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Unraveling characterizations of bacterial community and spoilage profiles shift in chilled pork during refrigerated storage

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Abstract

Changes in bacterial community composition and bacterial counts of chilled pork during storage at 4 °C were unraveled by culture-dependent method and culture-independent method. Physical and chemical analyses including drip loss, total volatile basic nitrogen (TVB-N), pH, and surface color were also performed to estimate its quality and shelf life. A total of 37 phyla, 575 genera and 843 species were identified in all samples by high-throughput sequencing technology during 10 days' storage. At the phylum level, Proteobacteria and Firmicutes were the dominant phylum. At the genus level, *Pseudomonas* spp., *Acinetobacter* spp., *Pantoea* spp., *Brochothrix* spp. and *Raoultella* spp. were the dominant genera with their average relative abundance above 5%. In addition, 12 species with average relative abundance more than 1% were found. These dominant bacteria were main pathogenic or spoilage bacteria, and seriously affected the quality of chilled meat. Based on the results of total viable counts (TVC), TVB-N level and sensory evaluation, the shelf life of chilled pork stored at 4 °C was no more than 3 days. Through the analyses of the TVC and the microbial community structure during the spoilage of chilled pork, the main microorganisms causing spoilage were revealed, which will guide significance for further control microbial quality of chilled pork.

Keywords: bacterial community; chilled pork; high-throughput sequencing; spoilage profiles.

Practical Application: The quality deterioration of chilled pork possibly is due to the combined effect of microbial spoilage and biochemistry caused by certain enzymes from chilled pork. However, the microbial profile, microbial community structure and succession in chilled pork has not been described clearly. Therefore, it is necessary to investigate the microbial profile of chilled pork, particularly spoilage bacteria, during chilled storage, which will contribute to control the source of contamination and inhibit bacteria in the preservation process. In this study, conventional culture-dependent method combined with high-throughput sequencing technology as culture-independent method were performed to unravel bacterial spoilage profiles in chilled pork during storage. Meanwhile, the fresh quality shift of chilled pork such as pH, color, TVB-N and sensory evaluation was also analyzed in chilled pork form fresh to spoilage. These results will enhance the understanding of the relationship between microorganism and the spoilage, and spoilage profiles shift in chilled pork during refrigerated storage.

1 Introduction

Chilled fresh pork is the most common types of raw pork on the market, which is subjected to a fast cooling process (0 to 4 °C for 24 h) after slaughter. After the process of rigidity, deliquescence, ripening and myofibrillar fragmentation, the chilled pork has a better meat quality in tenderness, juiciness, flavor and color than that of heated fresh pork. Furthermore, spoilage microorganisms in chilled pork will grow slowly and enzyme activities from meat are inactivated partly during chilled storage (-1.5 to 5 °C), which is conducive to preserving meat from microbial spoilage and biochemical deterioration, resulting in a longer shelf life compared with fresh pork sold at room temperature (Pellissery et al., 2020). Thus, the sensory, tenderness, flavor and food safety of chilled pork is more easily accepted by consumers. Recently, the chilled pork is gradually more and more popular in China, and chilled pork consumption has reached 27 million tons in 2018 years in China.

However, meat structure and biochemistry undergone significant changes such as tenderness and color along with chilled storage, resulting in losses in quality and perceived value. It has been reported that the quality deterioration of chilled pork possibly is due to the combined effects of microbial spoilage and biochemistry caused by certain enzymes from chilled pork, in which the succession of microbial communities is considered as a crucial factor resulting in meat deterioration (Yang et al., 2018). Furthermore, the spoilage potential of microorganisms changes with the alterations of the microbial composition during storage, ultimately affecting the process of spoilage (Odeyemi et al., 2020). However, the microbial profile, microbial community structure and succession in chilled pork during chilled storage has not been described clearly. Therefore, it is necessary to investigate the microbial profile of chilled pork, particularly spoilage bacteria, during chilled storage, which will contribute

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to control the source of contamination and inhibit the growth of bacteria in the preservation process.

Due to certain microorganism are "viable but non-culturable" in culture medium unfortunately, the culture-dependent methods have been unsuitable for microbial profile analysis in chilled meat. Recently, molecular biology-based techniques by culture-independent have been widely applied, such as denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism analysis (RFLP) and random amplified polymorphic DNA fingerprinting analysis (RAPD). Nowadays, with technology reform and innovation, the emergence of high-throughput sequencing (HTS) technology widened the scope of microbial analysis, which can provide more detailed information compared with RFLP, RAPD and DGGE, particularly in the analysis of complex substrates with considerable microbial community diversity (Dalmasso et al., 2016). Notablely, the HTS technology could improve quality and speed of sequencing. Recently, the HTS technology had been used to investigate microbial community diversity and structure of sausage (Wang et al., 2017, 2021), douchi (Yang et al., 2016) and milk (Aldrete-Tapia et al., 2018) and so on. High throughput analysis by 16S rRNA amplicon sequencing has gradually become the preferred method for studying microorganisms in food.

