PE8.35 Detection of bacterial community dynamics of frozen chicken during storage by 16S rDNA-ARDRA 397.00

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Abstract ARDRA (amplified ribosomal DNA restriction analysis) technique was applied to investigate bacterial diversity of frozen chicken during storage. Metagenome DNA from two batches of chicken sample of 60 and 90 days storage respectively was extracted to build bacterial 16S rDNA library ¢ñand ¢ò, from which 57 and 71 positive clones were chosen randomly from each corresponding library to analyze amplified ribosomal DNA restriction polymorphism by double digestion of Afa I and Msp I followed by UPGMA cluster analysis. A total of 47 and 55 distinctive bands were given by 16S rDNA-ARDRA, while 44 and 65 OTUs, accounting 77.20% and 91.50% of total clones, were obtained from library ¢ñand ¢ò, respectively. Richness index, Shannon-Wiener index, Simpson index, and Evenness index of library ¢ñand ¢ò were 13.36, 3.64, 0.97, 0.91 and 16.19, 4.15, 0.98, 0.98, respectively, suggesting that the diversity and structures of bacteria community of chicken carcass were complex, and bacterial diversity decrease with the storage time.

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Index Terms- chilled chicken; ARDRA; bacterial diversity; community dynamics

I. INTRODUCTION

Increasing people focus on chicken consumption because chicken is rich in proteins and unsaturated fatty acids, with fine taste and low fat content. It is an inevitable trend that a large amount of chicken carcass was frozen after coming out of slaughter house for later consumption or processing. However, meat and meat products ware contaminated easily by microorganisms during processing, transportation and storage. Thus comprehensive study on the dynamics of microbial community of chicken carcass is of great fundamental and economic value. Traditional isolation and identification procedure, such as pure culture, colony count etc., had some defects. Recent studies demonstrated that only 0.1¨C3% of the total bacterial population could be cultivated in meat and meat products[1]. As a consequence, comparative community and microbial similarity analyses using the above mentioned methods frequently underestimated the scale of the bacterial diversity. Numerous studies have stimulated extensive interest to investigate the structure and genetic diversity of microbial community in meats by uncultured molecular methods. Microbial community and specifics spoilage organisms in chilled meat were investigated mainly on the changes of bacterial diversity and main flora in chilled pork during storage using PCR-DGGE, with Pseudomonas sp., accounting up to 90% of the spoilage flora, disclosed as the dominant species in chill-stored meat[2][3][4][5]. However little information is available on the dominant spoilage microorganisms from chilled chicken, especially the micro flora at the molecular level. ARDRA technique has been employed to distinguish bacterial species by analyzing 16S rDNA sequences, and every 16S rDNA restriction fragment polymorphism represented an Operational Taxonomic Units (OTU). Due to its sensitivity and time-efficiency, it was designated to investigate bacterial diversity of samples [6]. Therefore, the aim of the present study was to investigate bacterial diversity and community dynamics of chilled chicken by ARDRA technique.

II. MATERIALS AND METHODS

A. Sampling Under aseptical condition, two batches of chicken sample were taken from local chicken storage room. Chicken sample ¢ñwere stored at -20°° for 90 days while sample ¢ò was 60 days.

B. DNA extraction and PCR reaction Metagenome DNA of bacteria from chicken sample ¢ñand ¢ò was extracted by modified CTAB method[7]. The DNA was assayed by agarose gel electrophoresis. Universal

primers Eu27F (5'-GAGAGTTTGATCCTGGCTCAG-3') and 1492R 5'-CTACGGCTACCTTGTTACGA-3' were used to amplify the bacterial 16S rRNA gene. PCR reaction system (50 ml) included 5 ¦Ìl of ten-fold PCR buffer, 4 ¦Ìl of dNTP (2.5 mmol/L), 4 ¦Ìl of MgCl2 (25 mmol/L), 1 ¦Ìl of Taq polymerase, 5 ¦Ìl of DNA solution, 1 ¦Ìl of primers Eu27F and 1492R and 30 ¦Ìl of UV-sterile water and PCR was performed under the following conditions: 94 ° for 5 min, followed by 30 cycles of 60 s at 94 °, 50° for 60 s, 72 ° for 90 s and finally 72 ° for 10 min. The results were analyzed by 1.0% agarose gel electrophoresis.

