# IDENTIFICATION OF CRITICAL CONTAMINATED SOURCE DURING PRODUCTION OF CHILLED PORK USING PCR-DGGE

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## Introduction

The bacterial diversity and the contaminated source during slaughter were the major factor to the shelf life and meat safety in meat. The contaminated microorganism were great distinct in different corporation and different country (Sumner, Petrenas, & Dean, 2003; Zweifel, Baltzer, & Stephan, 2005). It is critical for meat industry to identified the critical contaminated source to the production of chilled pork during slaughter and storage. Contaminated bacteria diversity between slaughter and storage should be chiefly investigated and then control them. The bacterial diversity of meat under different storage conditions have been extensively studied according to traditional cultivation methods (Jay, Vilai, & Hughes, 2003), by which only 0.1% to 3% of the total bacterial population can be cultivated. However, it is very difficult to do this due to inability to detect some bacteria on the known media and complexity of sources contamination such as water, soil, feces and environment.

The method of denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments, which was proposed by Muyzer, de Waal, & Uitterlinden (1993), can overcome these problems by reflecting the microbial diversity in different period. Therefore, it has been used as a tool to analysis microbial diversity. In recent years, this technique has been applied in many fields such as sausage, cheese, food waste, kimchi, soil and pig feces. Therefore, the objective of this study was to explore the bacterial diversity of contaminated source and identify the critical contaminated source to the production of chilled pork by the method of PCR-DGGE of 16S rRNA gene.

## Materials and Methods

Pork loin steaks (weight: 100 to 250 g) were removed from pork carcasses at 24 h postmortem in a commercial meat plant in middle China. All the steaks were tray-packaged and stored at 4℃ and 10℃ for 4 days respectively. Contaminated microorganism during slaughter were at four point of slaughter that is behind skinned, at the end of slaughter line, carcass chilled and segment meat by swab methods. The samples of contaminated source were contaminated microorganism at the chopping board, knife and water of wash knife by swab methods.

Both ten grams of meat sample and swab samples were diluted in 100 ml peptone water. Ten milliliters of dilution was centrifuged for 3 min at 12,000 × g and the sediment was used for further analysis. Bacterial DNA was extracted using GenElute$^{TM}$ Kit (Bacterial Genomic DNA Kit, Sigma,USA) according to the manufacturer's instructions, and then suspended in 100 μl of TE.

Primers U968-GC (5' CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG GAA CGC GAA GAA CCT TAC) that contained GC clamp and L1401 (5' GCG TGT GTA CAA GAC CC) (Nübel, Engelen, Felske, Snaidr, & Wieshuber, 1996) were used to amplify the V6–V8 regions of the bacterial 16S rRNA gene. PCR reaction system (50 μl) included 0.25 μl of Taq polymerase (1.25 U), 1 μl of primers U968-GC and L1401 (5 pmol), 1 μl of DNA dilution (approximately 1 ng), 5 μl of ten-fold PCR buffer, 3 μl of $MgCl_2$ (50mM) and 39.75 μl of UV-sterile water and it was programmed with 35 cycles of 94℃ for 30 s, 56℃ for 20 s, and 68℃ for 40 s. The results were analyzed by 1.2% agarose gel electrophoresis.

DGGE was performed according to the procedure of Nübel et al (1996) with a small modification. Briefly, an 8% polyacrylamide gel (acrylamide: bisacrylamide 37.5:1) containing a denaturing gradient of 38-48% urea-formamide was electrophoresed at 200 V for 5 min, and then at 85V for 16 h at 60℃. DGGE gel was stained with $AgNO_3$. Similarity indices were calculated for the DGGE profiles. The DGGE gel was scanned at 400 dpi and similarity indices (i.e., Pearson product-moment correlation coefficient) were calculated using the software of Molecular Analyst 1.12 (Bio-rad).