In this study, conventional culture-dependent method combined with HTS technology as culture-independent method were performed to unravel bacterial spoilage profiles in chilled pork during storage. Meanwhile, the fresh quality shift of chilled pork such as pH, color, TVB-N and sensory evaluation was also analyzed in chilled pork form fresh to spoilage. These results will enhance the understanding of the relationship between microorganism and the spoilage, and spoilage profiles shift in chilled pork during refrigerated storage.

2 Materials and methods

2.1 Sample preparation

The fresh pork was supplied by a local market (Chengdu, China) no more than 2 h after post-slaughter. Cut the pork into 30 slices with about 500 g, and then all unpacked samples were immediately stored at 4 °C. Three portions were periodically sampled for meat quality, sensory evaluation and microbiology analysis after storage on day 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, which were marked as D1, D2, D3, D4, D5, D6, D7, D8, D9, and D10 in reference to the storage date.

2.2 Physical and chemical analyses

The physical and chemical analyses including fluid losses, TVB-N, pH, color (L*, a*, b*) and thiobarbituric acid reactive substances (TBARS) were performed. Fluid losses were determined by weighing the pork before storage and after storage (Bedane et al., 2018), and the Formula (1) as follows:

$$Fluid\ losses(\%) = \frac{Weight\ before\ storage - Weight\ after\ storage}{Weight\ before\ storage} \times 100\% \quad (1)$$

The TVB-N was measured according to the methods as described by Wang et al. (2015a). pH measurement was conducted with a pH meter (NKW K21, Germany) with automatic temperature compensation (NTC) electrode according to the methods as described by Wang et al. (2018). Briefly, inserting the electrode directly into pork samples, six readings were obtained from 6 different random locations and then averaged. The color values of pork was measured by using a colorimeter (CS-220, China)according to the methods as reported by Wang et al. (2019). Lightness (L*), redness (a*) and yellowness (b*) were determined at three random locations on each sample. The value of TBARS was determined using a spectrophotometer (Mepod, UV-2300, China) according to the methods as described by Vilarinho et al. (2018).

2.3 Sensory evaluation of freshness

Sensory evaluation freshness of chilled pork samples was performed as described by Cavalheiro et al. (2019). All samples were coded with three-digit numbers and presented to 11 sensory panelists who has been trained according to in a random order. The color, the amount of liquid, odor intensity, overall appearance liking and freshness appearance on hedonic scale from 1 (not fresh) to 20 (very fresh) were evaluated for freshness by assessors.

2.4 Bacterial growth monitored by culture-dependent method

Bacterial growth in the pork samples was monitored by plate counts. At each sampling date, 25 g chilled pork was removed aseptically from each group to sterile bags containing 225 mL of 0.85% saline (Chen et al., 2019). Then the mixture was homogenized for 2 min using a sterile homogenizer (Scientz-11L, China). Thereafter, serial decimal dilutions were undertaken using 0.85%. 1 mL of serial dilution was taken and mixed with 15~20 mL Plate Count Agar medium (PCA, Hai Bo Biological Technology Co. Ltd, Qingdao, China) and then incubated at 36 ± 1 °C for 48 h. The results were expressed as lg CFU/g of sample.

2.5 Bacterial community diversity and succession analyses

DNA extraction

Total bacterial genomic DNA extraction was conducted according to previous method, with some modifications (Yang et al., 2018). The bacterial DNA of samples was extracted using the E.Z.N.ATM Mag-bind Soil (OMEGA, USA) according to the manufacturer's instructions. The DNA concentration was measured using BioSpec-nano (Shimadzu, Japan) and the DNA quality was confirmed by 0.8% agarose gel electrophoresis.

PCR reaction and sequencing

PCR amplifications of the V4 region of bacterial 16S rRNA gene were implemented with the primer pairs 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Using the diluted genomic DNA as template, specific primers with barcode were used for PCR according to the selection of sequencing region.

Amplifications were performed in 25 μL reaction mixture containing a 1x PCR buffer, 1.5 mM $MgCl_2$, 0.4 M dNTPs, forward and reverse primers of 1.0, 0.5 U KOD-plus-Neo enzyme (TOYOBO) and 10 ng template. The cycling conditions were: denaturation at 94 °C for 60 s, 30 cycles of denaturation at 94 °C for 20 s, annealing at 54 °C for 30 s and elongation at 72 °C for 30 s, followed by a final extension of 72°C for 5 min. Three PCR techniques were repeated for each sample.

After amplifications, PCR products were mixed with 1/6 volume of 6×loading buffer and detected by 2% agarose gel electrophoresis. PCR products were purified by using QIAquick Gel Extraction Kit (QIAGEN). The final PCR products were quantified through Qubit@2.0 Fluorometer (Thermo Scientific). After the constructed library passed the quantitative and library tests, the Hiseq 2500 platform of Rhonin Biosciences was used for PE250 mode sequencing.

Bioinformatics analyses

Based on Usearch software, UPARSE pipeline was used to cluster operation taxonomic units (OTUs) at an identity threshold of 97%. UCLUST taxonomy and SILVA database were used for annotation analysis. Representative sequences were performed multiple alignments by PyNAST. Alpha diversity was evaluated through the R programming Language with vegan package. Venn diagram was performed to depict the similarity and difference between the communities each samples (Liu et al., 2017).