C. Construction library The amplification products were connected with pMD18-T vector after purification. And then the connected vectors were transformed into competent cells Escherichia coli DH5¦Á. Library ¢ñand ¢ò were constructed through selecting positive clones on plate containing ampicillin.

D. Detection positive clones of 16S rDNA libraries Positive clones from library ¢ñand ¢ò were obtained randomly to analyze insertion of 16S rDNA by colony PCR. The PCR amplification was carried out in a thermal cycler T-gradient (Eppendorf, Germany) in a final volume of 50 ¦Ìl, using 5 ¦Ìl of ten-fold PCR buffer, 4 ¦Ìl of dNTP (2.5 mmol/L), 4 ¦Ìl of MgCl2 (25 mmol/L), 1 ¦Ìl of Taq polymerase, 1 ¦Ìl of primers M13F (5'- TGT AAA ACG ACG GCC AGT-3') and M13R (5'-CAG GAA ACA GCT ATG ACC-3') and 30 ¦Ìl of UV-sterile water. It was the same with the above program. The results were analyzed by 1.0% agarose gel electrophoresis.

E. ARDRA analysis The high frequency endonucleases Afa I + Msp I (Takara, China) were used to digest the 16S rDNA. A 20 ¦Ìl-aliquot holding 2 ¦Ìl ten-fold buffer, 2 ¦Ìl 0.1% BSA, 6 ¦Ìl of sterile deionized water, 8 ¦Ìl of amplification products, 1 ¦Ìl of Afa I and MspI, respectively, was incubated at 37 ° for 4 h. Endonuclease digestion was ceased by 2 ¦Ìl of ten-fold loading buffer. The DNA fragments were analyzed by 5% polyacrylamide gel electrophoresis. Electrophoresis maps were clustered using NTSYS-PC2.10e software. Richness indices (dMa), Shannon-Wiener indices(H'), Simpson indices(D) and Evenness(E) indices of bacterial communities of library ¢ñand ¢ò were calculated by the following formulas[8][9].

Formula

$$H' = -\sum_{i=1}^{s} p_i \ln p_i,\ p_i = \frac{n_i}{N}$$

$$D = 1 - \sum_{i=1}^{s} p_i 2$$

$$d_{Ma} = \frac{S-1}{\ln N}$$

$$E = \frac{H'}{\ln S}$$

Where S, ni , N denoted the total type of RFLP of 16S rDNA, band i, sum of clones, respectively.

III. RESULTS AND DISCUSSION

3.1. Amplification and purification of 16S rDNA Amplification of 16S rDNA sequences of bacteria from chicken sample ¢ñand ¢ò resulted in 1500 bp fragments of DNA. This indicated that PCR products of the specific size could be analyzed further (Data not shown).

3.2 Construction of 16S rDNA libraries The 16S rDNA libraries of chicken sample ¢ñand ¢ò were built using ampicillin selection and blue-white spot double selection system. Partial positive clones from library ¢ñand ¢ò were picked randomly to test insertion of target sequence by colony PCR. Figs. 1 and 2 showed the amplification products of some positive clones from two libraries, which disclosed that the PCR products had the size of 1500 bp. High quality bands of the uniformity and brightness could be applied to the following endonuclease digestion.

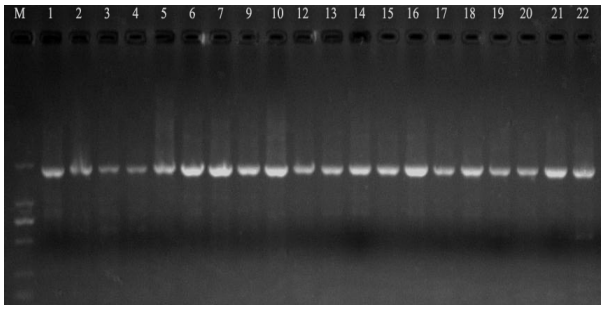

Fig.1 amplification products profiles of some positive clones of libraryl (note M: DL2000 Marker)

