HIGHLY DIVERSE MICROBIAL COMMUNITIES IN VACUUM-PACKD CHILLED PORK DURING STORAGE

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Abstract – A total of 28,216 bacterial sequences and 54,097 fungal sequences were obtained for the assessment of microbial diversity in vacuum-packed pork during chill storage. More than 200 bacterial genera belonging to eighteen phyla and over 100 fungal genera belonging to four phyla were observed, and most of them could be associated with contamination via fecal, air and/or water during slaughtering and subsequent handling. Microbial and fungal populations changed greatly during storage, of which the seventh day was a critical time point for microbial diversity. *Micrococcaceae*, *Flavobacteriaceae*, *Enterobacteriaceae*, *Lactobacillaceae* and *Carnobacteriaceae* were the major components that may be associated with the spoilage of meat. Although the potential impact of detected microbes on meat hygiene and/or safety is unknown, effective decontamination of the whole chain is always important for meat industry to guarantee meat safety and improve shelf-life of fresh meat.

Key Words – Pyrosequencing, fresh meat, bacteria, fungi

I. INTRODUCTION

Meat hygiene is determined by numerous environmental factors, which could result in meat spoilage and safety problems. For chilled meat, the growth of microorganisms, including bacteria and fungi, is the main cause for the reduction of freshness and progress of spoilage [1-3]. Bacteria in meat have been extensively studied, but fungi were less concerned [4].

The microbial diversity and main flora in fresh meat have been widely investigated with traditional cultivation methods. In recent years, as sequencing techniques developed, the bacterial diversity in beef steaks and a Chinese meat product (Zhenjiang Yao Meat) were examined with high-throughput barcoded parallel 454 pyrosequencing and their data showed that the bacterial phylotypes were more complex than previous studies [5,6]. In these two studies, high-throughput sequencing was shown to be powerful to give an insight for understanding the changes in microbial populations in meat and meat products during production or storage.

In the present study, parallel pyrosequencing with 16S rDNA and 18S rDNA was applied to characterize bacterial and fungal changes in vacuum-packed chilled pork. The results were expected to provide insights for understanding microbial changes in fresh pork during production and subsequent chill storage in terms of the precaution for meat safety and the improvement of meat hygiene.

II. MATERIALS AND METHODS

Sampling. A total of 20 pig carcasses were selected from a single slaughtering line in a commercial slaughterhouse (Henan, a capacity of 3000 carcasses per day). After 20 h chilling in a 0℃ chiller, carcasses were commercially fabricated. The cut "hind leg" was vacuum-packed in heat-shrink bags. Vacuum packed cuts were stored at 0℃ up to 21 days and sampled after 1h (d 0) 7d, 14d, 21d after vacuum packaging (5 bags per time point). On each sample occasion, 100 g of muscle on the exposed surface of cut with thickness up to 2 cm were removed for DNA extraction.

Total bacterial genomic DNA extraction. Meat subsamples were homogenized in peptone saline for 30 s in a blender. Twenty milliliters of the homogenate were centrifuged at a speed of

10,000×g for 10 min. The pellets were resuspended in lysis buffer and broken with 100 mg of zirconium beads (0.1 mm) in Mini-beadbeater for 2 min. Microbial DNA was extracted with DNeasy tissue kit (QIAGEN, Germany) according to the manufacturer's instructions. The 5 DNA subsamples from each group were recombined and DNA concentration was measured under a Nano-drop 1000 spectrophotometer.

Pyrosequencing for 16S rDNA. The bacterial diversity was analyzed by pyrosequencing of the amplified 16S rDNA V4-V5 variable region [7].

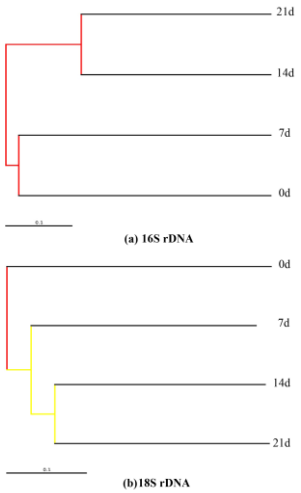
Pyrosequencing for 18S rDNA. The fungal diversity was analyzed by pyrosequencing of the amplified 18S rRNA V4 variable region [8].