2.6 Statistical analyses

Data were given as mean values accompanied with the standard deviation. Duncan's multiple range test (significance $p<0.05$) was employed for the independence of error terms using the SPSS Statistics software (IBM, Chicago, Ill., U.S.A.).

3 Results and discussion

3.1 Fluid losses, TVB-N, pH, color and TBARS value of chilled pork during storage

The meat quality of chilled pork was monitored during storage, based on fluid losses, TVB-N, pH, and color analysis as shown in Figure 1. The fluid losses were steadily increased and reached to 11.71% on the 10th day storage at 4 °C (Figure 1A). The decrease of water-holding capacity may be caused by proteolysis

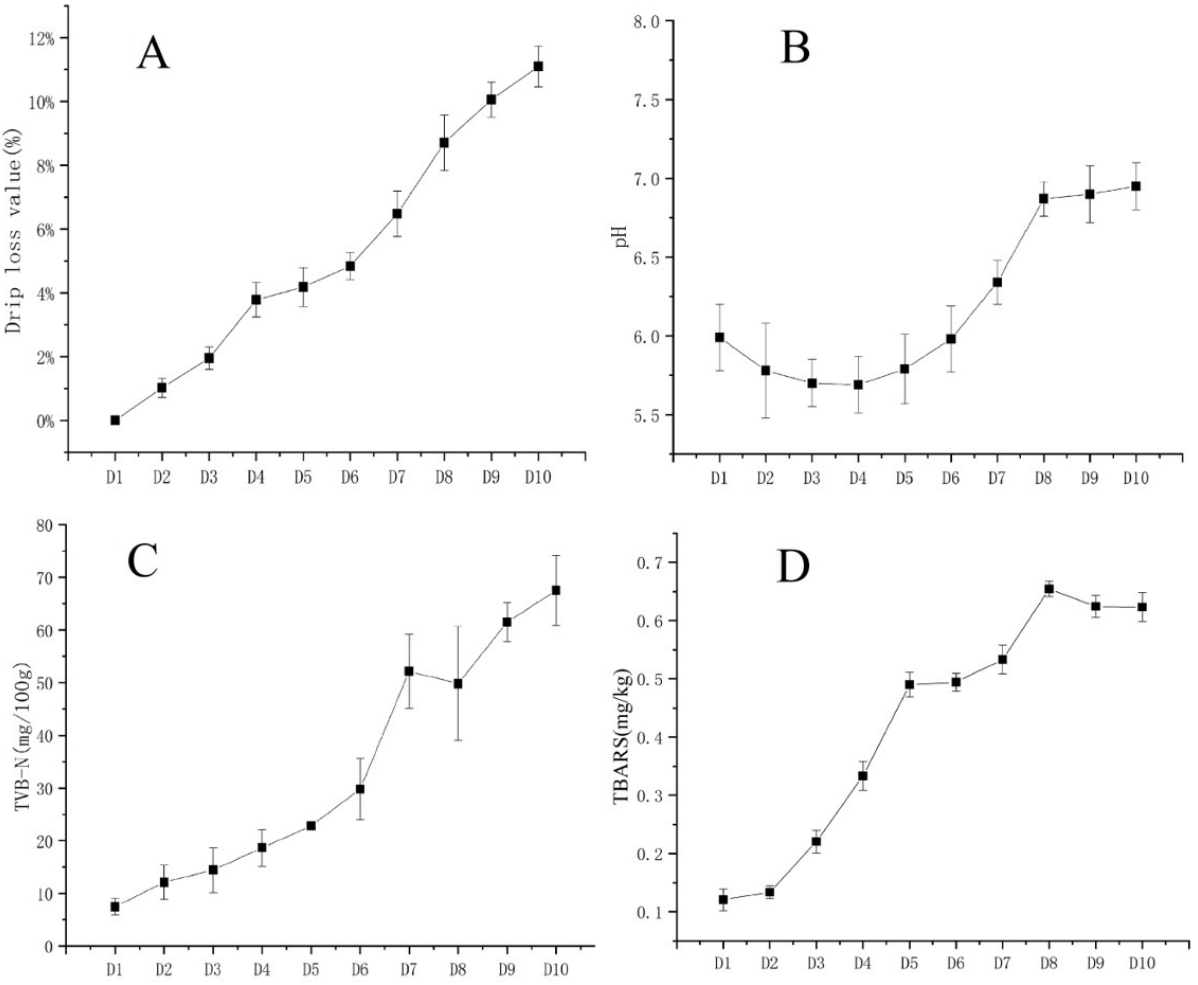

Figure 1. The changes in fluid losses (A), pH (B), TVB-N content (C) and TBARS value (D) of chilled pork during storage at 4 °C.

of cytoskeletal proteins. A partial insight into freshness can be made through measuring water holding capacity as a percentage of liquid losses during storage, and the relationship between water-holding capacity and freshness is usually consistent across chilled storage durations. The decrease of water-holding capacity indicates that the freshness of chilled pork is decreasing along with storage.

The pH values of chilled pork fluctuated during storage varied from 5.69 to 6.87 as shown in Figure 1B. The pH values gradually reduced from 5.99 to 5.69 in first 4 days, which was mainly attributed to lactic acid produced by anaerobic respiration of the cells during the process of rigidity after post slaughter. Then this trend was transformed and pH values increased rapidly to 6.87 on the 8th day and slowly increased to 6.95 at the last 3 days, which was perhaps attributed to the alkaline substances from protein breakdown, resulting in a buffering effect on the organic acids.

