EFFECT OF MICROBIAL CONTAMINATION CONDITIONS OF SLAUGHTERING AND SPLITTING PROCESS ON MICROBIAL DIVERSITY OF CHILLED VACUUM-PACKAGED BEEF DURING STORAGE

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Abstract - The purpose of this study was to analyze the bacterial diversity associated with vacuum packed chilled beef under different processing conditions and to determine the main sources of the contamination. The level of contaminated microorganism of the different slaughter processes in 3 beef slaughter plants (A, B and C) were investigated using denaturing gradient gel electrophoresis (DGGE) method. The microorganism samples were from the surface of all the critical control points and the chilled beef during storage. The results showed that species of *Lactobacillus spp.*, *Carnobacterium*, *Psychrobacter*, *Brochothrix thermosphacta*, *Leuconostoc spp.* were the common dominant spoilage-related microorganisms in vacuum-packaged chilled beef from different plants. The contamination of the contact surfaces including gutting knives, worker's hands, worker's aprons, deboning knives, trimming knives, trimming board and belt conveyors were the main sources of the microorganisms. Thus, good control of the main surfaces is a better way to extend the shelf life of vacuum packaged chilled beef.

Key words - chilled beef, bacterial diversity, contamination sources

I. INTRODUCTION

Chilled beef is the main consuming pattern of beef products in China. Currently, beef processors and retailers usually use vacuum-packaging for chilled beef transportation. However, the shelf-life of vacuum-packaged beef in China is only about 4 to 6 weeks which is about 2 ~ 4 weeks shorter than that in America. For chilled vacuum-packaged beef hygiene, the microorganism contamination during slaughtering and processing process in plants is a main limiting factor. Microbiological spoilage progress is determined by numerous environmental factors, which could result in meat spoilage and reduction of freshness [1]. The type of bacteria and their loads mainly depend on the initial meat contamination during slaughter and processing, such as stripping, deboning, and trimming etc., which influences the rate of spoilage [2]. However, to our knowledge, few have investigated the level of microbial contamination at different slaughter processes in Chinese plants.

Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) overcomes the limitations of traditional cultivation methods and has become a popular method to detect microbial diversity and main flora of meat. This technology has been successfully applied to investigate the microbiology of meat products in the last decade [3].

In the present study, PCR-DGGE was used to investigate bacterial origins and changes for vacuum-packed chilled beef during slaughter and storage in three beef slaughter plants, including all the critical control points. The results were expected to provide in-sights into the understanding of microbial changes in fresh beef during production and during

subsequent chill storage in terms of any precautions for meat safety and for the improvement of beef hygiene.

II. MATERIALS AND METHODS

A. Microbial sampling

Three big beef slaughter plants were selected for microbial sampling. Microbial samples were collected from both the processing environment and the chilled beef during storage. The environment sampling points were the main contact surfaces during slaughtering and parts dividing including the knives, worker's hands, worker's aprons, boards and belt conveyors. Three LL muscles with pH 5.6 to 5.8 were collected after aging for 72 h, and transported to lab at 4 °C under vacuum package. Each muscle was then cut into 12 pieces, vacuum packaged again and stored at 2 ± 2 ℃ for microbial sampling each week until spoilage.

B. Polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE)

Total bacterial DNA was directly extracted directly from the microbial samples using a GenElute™ Kit (Bacterial Genomic DNA Kit, Sigma, USA) according to the manufacturer's instructions and then dissolved in 200 μL of eluting solution [4, 5]. All DNA samples were purified using a DNA purification kit (Solarbio®, China) and stored at -20℃ for analysis.

Nested PCR and touchdown PCR reactions were followed as described by Hu et al (2008) (6). PCR products were analyzed by DGGE by using a Bio-Rad D-code apparatus. Samples were applied to 8% (wt/vol) polyacrylamide gels in 0.5×TAE buffer. Parallel electrophoresis experiments were performed at 60°C by using gels containing a 30 % - 60 % urea-formamide denaturing gradient (100% corresponded to 7M urea and 40% (wt/vol) formamide). Electrophoresis was performed for 5 min at 120V and then at 75 V for 14 h in 1×Tris-acetate-EDTA buffer. The gels were stained with ethidium bromide for 5 min, rinsed for 15 min in distilled water, observed and photographed by the Bio-Rad Gel Doc system (BioRad, Milano, Italy).

The main DGGE bands were excised, transferred into 40ml of sterile water, and incubated overnight at 4°C to allow diffusion of the DNA. The extracted DNA was re-amplified using the same PCR protocol as described for DGGE with primers L1401 and U968 (without the GC clamp). There-PCR products were inserted into a pMD-18Tvector (TaKaRa, Dalian, China). The following processes were performed according to the manufacturer's instructions and were sequenced (Sangon BiotechCo., Ltd., Shanghai, China). All sequences were compared with those in the GenBank database (www.ncbi.nlm.nih.gov/blast/) with the BLAST search program. Sequences with a percent identity of 97% or higher were considered to represent the same species.