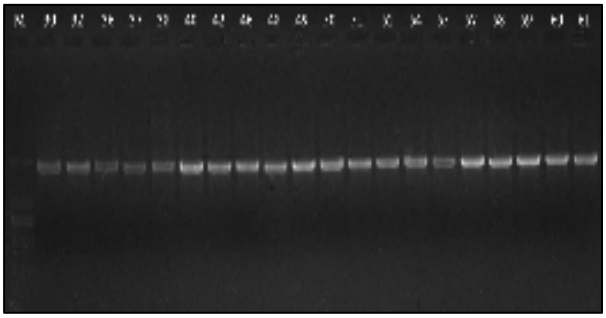

Fig.2 amplification products profiles of some positive clones of libraryⅡ(note M: DL2000 Marker)

3.3 ARDRA analysis PAGE fingerprint profiles of endonuclease digestion of partial clones from library ¢ñand ¢ò was displayed in Fig. 3 and 4. It was clear that four-eight digestion sites were included in the amplicons analyzed and the restriction fragments were of size of 100-800 bp. Besides, 47 and 55 distinctive bands were obtained from library ¢ñand ¢ò, respectively, corresponding to 44 and 65 OTUs, accounting 77.20% and 91.50%, in each library. The evidences fully demonstrated that bacteria diversities and community structures of 16S rDNA library of frozen chicken were complex.

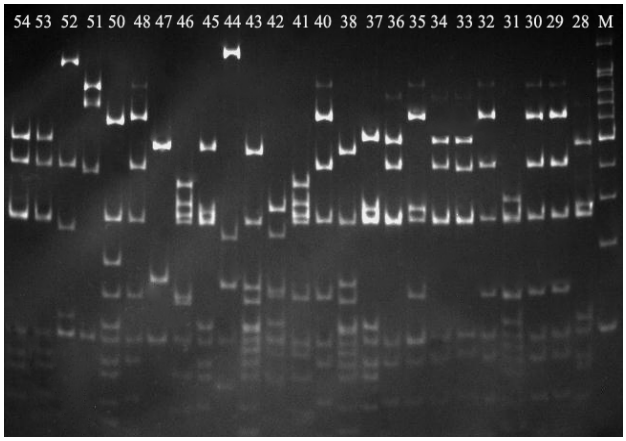

Fig. 3 PAGE fingerprint profiles of endonuclease digestion of some clones from libraryⅠ (note M:100bp DNA Ladder)

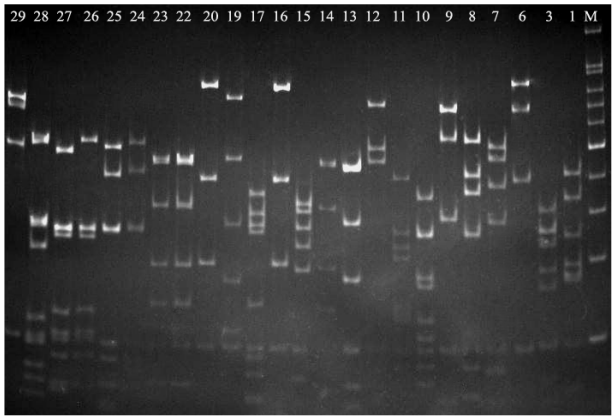

Fig. 4 PAGE fingerprint profiles of endonuclease digestion of some clones from libraryⅡ
(note M:100 bp DNA Ladder)