The TVB-N value also increased steadily during storage as shown in Figure 1C. A sharp increase in the TVB-N values was found after 5 days and reached up to 16.53 mg/100g on the 4th day, which reached the threshold of acceptability of 15 mg/100g according to the China national food safety standard GB 2707-2016. The TVB-N as an important quality indicator of fresh meat in China (Wang et al., 2015b) is mainly composed of ammonia, amine and trimethylamine produced by microorganisms and enzyme activity (Casaburi et al., 2015). These results indicate that the chilled pork stored at 4°C without packaging after 3 storage days would been unsuitable for consumers according to Chinese National Food Safety Standard GB 2707-2016, suggesting that its shelf life is no more than 3 days.

The changes in meat color of chilled pork during storage were displayed in Table 1. The L* value decreased from initial 33.77 to 23.61 during the first 6 days, and then gradually increased back to 32.26 on the 10th day. Similar results were reported by Chen et al. (2019). The increase of L* value might be the result of more free water generated during storage, affecting the scatter coefficient. The a* value increased from initial 24.80 to 30.42 during the first 6 days, and then decreased rapidly to 13.21 during the last 4 days. The yellowness (b*) value increased from -8.31 to 4.14 during the first 4 days, and then gradually reduced to -8.73 on the 10th day, which indicates that the chilled meat is darkening in color (Raines et al., 2009). The increased *a*∗ and *b*∗ values of chilled pork during the first 4 days could be associated with the oxidation of myoglobin and oxymyoglobin to metmyoglobin (Belem et al., 2019). Meat color as a critical indicator of freshness and quality of meat has direct influence on the acceptance of meat and consumers' purchase decisions. A bright red color is considered a positive attribute for chilled meat, and there is a positive relationship between a* value and consumer acceptability (Holman et al., 2016). Storage temperature has an effect on the rate of discoloration, and an increase of storage temperature was responsible for the reduction in color stability, due to damage of inherent muscle fibre and leaching of metmyoglobin reducing enzymes (Chen et al., 2019).

The lipid oxidation of chilled fresh pork was indicated by TBARS values as shown in Figure 1D. The TBARS value increased continuously from 0.12 on the 1st day to 0.65 on the 8th day during pork storage, and then stabilized during the last 3 days. Some studies found that TBARS values of beef increased up to 2.6~3.11 MDA mg/kg after 20 weeks' storage, which was still acceptable to consumers. The results were far below this value, which may be because the low lipid content in the samples. Besides, lipids can be oxidized by three main ways that include complex reactions: autoxidation, enzymatic-catalysed oxidation and photo-oxidation (Domínguez et al., 2019). In this study, this samples were stored in a lightless refrigerator, which caused the low TBARS values.

3.2 Consumer panel evaluation

As shown in Figure S1, the appearance of chilled pork gradually deteriorated during storage at 4 °C. The appearance of the chilled pork was high acceptable during the first 3 days. However, the surface color of the meat gradually lost luster on the 4th day, and the color of the meat became significantly darker on the 6th day. Furthermore, the chilled pork lost elasticity and completely lost its edible value. The color, the amount of liquid, odor intensity, overall appearance liking and freshness appearance were evaluated by the consumer panel as 5 evaluation indexes. The sensory score radar map was made according to the results of sensory evaluation by the consumer panels as shown in Figure 2. The distance between D4 and D5 in the radar map was the largest, indicating that the sensory changes of meat were the most obvious during the 4th to 5th day, and the biggest change was in odor. Among the 5 indicators, the variation of fluid loss value was the smallest, indicating that fluid loss is the least weighted factor for sensory evaluation. Therefore, it might be useful to use sensory evaluation together with TVB-N values and microbial results to judge shelf life.

3.3 Enumeration of bacterial communities

TVC counts were enumerated on plate count agar and the results are shown in the Figure 3. The TVC counts were 3.3 lg CFU/g in the first sample, indicating a good hygienic quality of tested samples. As storage time extended, the TVC counts in samples markedly increased and reached up to 5.80 lg CFU/g on the 3rd day. Subsequently, the TVC counts in chilled meat samples exceeded the 6 lg CFU/g on the 5th day, the microbial shelf life

Table 1. Changes in chromatic aberration of chilled pork during storage at 4 °C.

Sample	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
L*	33.77 ± 0.92	34.04 ± 1.53	33.45 ± 2.11	30.17 ± 1.45	33.14 ± 1.21	23.61 ± 1.75	30.08 ± 1.34	30.28 ± 1.78	31.58 ± 0.92	32.26 ± 1.65
a*	24.80 ±0.81	26.65 ± 0.76	25.24 ± 1.57	25.06 ± 1.21	23.22 ± 1.07	30.42 ± 1.57	27.61 ± 1.56	25.37 ± 1.92	15.11 ± 0.70	13.21 ± 1.22
b*	-8.31 ± 1.23	-0.31 ± 0.89	2.88 ± 1.53	4.14 ± 0.98	-1.29 ± 1.11	-3.40 ± 1.75	-9.15 ± 1.55	-7.53 ± 1.87	-7.66 ± 0.93	-8.73 ± 1.10

Values are mean ± standard deviation of 3 replicates.

