METAGENOME-BASED CHARACTERIZATION OF MICROORGANISMS FROM YELLOW-FEATHER BROILER, A HIGHLY POPULAR MEAT IN ASIA

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Abstract –The spatial and temporal variation in the microbial community of yellow-feathered broiler carcasses under penetrated-air packaging (PAP, air filling) and modified-atmospheres packaging (MAP, 80% CO_2 & 20% N_2) was investigated by whole genome sequencing-based metagenomics. The results revealed that MAP prolonged shelf life by 4 days, when the numbers of total plate counts and lactic acid bacteria reached more than 7 log CFU/g. *Aeromonas*, *Acinetobacter*, *Escherichia* and *Streptococcus* occupied the microbial communities in initial broiler carcasses, and MAP dramatically increased the diversity of microorganisms during storage compared to PAP. Clear shifts of the dominant bacteria species were also observed, with the top genera of *Aeromonas*, *Lactococcus*, *Serratia* and *Shewanella* in MAP, whereas the microbial communities in PAP were largely dominated by *Pseudomonas*.

Key Words – bacteria diversity, packaging, spoilage

I. INTRODUCTION

The yellow-feathered broiler is a traditional poultry breed in Asia that is well known for its unique meat flavor. In 2015, the production of this broiler in China has reached more than 4.4 billion heads. Traditionally, the live broilers approved by the purchaser were individually sold and immediately slaughtered in wet markets. However, the traditional consumption pattern has recently been prohibited by the Chinese government due to outbreaks of animal influenza. A new pattern of "slaughtered in large-scale plants and sold with chilled meat" has been advocated nationwide and implemented for yellow-feathered broilers. In this pattern, there are usually more than 60 hours between live broiler slaughtering and meat cooking. Thus, innovative solutions are required to inhibit bacteria growth and extend the shelf-life of chilled meat. Previous work has provided preliminary evidence that modified-air packaging (MAP) could prolong the shelf-life of chilled yellow-feathered meat. However, how to induce the diversity and metabolic profile shift of microorganisms during storage by the packaging patterns is still unknown.

II. MATERIALS AND METHODS

The yellow-feathered broilers carcasses that had not been packaged and stored (0 d) were regarded as control samples. The carcasses were packaged with penetrated-air packaging (PAP, air-filling) or modified-atmospheres packaging (MAP, 80% CO_2 /20% N_2) and then were stored at 4℃ for 4 d and 8 d, respectively. The total microorganisms from broiler carcasses were collected by carcass-washing methods. Total DNA of microorganisms was directly extracted using DNA Microbiome Kit (Qiagen) following the manufacturer's recommendations. Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations. Library preparations were sequenced on an Illumina HiSeq 4000 platform and paired-end reads were generated.

III. RESULTS AND DISCUSSION

The numbers of two different kinds of representative bacteria on carcasses exceeded 7.0 log CFU/g after storage for 4 d and 8 d for PAP and MAP, respectively, which was widely considered as the threshold of microorganism counts during meat spoilage (Figure 1). Compared to PAP, MAP prolonged the shelf life by 4 days, which was in line with previous finding [1].

The heat map shown in Figure 2 revealed the dynamics of the 35 most abundant bacteria along with the packaging patterns.The dominant bacteria genera in control samples mainly included *Streptococcus*, *Enterobacter*, *Empedobacter*, *Micrococcus*, *Enhydrobacter* and *Aeromonas*. Compared with control treatment, PAP caused a shift in microorganism diversity, with an increase in abundance of *Pseudomonas*, *Arthrobacter*, and *Janthinobacterium* and a decrease in *Aeromonas*, *Citrobacter* and *Klebsiella*. In contrast, MAP dramatically increased the relative abundance of

Carnobacterium, *Cagococcus*, *Enterococcus*, *Lactobacillus* and *Serratia*, as well as *Hafnia*. *Shewanella* and *Weissella*, which are commonly associated with food spoilage, showed lower abundance in both control and PAP treatments.