Data Analysis. After pyrosequencing, all reads were screened and filtered using QIIME 1.6.0 software [9]. Operational taxonomic units (OTUs) were picked only if they had similarity values of 97% or higher. The representative sequences were compared to the RDP classification (Ribosomal Database Project, http://rdp.cme.msu.edu/wiki/index.php/Main_Page) to obtain the taxonomy assignment [10].

III. RESULTS AND DISCUSSION

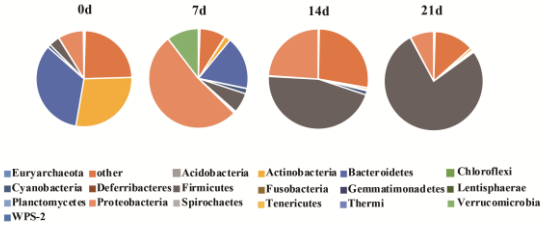
Phylogenetic tree analysis indicated that the 14 day samples had a highly similar bacterial and fungal diversity to 21 day samples. The 0 day samples showed a similar bacterial diversity to 7 day samples (Fig. 1a&b).

Figure 1. Phylogenetic tree analysis on 16S rDNA and 18S rDNA data



For bacteria, eighteen phyla were found in in vacuum packed pork across all the time points. The vast majority of sequences belong to four major phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*. *Actinobacteria* and *Bacteroidetes* were the most predominant microbiota at day 0 (28.17% and 33.75%, respectively), but they were substantially decreased afterwards. *Firmicutes* increased greatly at day 14 and day 21 (45.77% and 77.04%, respectively). The abundance of *Proteobacteria* increased from 8.57% at day 0 to 52.32% at day 7, but it decreased to 7.69% at day 21. *Verrucomicrobia* showed a similar tendency to increase from 0.03% at day 0 to 10.40% at day 7, but it was not detectable at day 14.

Figure 2. Changes in abundance of bacterial phyla based on 16S rDNA sequencing

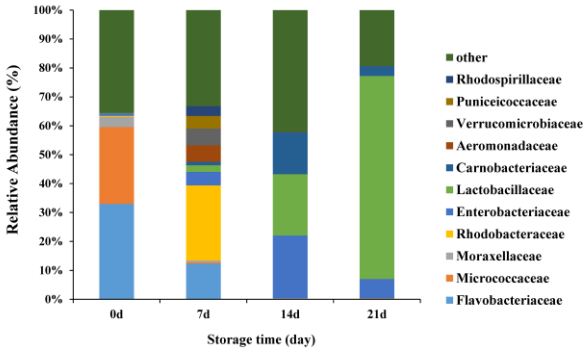


When compared at family level, the major components of bacteria in the first 7 days were more diverse and different from those at day 14 and day 21 (Fig. 3). *Micrococcaceae* and *Flavobacteriaceae* accounted for 26.59% and 32.99% of total sequences at day 0. *Micrococcaceae* is a type of aerobic bacteria and *Flavobacteriaceae* is a type of amphimicrobe, both of them exist widely in the environment and may contaminate meat during animal slaughtering and postmortem handling by tools and workers [5]. After vacuum packaging, the abundance of such bacteria decreased greatly, which could be due to the death or the growth inhibition of the bacteria.

At day 7, *Flavobacteriaceae*, *Rhodobacteraceae*, *Enterobacteriaceae*, *Verrucomicrobiaceae*, and *Lactobacillaceae* contributed to 12.34%, 25.94%, 4.70%, 5.84% and 2.28% of total sequences. At d 14, *Carnobacteriaceae*, *Lactobacillaceae*, and *Enterobacteriaceae* were the predominant populations accounting for 14.54%, 21.15% and 21.63% of total sequences. At d 21, *Lactobacillaceae*, *Carnobacteriaceae* and

