ‘replacement’ of the dominant species.

5. Conclusions

A. pullulans S-2 can be used as a microbial antagonist to prevent and control postharvest tomato diseases. It affected the composition of the fungal community and the growth and decline rate of various bacteria to achieve the ‘replacement’ of the dominant flora. In addition, it can maintain the qualities of postharvest tomatoes, thereby reducing the incidence of diseases. This study found several potentially beneficial bacterial taxa from tomato surfaces during storage. Further studies about the role of these bacteria on host and biocontrol efficacy of the combination of potentially beneficial bacteria and *A. pullulans* S-2 will be needed. This study should provide a better understanding of the mechanisms which microbial antagonists control diseases.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

All raw sequence data have been made available in the NCBI Sequence Read Archive (SRA) database under the BioProject PRJNA730651.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Hongyin Zhang, Xiaoyun Zhang, Qiya Yang and Kaili Wang developed the ideas and designed the experimental plans. Yu Shi, Qianhua Zhao, Qiya Yang and Siqi Zhou performed the experiments. Yu Shi wrote the manuscript. Solairaj Dhanasekaran, Joseph Ahima and Samir Droby assisted in writing the manuscript. The authors read and approved the final manuscript.

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Figures

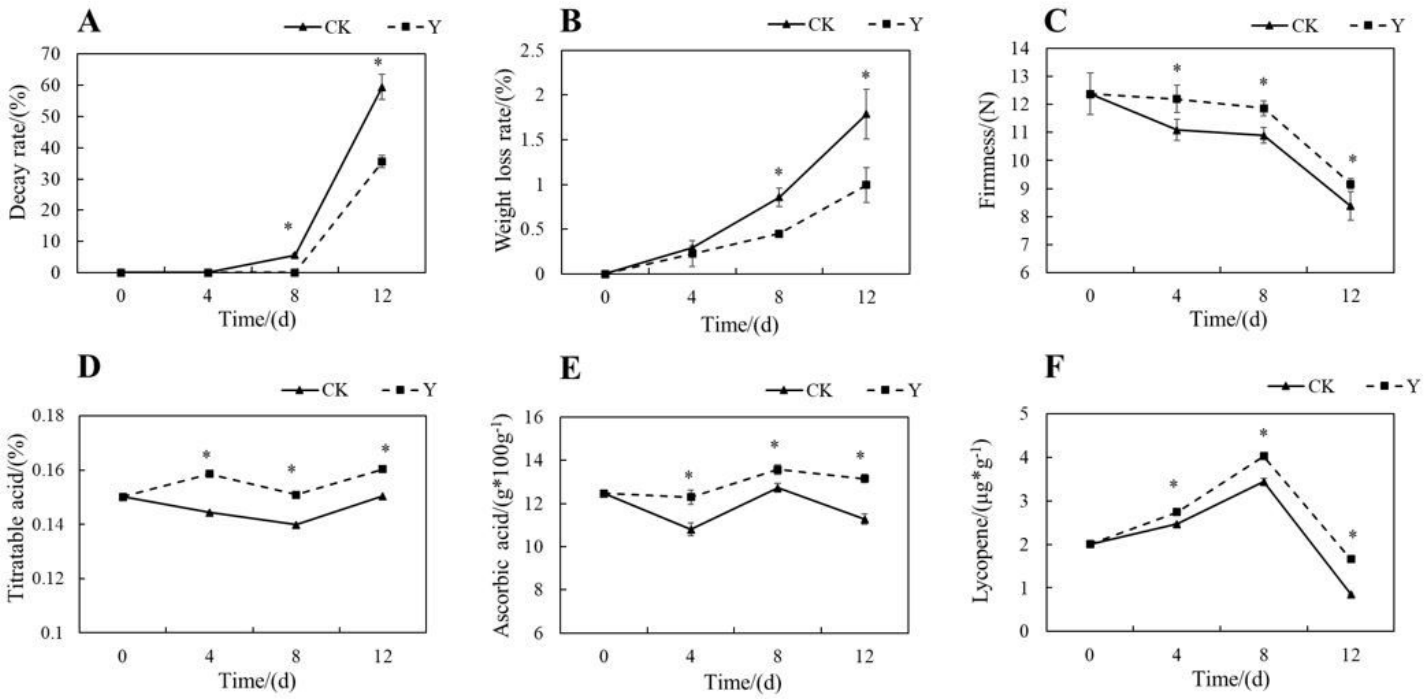

Figure 1

Effect of 1 × 108 cells/mL A. pullulans S-2 on the overall change of decay rate (A), weight loss (B), firmness (C), titratable acid (D), ascorbic acid (E) and lycopene (F) of tomatoes during storage for 12 days. Values are the means ± SD (n = 3), * = P < 0.05.

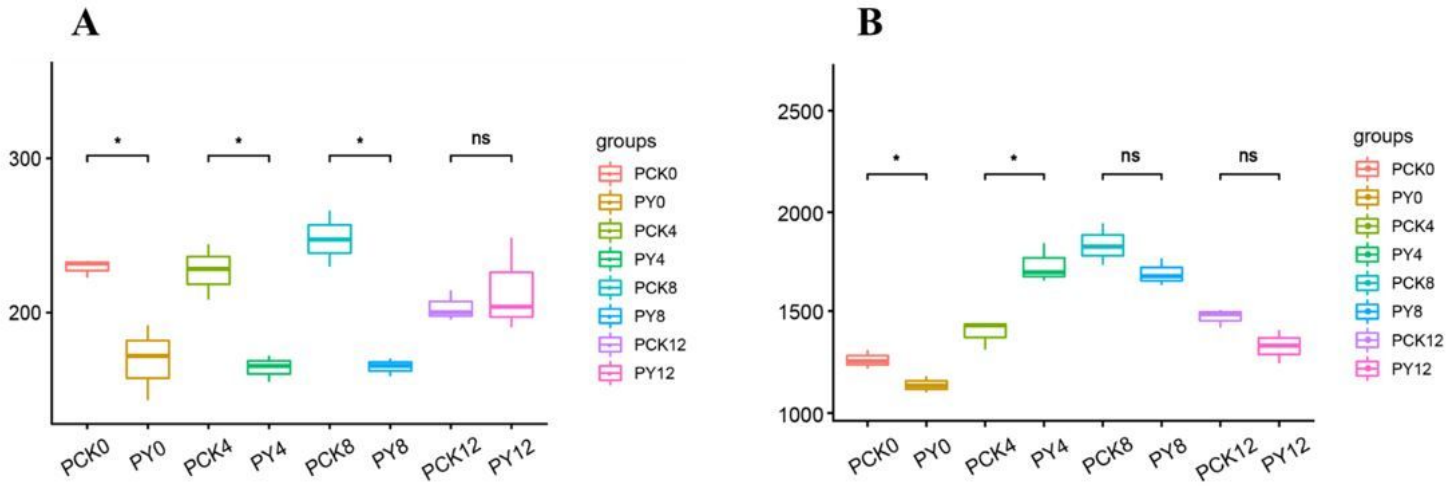

Figure 2

Box plots showing the fungal (A) and bacterial (B) diversity (Ace index) of the tomatoes at different storage times, * = P < 0.05, ns = not significant.