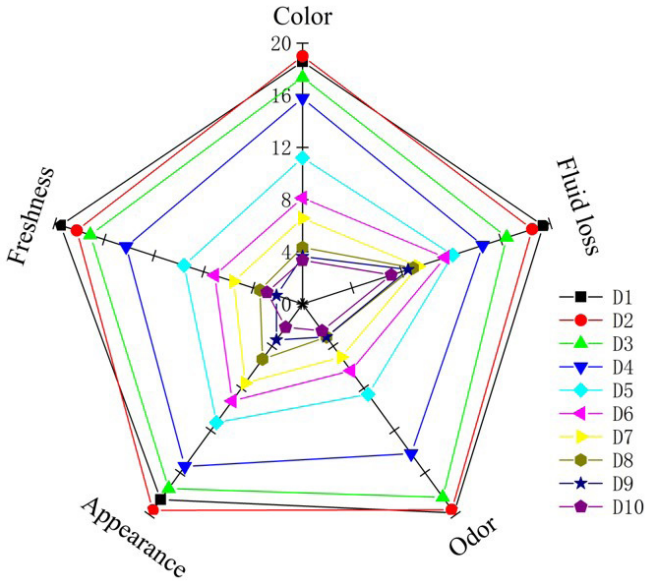

Figure 2. Radar map of sensory evaluation of chilled pork during storage at 4 °C.

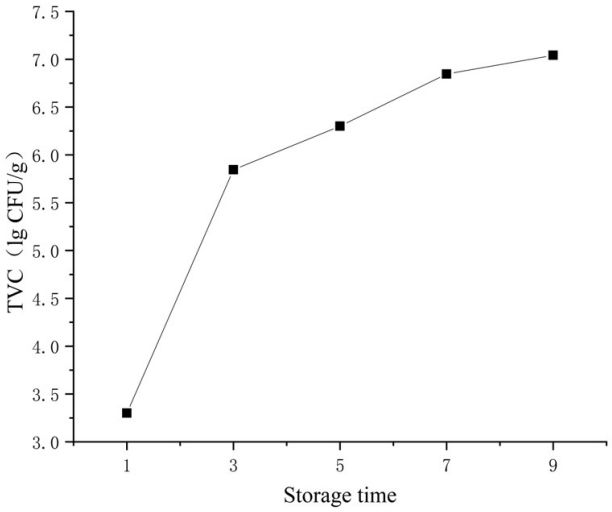

Figure 3. The changes of total viable counts (TVC) in chilled pork during storage at 4 °C.

should be considered to have ended and the meat is unacceptable. Finally, it reached 7.04 lg CFU/g on the 9th day. The growing trend during storage of viable bacteria in all samples indicates that chilled meat has a shelf life of about three days. The shelf life evaluated by TCV values is well consistent with the result evaluated by sensory evaluation together with TVB-N values.

3.4 Bacterial community diversity and succession identified by high-throughput sequencing technology

Sequencing data and Alpha diversity analysis

A total of 337567 effective sequences with an average length of 308.2 bp from chilled pork samples during storage at 4°C were obtained by HTS technology, which were clustered into 8404 OTUs with the 97% identity level. Alpha-diversity is shown in the scatter plot (Figure 4). The Chao1 index was used as abundance estimator. Bacterial diversity was evaluated by Shannon and Simpson indices. The phylogenetic diversity analysis was conducted by phylogenetic diversity (PD) value, which is the sum of all the branches of phylogenetic tree. As shown in Figure 4, the bacterial diversity and abundance of chilled pork declined gradually through storage. The Chao1 and Shannon indices were decreased from on the 3rd day to 4th day, and from on the 6th day to 7th day, respectively. The probable cause of the result may be that the thermophilic bacteria were gradually reduced by cryopreservation environment in the early storage, and then dominant bacteria of putrefactive inhibited the growth of other bacteria in the later storage. This conclusion is well consistent with those results reported by Suo et al. (2017).

The Venn diagram was carried out to evaluate the distribution of OTUs among the different samples as shown in Figure S2. By comparing OTUs in samples D1, D3, D5, D7 and D9 during the storage period of chilled pork, it was found that 121 OTUs were common among 5 samples. However, each sample has a number of unique OTUs, in which 318, 335, 336, and 80, 79 unique OTUs were found in sample D1, D3, D5, D7, and D9, respectively. The shared OTUs mainly belonged to Proteobacteria and Firmicutes at phylum level, and *Pseudomonas* spp., *Acinetobacter* spp. and *Pantoea* spp.at the genus level. These results revealed that the succession of bacterial communities changed greatly during storage.

Bacterial community structure and succession

Based on the HTS results, a total of 37 phyla, 84 classes, 181 orders, 299 families, 575 genera and 843 species were received from bacterial sequences of all samples. Relative abundance of bacterial community proportions at phylum, genus and species level of the top 10 are shown in Figure 5.