C. Statistical analysis.

DGGE gels were analyzed using Quantity One software (version4.3.1, Bio-Rad).

III. RESULTS AND DISCUSSION

Bacterial diversity and dominant species in chilled beef from different plants

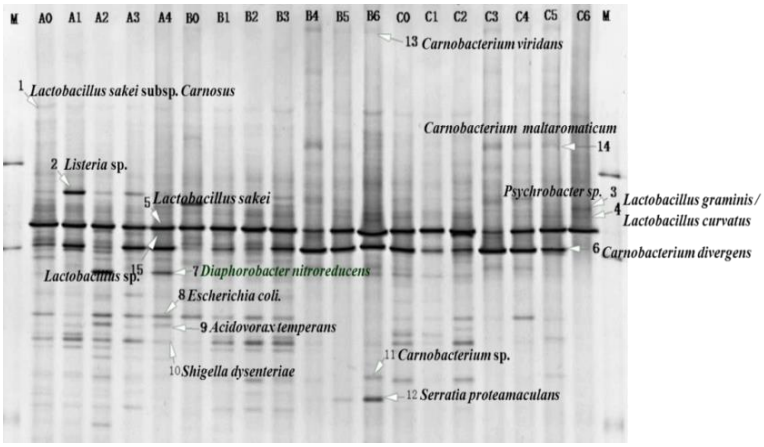

Fig. 1 DGGE fingerprinting of PCR products of micro-flora from vacuum-packaged chilled beef stored at 2℃

M：marker；A0- A4: samples from plants A which were stored for 0 - 4 weeks; B0-B6: samples from plants B stored for 0 - 6 weeks; C0-C6: samples from plants C stored for 0 - 6 weeks.

Changes in the bands in the DGGE lanes showed the complexity and variability of bacterial flora in different samples (Fig.1). One obvious result is that the bacterial community varied between the plants (Fig.1). The highest diversity of flora was found for plant A, and the lowest for plant C. The initial bacterial community in the chilled beef from different plants was also different (Fig.1). This was probably due to the different controls in different factories.

There was a same tendency in 3 plants that the microbial diversity declined and similarity increased with the storage time prolonged. At the last storage period, the number of the bands declined and some bands became dominant. Species of *Lactobacillus sakei* subsp. Carnosus (band 1), *Lactobacillus sakei* (band 5), *Carnobacterium divergens* (band 6) and *Escherichia coli.*(band 8) were the common dominant spoilage

bacteria in different samples. But, there were also some peculiar bacteria which were only detected in one or two plants. For example, those of *Listeria* sp. (band 2), *Acidovorax temperans* (band 9), *Shigella dysenteriae* (band 10) and *Lactobacillus* sp.(band 15) only existed in the samples from plant A.

A. Relationship of bacterial community between environment and chilled beef during storage

Fig.2 showed that there were some same bands in beef samples and environment samples. And most dark band (band 2, 3, 4, 5) in the beef at the end of the storage could be found in the environment samples. This indicated that the dominant bacteria probably came from the environment during processing. This would also support the opinion that some of the microorganisms originate from the environment that the animal was in before or during slaughtering and processing [2, 8].

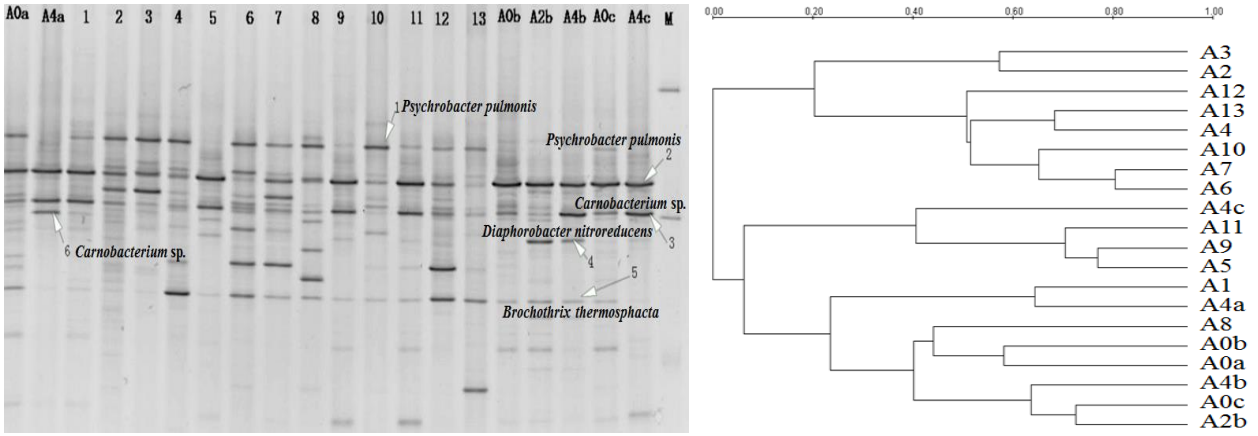

Fig. 2 DGGE fingerprinting and profile similarities of contaminated resources and stored beef samples from plants A

a、b、c: three beef samples collected from plant A; 1-13: peering knives, gutting knives, slaughtering worker's hands, deboning boards, deboning knives, deboning worker's aprons, deboning worker's hands, trimming boards, trimming knives, trimming worker's aprons, trimming worker's hands, belt conveyors and packing contacts.