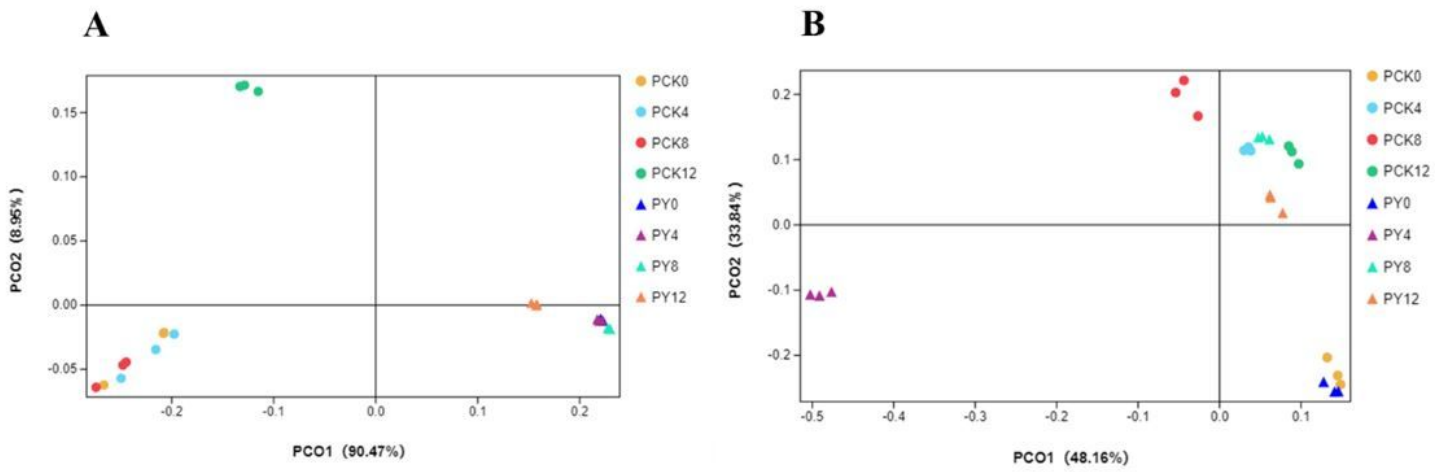

Figure 3

The PCoA (principal co-ordinate analysis) analysis for fungal (A) and bacterial (B) communities based on Weighted UniFrac at different storage times.

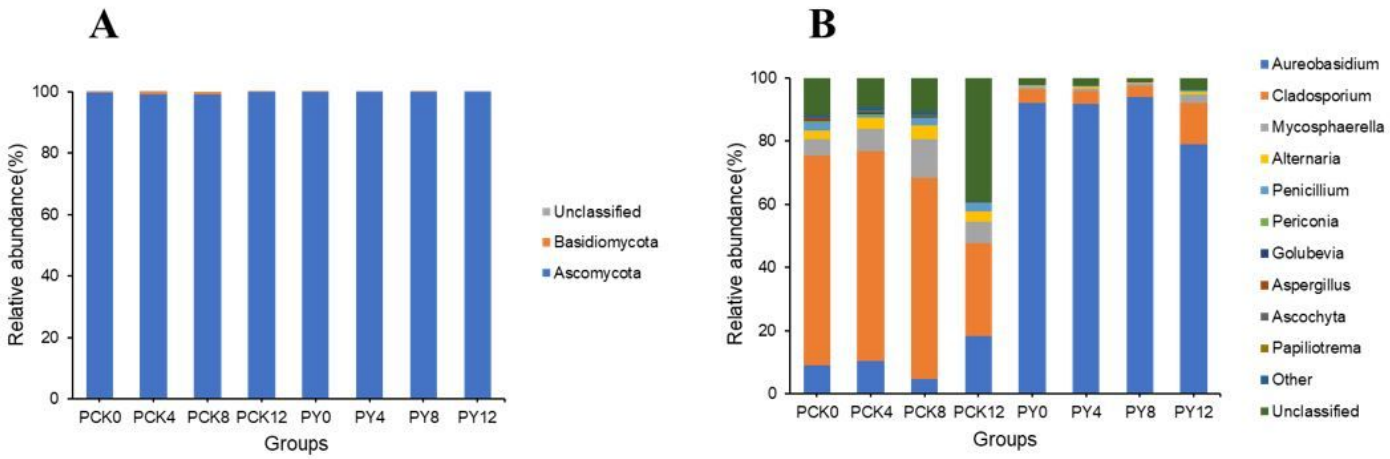

Figure 4

Fungal community composition at the level of Phylum (A) and Genus (B). The relative abundance of the top 10 in all samples is displayed in detail. The rest of the taxa are merged into the other category; the tags that cannot be annotated are merged into the Unclassified category.