At the phylum level, more than ten phyla were observed, namely Proteobacteria, Firmicutes, Bacteroidetes, Acidobacteria, Actinobacteria, Chloroflexi, Epsilonbacteraeota, Planctomycetes, Thaumarchaeota and Deferribacteres, as shown in Figure 5A. Proteobacteria maintained predominant bacterial phylum throughout the storage, particularly in the later storage in which its relative abundance reached up to 89.41% and 88.01% in D9 sample and D10 sample, respectively. The second dominate phylum was Firmicutes with an average relative abundance of 16.51% in 10 samples and its highest relative abundance reached 40.28% on the 8th day of storage. The abundance of Bacteroidetes increased from initial 8.31% to highest 15.26% on the 3rd day, and then decreased to 1.70% at the end of the storage. In addition, a small amount of Acidobacteria was observed in all samples, which increased from 1.26% to 1.81% in the first 3 days, and then decreased to 0.26% on the 10th day. The abundance of other 6 phyla were always less than 1.00% during storage, except for Chloroflexi which reached 1.11% on the 5th day. A similar result was observed by Kaur et al. (2017), who reported that Proteobacteria and Firmicutes dominated in vacuum packaged beef stored at - 0.5°C.

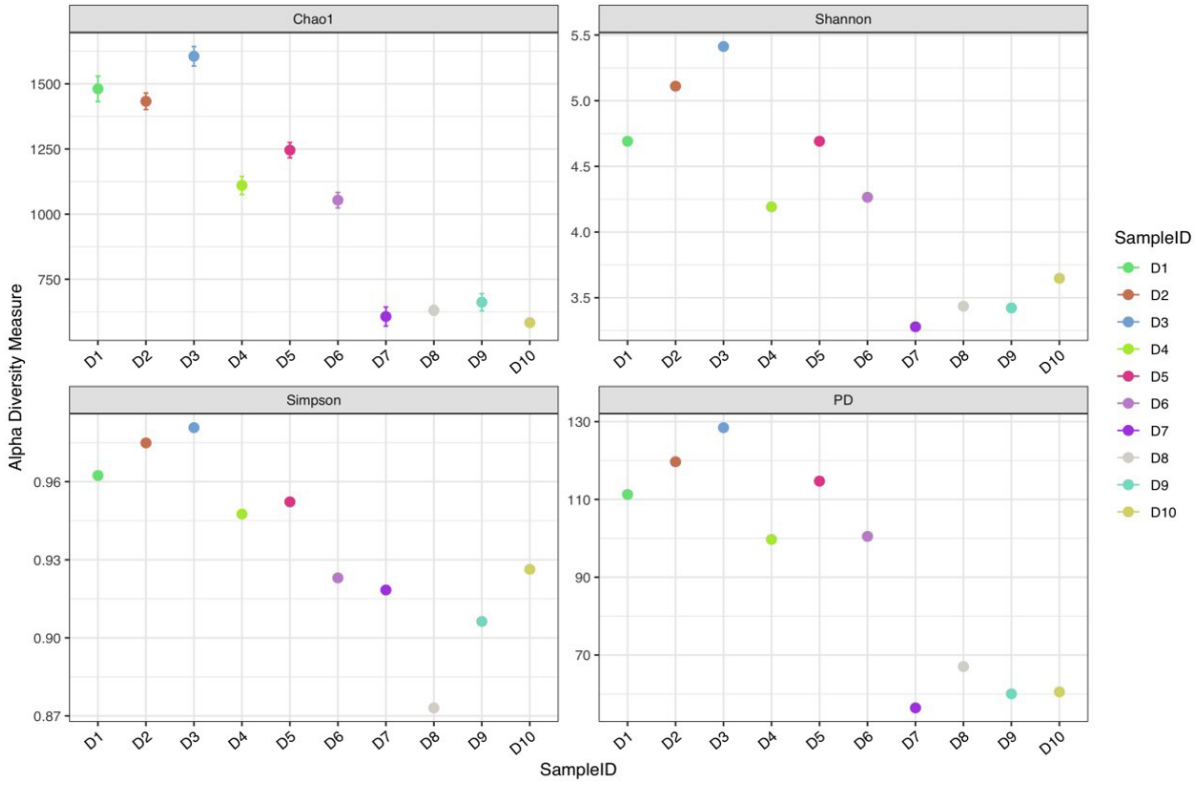

Figure 4. Alpha-diversity indices scatter plot. *Chao1* index shows the abundance of bacteria. *Shannon* index, *Simpson* index and PD value shows the diversity of bacteria. *Simpson index* in here is defined by *1 - Simpson's index,* higher values indicate higher bacterial diversity.