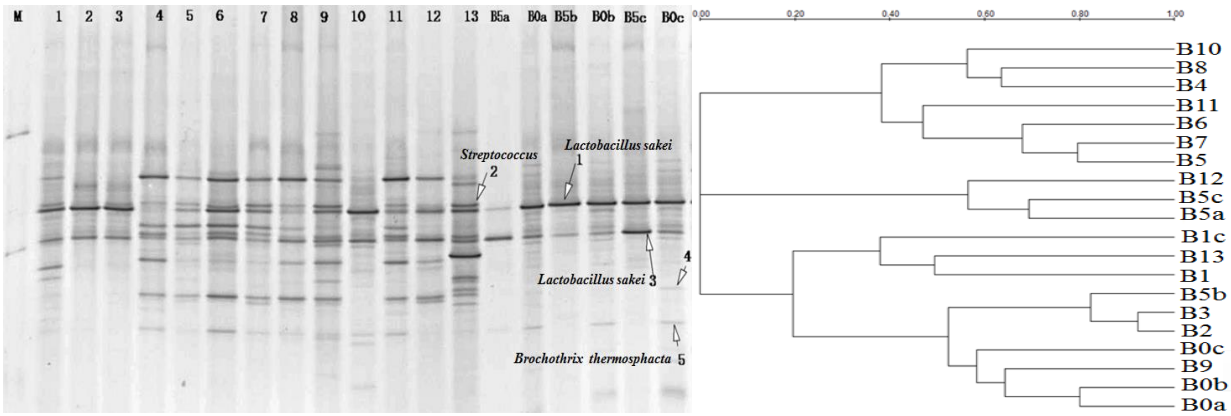

Fig. 3 DGGE fingerprinting and profile similarities of contaminated resource and stored beef sample from plant B

Letter of a, b, c and number of 1-13had the same mean as that of Fig.2

The profile similarities (Fig.2) showed that the contact surfaces of peeling knives, deboning knives, trimming boards, trimming knives and worker's hands had the highest similarity (40%) of the bacterial diversity with the chilled beef at the end of storage. And the trimming boards had the highest similarity (45%) with the initial bacterial community. So, these main contact surfaces during processing were the main contamination resources of the dominant spoilage bacteria.

Similar tendency was found in samples collected in plants B (Fig.3). The dominant bacteria in beef could also be found in the environmental samples. And the diversity in beef samples decreased with storage time. The similarity analysis showed that the trimming knives had the highest score of 58 % with the initial bacterial community and conveyor belt got the highest one of 57 % with samples a and c at the end of the storage. While, there was a 62 % similarity between workers' hands and

gutting knives with beef sample c at the end of storage. So, it could be concluded that the worker's hands, gutting knives, trimming knives and the conveyor probably were the main contamination points in plant B.

With the same method, samples collected in plants C were also proved to have the similar tendency with plant A and B (data were not shown). High bacterial community similarity of 60 % existed between beef samples at the beginning and the surfaces of worker's hands, trimming knives, worker's aprons. While, those of worker's hands, worker's aprons and trimming knives during deboning and trimming had the high similarity of 20 % to 48 % at the end of shelf life of 6 weeks.

So, based on the results of the 3 plants, it can be concluded that there were differences in environment controls among different plants. The initial and finial bacterial community in vacuum packaged chilled beef during storage differed as result of the different controls. There was a great relationship between the surface conditions and the bacterial community of the chilled beef. The main surfaces included worker's hands, gutting knives, worker's aprons, trimming knives and belt conveyor, especially during parts dividing which had the highest similarity with the bacterial community of chilled beef at the end of the shelf life.

IV. CONCLUSION

Species of Lactobacillus sakei subsp.Carnosus, Psychrobacter pulmonis/Psychrobacter faecalis, Lactobacillus graminis/Lactobacillus curvatus, Lactobacillus sakei, Carnobacterium divergens, Carnobacterium viridans / Carnobacterium maltaromaticum,and Brochothrix thermosphacta were the common dominant spoilage related microorganisms in vacuum packaged chilled beef. The contact surfaces of gutting knives, worker's hands, worker's aprons, deboning knives, trimming knives, trimming board and belt conveyor were the main sources of the microorganisms. As the last process, those surfaces during trimming were most important to chilled beef. So, further control for above surfaces is very important for shelf life extending of chilled beef.

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