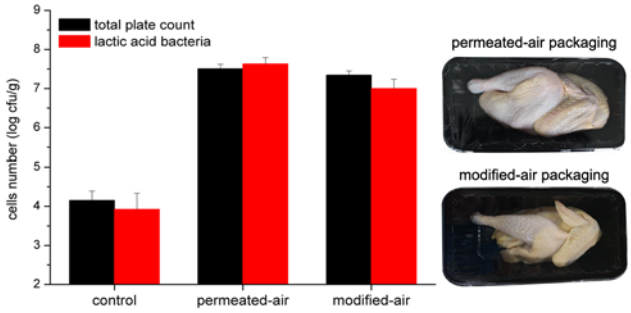

Figure 1 Numbers of bacteria of broiler carcass

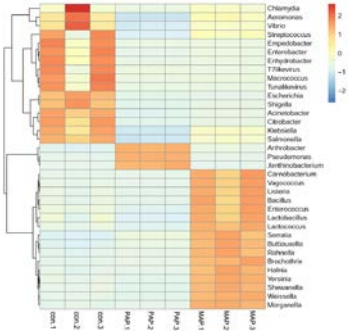

Figure 2 The heat map of relative abundance within each sample of the top 35 bacterial in genus level

The results of clustering tree (Figure 3) indicated that a high similarity in relative abundance was found between each duplicate in individual treatments, and low similarity was found between each treatment. The structure of the top 10 dominant bacteria was dramatically affected by the packaging patterns (Figure 4). Compared with control treatment, the abundances of *Escherichia coli* and *Aeromonas veronii* were dramatically reduced in MAP and PAP treatments, which have been demonstrated to show great lipase activity and proteinase activity [2]; whereas the abundances of *Lactococcus raffinolactis* and *Pseudomonas* sp. were sharply increased in MAP and PAP, respectively, especially for *Pseudomonas* sp., which occupied a dominant abundance in PAP at the end of shelf life. In contrast, *Serratia liquefaciens*, *Lactococcus raffinolactis*, *Aeromonas salmonicida* and *Carnobacterium maltaromaticar* together become the dominant bacteria in MAP at the end of shelf life [3].

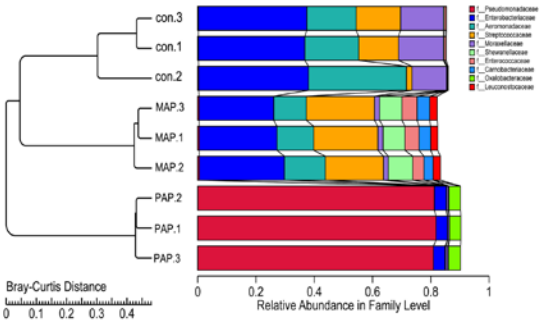

Figure 3Clustering tree based on the Bray-Curtis distance obtained from the relative abundance in family levels

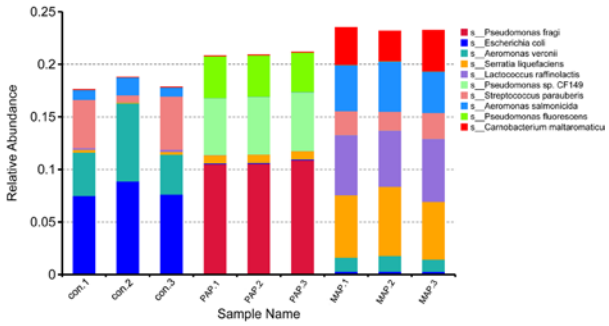

Figure 3 Top 10 bacteria obtained from the relative abundanc in species level

IV. CONCLUSION

Bacterial community shift was dramatically affected by packaging patterns in chilled broiler carcasses. Although our data have enabled new insights into community composition during meat spoilage process, more studies on dominant species are expected to be done in the future to confirm the potential link of off-odors between natural meat spoilage and dominant species metabolism on meat in situ.

ACKNOWLEDGEMENTS

This study was supported by the China Agriculture Research System (CARS-42), funded by the China Ministry of Agriculture.

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