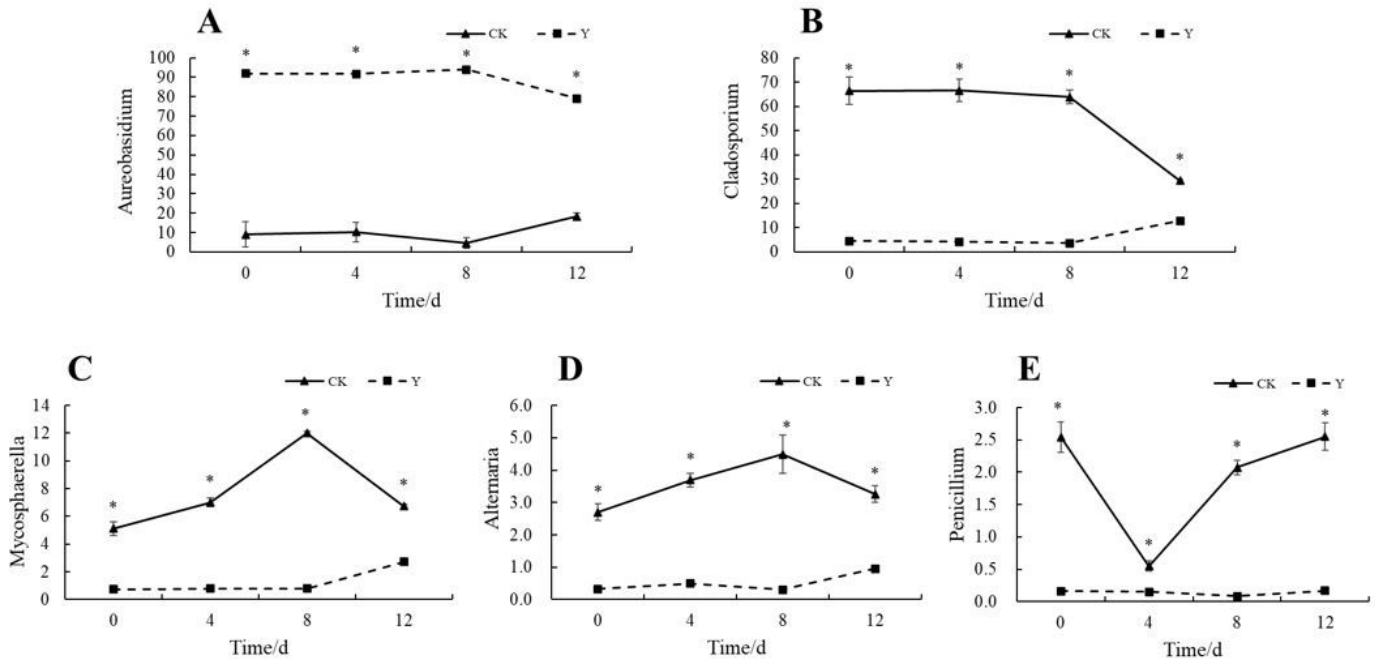

Figure 5

The change curves of several important genera of fungal during storage, * = P < 0.05.