Some Gram positive bacteria like Listeria monocytogenes, Gram negative bacteria such as Pseudomonas sp. and facultative anaerobes, including lactic acid bacteria, Brochothrix thermosphacta and Enterobacteriaceae, can survive and grow at low temperature. They have the potential to become dominant bacteria and construct stable community structure[4][10][11]. As a consequence, the microbial diversity of meat and meat products can be high. Endonuclease maps and OTUs acquired from libraries in present study also indicated that bacterial diversity of chilled chicken was high. Furthermore, in current study, double digestion with Afa I and Msp I was coupled with 5% polyacrylamide gel electrophoresis, while numerous previous studies, as well as our preliminary one, commonly used single-enzyme digestion plus agarose gel electrophoresis. Our electrophoregram demonstrated that double digestion coupled with polyacrylamide gel electrophoresis could significantly improve the sensitivity and accuracy of the electrophoretic bands (data were not shown), thereby it could enhance the resolution to the microbial diversity of samples. Bacterial diversity of disused thermal vents in Tibetan Yangbajing Region was analyzed and OTUs of library Sediment A, Water sample A and Sediment B accounting 29.5%, 30.1%, 29.2% were obtained, respectively[12]. OTUs, taking up 6.2%, 12.2%, 25.2%, were acquired in the study of microbial diversity of marine sediment of Beidaihe[13].

3.4 Cluster analysis based on 16S rDNA-PCR patterns
According to PAGE fingerprint profiles, 0¨M1 matrix was built, and positive clones from both libraries were used for UPMGA cluster analysis. Fig. 5 and 6 showed

the NTSYS dendrogram based on 16S rDNA-PCR patterns of positive clones of two library. Clones from library ¢ñand ¢òwere grouped into two clusters, similarity indices of which were 0.79 and 0.80, respectively. It is worth of noticing that clone 49 in library ¢ò was clustered individually.

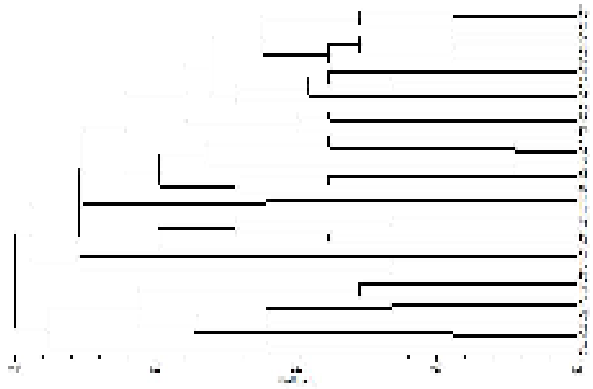

Fig.5 NTSYS dendrogram based on 16S rDNA-PCR patterns of some positive clones of libraryⅠ

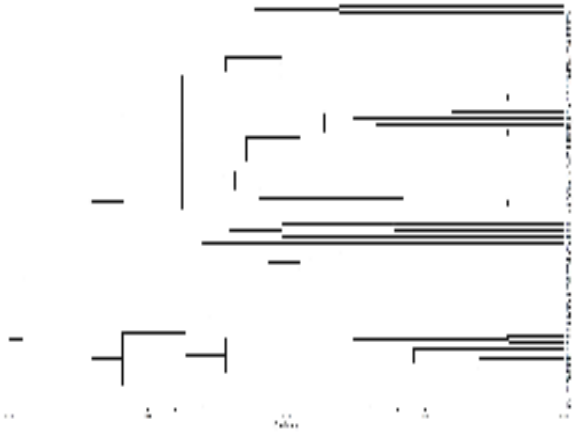

Fig.6 NTSYS dendrogram based on 16S rDNA-PCR patterns of partial positive clones of libraryⅡ

Species diversity was estimated from species presences (species richness, S), and also taking into account quantitative information[14]. Richness index, Shannon-Wiener index, Simpson index and Evenness index of bacterial communities of library ¢ñ and ¢ò were 13.36, 3.64, 0.97, 0.91 and 16.19, 4.15, 0.98, 0.98, respectively, suggesting that bacterial diversity of library ¢ò was higher than that of library ¢ñ. It indicated that bacterial diversity reduced and similarity index increased with the extension of storage time. This was in agreement with the previous study in which the similarity index was observed to increase and the diversity to decrease in chilled pork during storage[4].

IV. CONCLUSION

In this study, the dynamics of bacterial diversity in frozen chicken was investigated by 16S rDNA-ARDRA with the coupling of double endonuclease digestion and polyacrylamide gel electrophoresis, and more comprehensive community diversity was disclosed compared with previous studies. ARDRA technique is demonstrated as an effective tool to detect bacterial diversity and community dynamics of frozen chicken during storage.

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