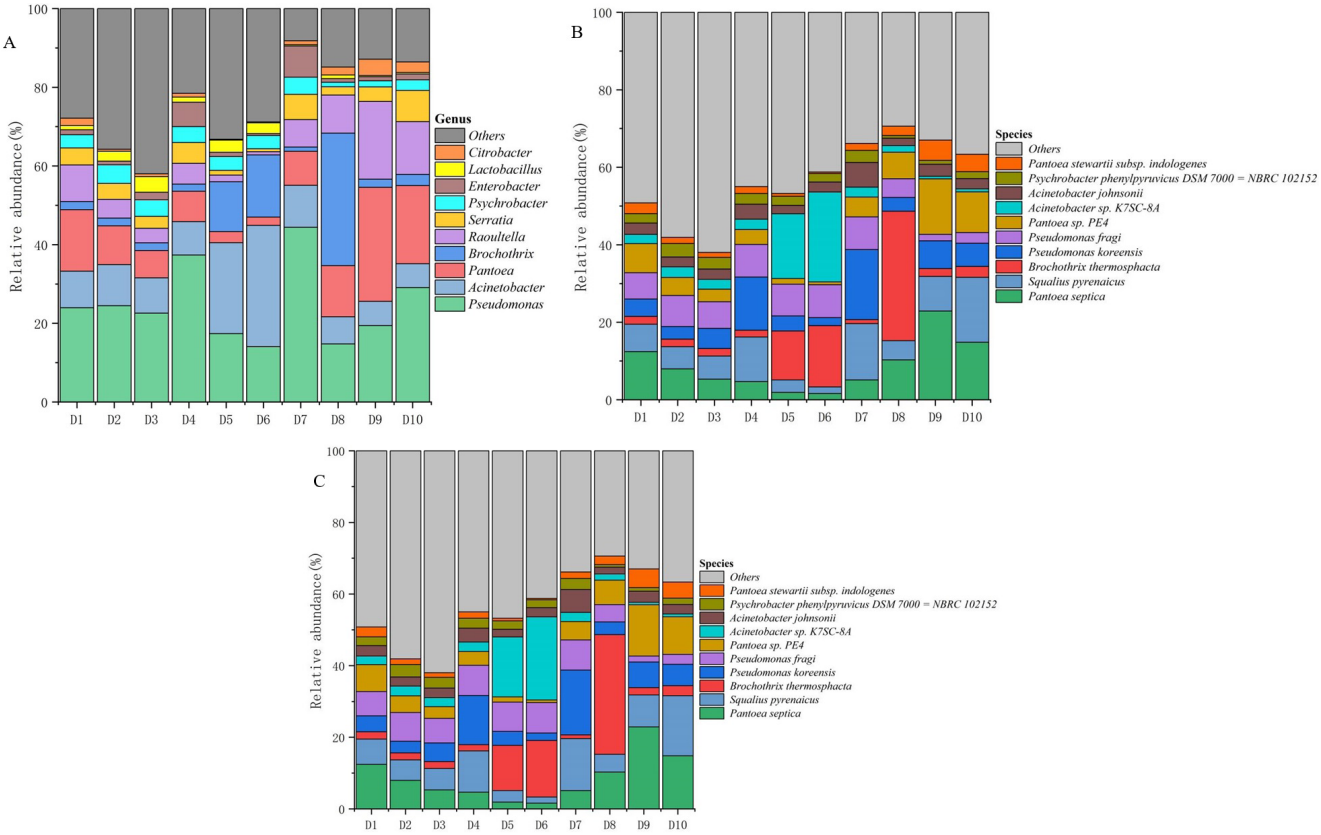

Figure 5. Dynamics in relative abundance of bacterial taxa based on 16S rDNA sequencing at phylum level (A), genus level (B) and species level (C) in chilled pork samples during 10 days' storage at 4 °C.

At the level of genus, the top 10 genera by mean relative abundance determined in chilled pork during storage at 4°C as shown in Figure 5B. *Pseudomonas* spp., *Acinetobacter* spp., *Pantoea* spp., *Brochothrix* spp., *Raoultella* spp., *Serratia* spp., *Psychrobacter* spp., *Enterobacter* spp., *Lactobacillus* spp. *and Citrobacter* spp., in which *Brochothrix* spp. and *Lactobacillus* spp. belonged to Firmicutes and other 8 genera belonged to Proteobacteria.

Pseudomonas spp. were the most dominant genus with an average relative abundance of 24.77% and reached a maximum abundance of 44.43% on the 7th day. *Pseudomonas spp.* with high spoilage potential, dominate under aerobic conditions has been regarded as a contributor to aerobic package meat, fruit, even drinks spoilage (Li et al., 2019). *Acinetobacter spp.* underwent a sharp increase from 8.48% on the 4th day to 30.83% on the 6th day, which were considered as pathogenic bacteria and may cause community-acquired pneumonia even death (Hamouda et al., 2011). The proliferation of *Acinetobacter spp.* led to more rapid microbial spoilage and product rejection by consumers. In the later of storage, the relative abundance of *Pseudomonas* spp. and *Acinetobacte*r spp. substantially decreased and was replaced by *Brochothrix spp.* with an abundance of 33.69% on the 8th day and *Pantoea spp.* with an abundance of 28.98% on the 9th day, respectively. *Brochothrix spp*. and *Pantoea spp.* were identified as spoilage bacteria. Therefore, their rapid proliferation accelerated the meat spoilage, resulting in muscle tissue breakdown, discoloration and sensory spoilage. Moreover, *Raoultella* spp. were detected on the 9th day of storage, accounting for 19.75% of the total abundance. *Raoultella spp.* can grow slowly at low temperature condition and can produce a large amount of histamine, which has a great impact on food safety (Kanki et al., 2002). *Serratia spp.* as the representatives' genus of psychrotrophic Enterobacteriaceae were identified in all samples with an average relative abundance of 3.89%, indicating a potential contribution to deterioration. The appearance of *Serratia* spp. is usually attributed to the contamination from processing environment. Low temperature would be conducive to the growth of *Psychrobacter* spp. which can improve the *Epinephelus coioides'* immune response (Sun et al., 2011). However, *Psychrobacter* spp. maintained a low growth during storage, which the average relative abundance during the first 7 days was 3.95% and 1.79% during the last three days, respectively. *Enterobacter* spp. are common food spoilage and pathogenic bacteria and may cause diseases even death, especially for children, which were found throughout the storage with the average relative abundance of 2.31%. *Citrobacter* spp. with an average relative abundance of 1.45% maintained a low growth during storage, which have been considered as pathogen bacteria and associated with many diseases including septicemia in animals even human being (Duceppe et al., 2019). Interestingly, *Lactobacillus* spp. were detected with average relative abundance of 1.66% during storage. *Lactobacillus* spp. were generally considered to be beneficial for fermentation in ferment foods (Wang et al., 2019) and can significantly decrease the production of TVB-N of chilled meat, indicating its contribution to the shelf life extension.