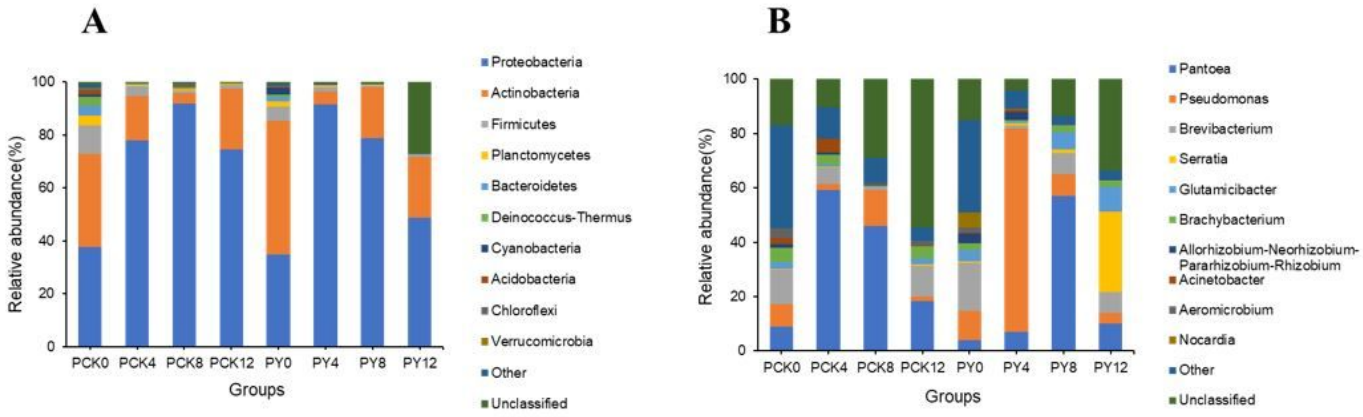

Figure 6

Bacterial community composition at the level of Phylum (A) and Genus (B).