## Results and Discussion

Fig.1 showed the AgNO3-stained DGGE profiles for all samples, which indicated the complexity and variability of contaminated bacterial flora in slaughter process (T1-T4), contaminated resource (T5-T7), and chilled pork (T8-T9). The bands on DGGE gel represented microbial species. The band density can give specific patterns of bacterial changes (Ercolini, 2004). Although band densities were not calculated in the present study, a distinct change was observed by calculating similarity indices. All samples had many the same bands such as band A and E. The bands of contaminated resource were almost in agreement with the stored samples, especially the sample of the knife surface (T6) and water of wash knife (T7). But compare to the stored and contaminated resource samples, the carcass surface were not enriched in the bands such as band B and C. The bacterial composition and its change were complex, and the bacterial diversity reduced from slaughter to storage in chilled pork. This was in agreement with previous studies that the microbiological diversity decreased after storage

(Olsson, Ahrné, Pettersson, &Molina, 2003).Contaminated resource especially the knife surface (T6) and water of wash knife (T7) play an important role in contaminated microbe in meat. The carcass surface failed to become the dominant microbial contaminated resource in chilled pork.

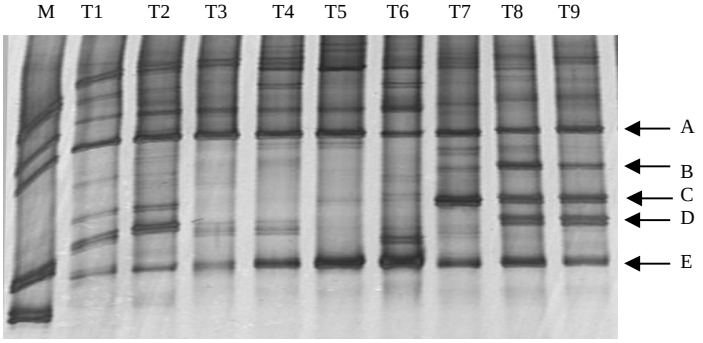


Fig. 1 DGGE profiles of PCR products of slaughter process (T1-T4), contaminated resource (T5-T7), and stored (T8-T9) samples' micro-flora. lane T1-T4, behind skinned, at the end of slaughter line, carcass chilled and segment meat; lane T5, T6, T7, at the chopping board, knife and water of wash knife; lane T8, T9, stored for 4 days at 4□ by tray-packaging, stored for 4 days at 10□ by tray-packaging; M: marker.

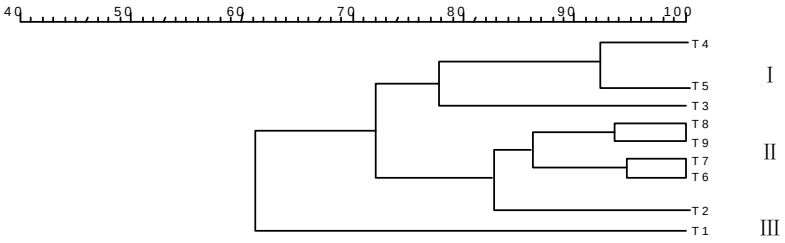


Fig. 2 Similarity index of DGGE profiles obtained from samples' micro-flora of slaughter process (T1-T4), contaminated resource (T5-T7) and storage at 4□ and 10□by tray packaging (T8-T9).

Fig. 2 shows the similarity indices of the 38-48% DGGE profiles. They were grouped into three clusters. Cluster□contained the samples contaminated resource involved in the surface of knife (T6)and water of wash knife (T7), and tray packaged for 4 days at 4□ and 10□. High similarities (90%) existed among these samples. Cluster □ was only the sample the contaminated bacterium behind skinned at the slaughter line. And the other samples were combined into cluster□. The similarity index between cluster □and cluster□, cluster□were both 60%-65%. The similarity index between cluster□and cluster□were 70%-75%. But for contaminated resource and tray packaged samples, the similarity index increased from 70% to 90% through contaminated resource and storage according to cluster□and cluster□. The increase in the similarity index implied the decrease of the bacterial diversity. The results indicated that contaminated resource especially the surface of knife (T6) and water of wash knife had a strong effect on the microbial communities in the storage of chilled pork.

**Conclusions**

In this study, the knife surface and water of wash knife were the critical microbial contaminated resource in meat. PCR-DGGE was useful to analyze the changes of contaminated microbial diversity and to provide real-time information about the contaminated resource during the produce of chilled pork.

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