The average abundance of top 10 species is shown in Figure 5C. *Pantoea septica* was the highest average relative abundance bacteria (8.72%) in samples of chilled pork. When pork was fresh (the first 3 days), *Pantoea septica* (sample D1, 12.46%) and *Pseudomonas fragi* (sample D2, 8.00% and D3, 6.84%) accounted for the highest proportion. *Pseudomonas koreensis* reached 13.73% in sample D4 and became the most abundant species, and the *Acinetobacter sp. K7SC-8A* became the most abundant bacterium over the next 2 days (sample D5, 16.73% and D6, 23.20%). Dramatically, *Pseudomonas koreensis* had the highest abundance again in the sample D7. Interestingly, from the heat-map of species level, *Brochothrix thermosphacta* can also survived in pork at 4 °C, and became the most abundant species on 8thday with its relative abundance of 33.42%. *Pantoea septica,* occupying for 22.91% in abundance of the sample D9, became the predominant species. The last, *Squalius pyrenaicus* was the most abundant bacterium in sample D10 (16.78%), followed by *Pantoea septica* (14.85%).

Although the abundance of bacteria varied irregularly, the bacteria with higher abundance were detected as the dominant spoilage bacteria in the whole storage process. In addition, the influence of non-dominant bacteria on meat quality and safety is still not negligible. Other bacteria species detected also played an important role in the spoilage of meat, namely, *Pantoea sp. PE4* (5.83%), *Acinetobacter johnsonii* (3.08%), *Psychrobacter phenylpyruvicus DSM 7000 = NBRC 102152 (2.28%), Pantoea stewartii subsp.indologenes* (2.25%).

There were some differences in these results and previous studies (Li et al., 2019; Pennacchia et al., 2011). This difference may be due to many complicated factors, such as environment, air, storage time, the source of meat, even the different parts of the pork (Li et al., 2019).

4 Conclusion

In this study, the characterizations of bacterial community and spoilage profiles shift in chilled pork during storage at 4°C were unraveled in detail. The bacterial diversity and composition and microbial community dynamics of chilled pork storage at 4°C were demonstrated by HTS technology. A total of 37 phyla, 575 genera and 843 species were identified during 10 days' chilled storage. Alpha diversity analysis showed that the abundance and diversity of bacteria in pork decreased during storage under aerobic conditions at 4°C. But there was a slight increase in bacterial diversity between the 8th day and the 10th day. At the phylum level, the predominant bacteria in samples were Proteobacteria and Firmicutes. And *Pseudomonas* spp., *Acinetobacter* spp., *Pantoea* spp., *Brochothrix* spp., and *Raoultella* spp. were the dominant genera which average relative abundances were higher than 5%. *Serratia* spp., *Psychrobacter* spp., *Enterobacter* spp., *Lactobacillus* spp. and *Citrobacter* spp. were the genera that average relative abundances were higher than 1%. Moreover, there were 10 species identified for the dominant species, namely, *Pantoea septica, Squalius pyrenaicus, Brochothrix thermosphacta, Pseudomonas koreensis, Pseudomonas fragi*, *Pantoea* sp. PE4, *Acinetobacter* sp. K7SC-8A, *Acinetobacter johnsonii, Psychrobacter phenylpyruvicus* DSM 7000 = NBRC 102152 and *Pantoea stewartii* subsp. *indologenes.* The growth of these bacteria was a major factor in deterioration of chilled meat. Based on the results of sensory evaluation, TVB-N values and together with microbial results, the shelf life of chilled pork storage at 4 °C was less than 4 days. Therefore, the analyses

of changes in bacterial community structure and bacterial enumeration, combined with spoilage profiles shift of chilled pork during storage at 4 °C will be great significance for the meat preservation technology aiming at controlling the dominant spoilage microorganisms.

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Supplementary Material

Supplementary material accompanies this paper.

Fig. S1. Changes in appearance of chilled pork during storage at 4 °C.

Fig. S2. Venn diagrams of the chilled pork samples during refrigerated storage at an identity threshold of 97%

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