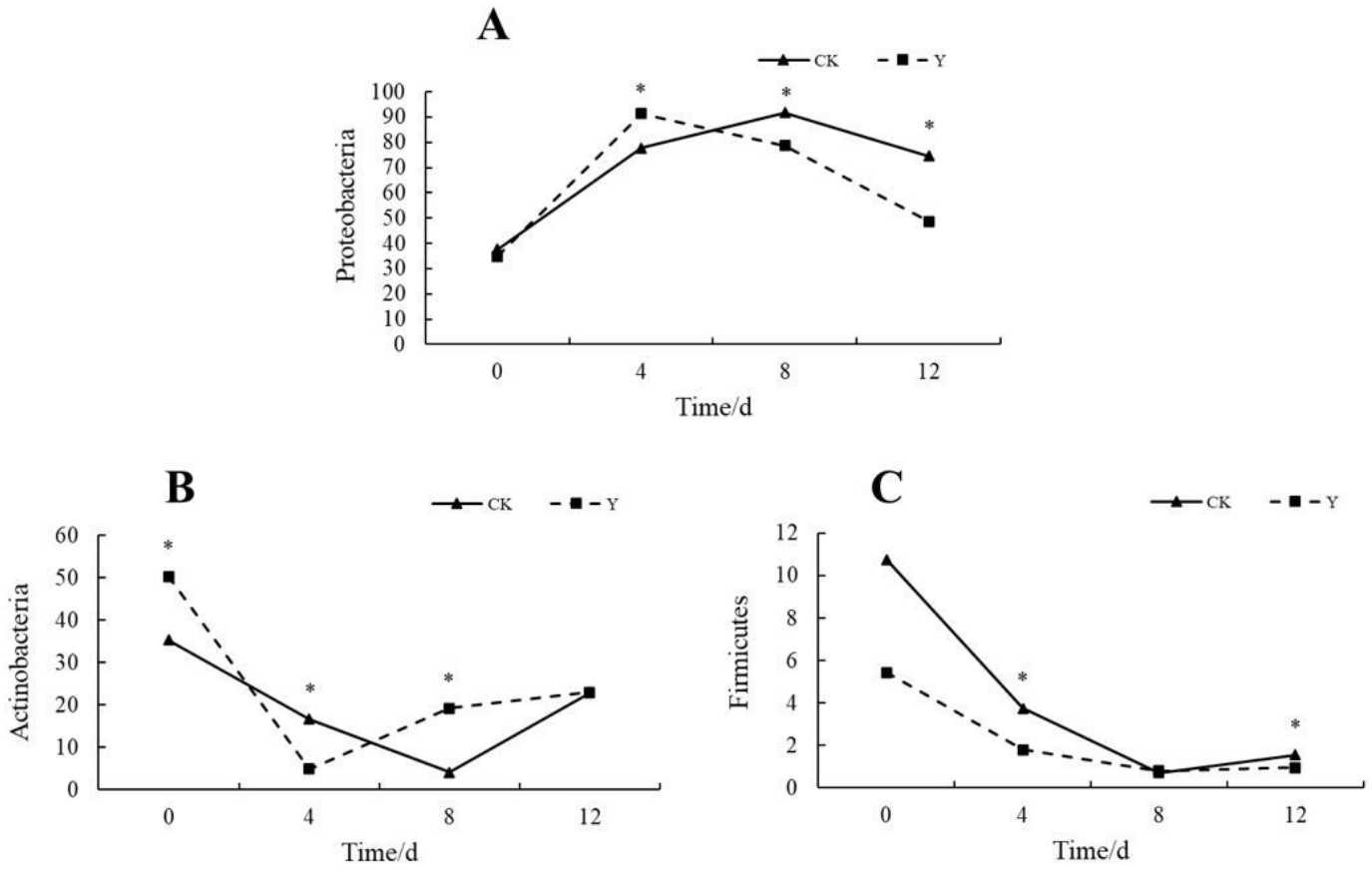

Figure 7

The change curves of several important phyla of bacteria during storage, * = P < 0.05.

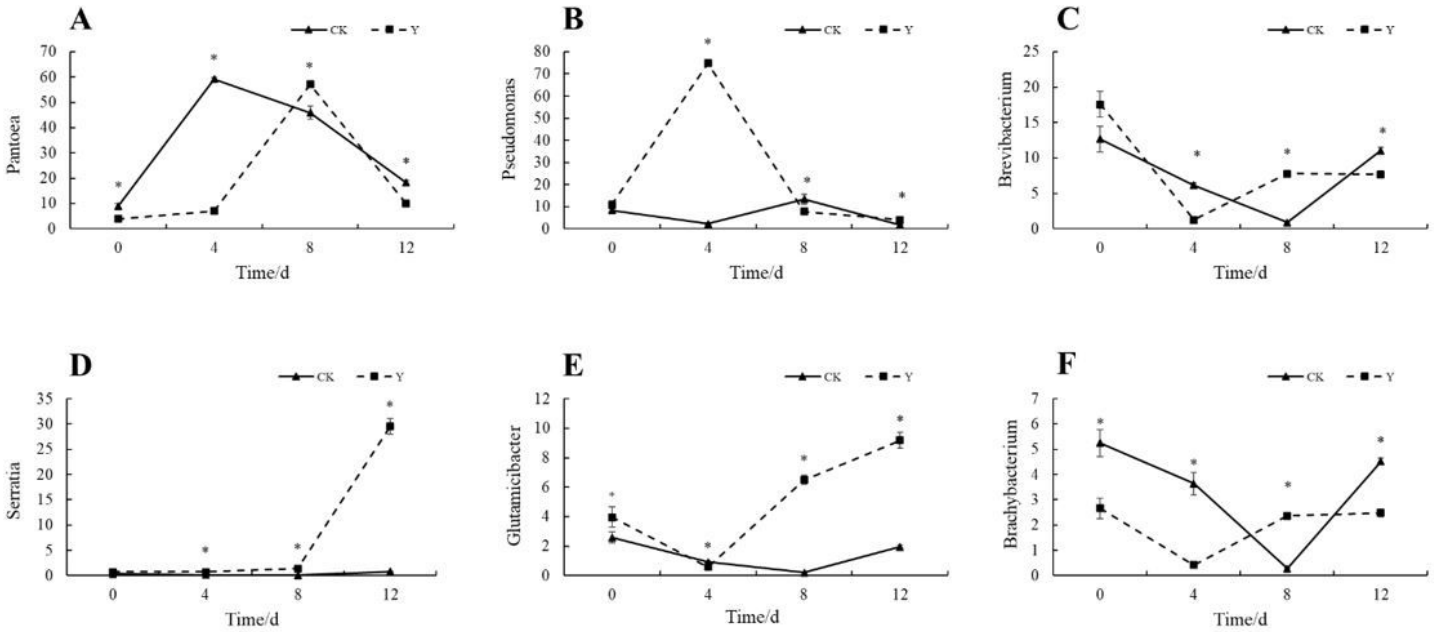

Figure 8

The change curves of several important genera of bacteria during storage, * = P < 0.05.

Supplementary Files

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- Additionalfile15.23.21.xlsx