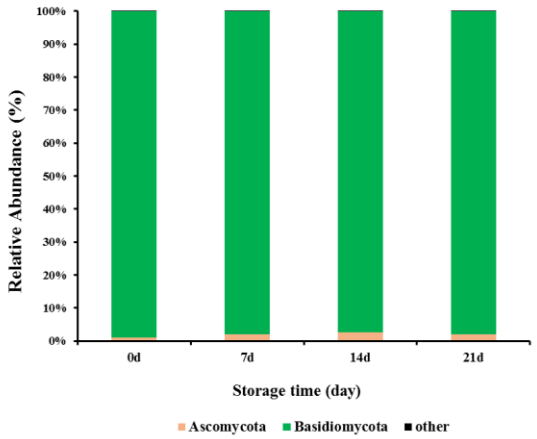
Enterobacteriaceae were still the prevalent populations consisting of 70.18%, 3.40% and 6.50% of total sequences. It has been shown that *Lactobacillus* may produce lactic acid to inhibit the growth of other families of bacteria in vacuum packed meat [11]. *Enterobacteriaceae* was shown to account for a decreasing proportion from day 7 to day 21. This agrees with Pennacchia *et al*. [12] who showed that *Enterobacteriaceae* was one of the major bacteria in vacuum-packed chilled meat. In addition, several low-abundance, animal-sourcing bacteria observed in the present study could be associated with contamination via fecal, air and/or water during slaughtering and subsequent handling. *Rhodobacteraceae* and *Verrucomicrobiaceae* were reported to be intestinal microorganisms in livestock and aquatic animals [13,14]. To the authors' knowledge, the present study is the first to detect the presence of *Mamiellaceae*, *Hyphomicrobiaceae* and *Chlamydomonadaceae* in pork. As yet the potential impact of such bacteria on meat hygiene and/or safety is unknown.

Figure 3. Changes in abundance of bacterial family based on 16S rDNA sequencing



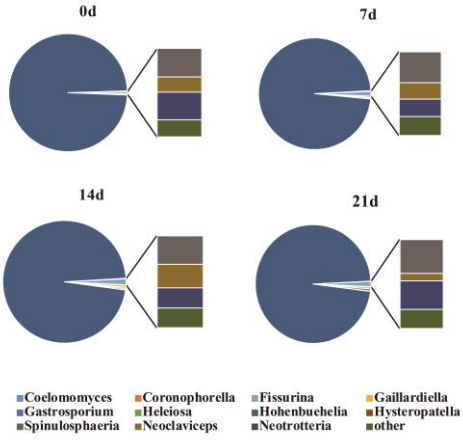
For fungi, four phyla were found across all the time points: *Chytridiomycota*, *Basidiomycota*, *Ascomycota* and *Blastocladiomycota* (Fig. 4). The majority of sequences across the four time points belong to *Basidiomycota* (greater than 97% across the time points), but the number of its reads decreased greatly from 18544 at day 0 to 4259 at day 21. The abundance of *Ascomycota* increased from 1.02% (191 reads) at day 0 to 2.69% (372 reads) at day 14, and then declined to 2.13% (93 reads). The other phyla were extremely low in abundance through storage.

Figure 4. Changes in abundance of fungal phyla based on 18S rDNA sequencing



At the genus level, a total of 24 genera were found across all the time points. Of these, *Hohenbuehelia* was always the predominant population across the time points (Fig. 5). In addition, *Hysteropatella*, *Heleiosa*, *Gaillardiella*, *Spinulosphaeria*, *Neotrotteria*, *Neoclaviceps* and *Gastrosporium* also made a great contribution to the remaining part. These fungi could be associated with contamination via fecal, air and/or water during slaughtering and subsequent handling. Although fungi (especially yeasts) have been shown to be potential agents of spoilage of fresh meat [4], the present study is the first to examine the 18s rDNA profile of fungi from fresh meats.

Figure 5. Changes in abundance of fungal genus based on 18S rDNA sequencing



IV. CONCLUSION

In summary, pyrosequencing was applied to explore the microbial community of vacuum-packed chilled pork and establish the range and diversity of this community. *Micrococcaceae*, *Flavobacteriaceae*, *Enterobacteriaceae*, *Lactobacillaceae* and *Carnobacteriaceae* were the major components that may be associated with the spoilage of meat. Other bacteria and fungi were also found that may be associated with contamination via fecal, air and/or water. Although the potential impact of such microbes on meat hygiene and/or safety is unknown, effective decontamination of the whole chain is always important for meat industry to guarantee meat safety and improve shelf-life of fresh meat.

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