PE8.11 Changes of bacterial diversity in Chinese bone and chicken strings during storage using T-RFLP analysis 77.00

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Abstract: Changes of bacterial diversity in Chinese bone and chicken strings during storage were analyzed using T-RFLP analysis. The results showed that bacterial diversity varied with different brands and storage time. Changes caused by factories or brands were much more than storage time. Similar predominant bacteria were found in all samples. The diversity probably got richer with storage time extending.

Keywords Bone and chicken string, 16SrDNA, Terminal restriction fragment length polymorphism (T-RFLP), Bacterial diversity, Terminal restriction fragment (T-RF)

I. INTRODUCTION

"Bone and chicken string" is one of the famous prepared chicken products with a large production and consumption in the People's Republic of China. However, it's a kind of fresh poultry food and easy to be contaminated. The microbial diversity and it's variation during storage are the major factors to the shelf life. Much have been done about that according to the traditional cultivation methods [2]. But only no more than 3% of total bacterial population can be cultured. Terminal restriction fragment length polymorphism (T-RFLP) analysis has been proven to be a consistent, high-resolution, and high-throughput cultivation-independent technique to monitor microbial community structures. Terminal restriction fragment (T-RF) patterns are generated from isolated DNA, targeting the conserved 16S rDNA gene [1]. In the article, all samples were analyzed with T-RFLP in order to determine the diversity of the bacterial community.

II. MATERIALS AND METHODS

Bone and chicken strings were obtained with intact package from supermarkets, which were produced in three factories (A, B, C) in Shandong province in east of the People's Republic of China. 250 grams of each brand products were taken out from more than three packages and tray-packaged aseptically immediately. All the samples were stored at 4 **°C** for further 4, 6 days respectively for analysis in the follows.

25 gram samples were taken aseptically from each package after 4, 6 days and were diluted with 225 ml of peptone salt solution (0.85% NaCl - 0.1% peptone) , then homogenized with a Stomacher (Bagmixer 400, France) for 1 min. Twenty milliliters of dilution was centrifuged for 2 min at 14,000*g* and the sediment was used for further analysis. Bacterial DNA was extracted using GenElute Kit (Bacterial Genomic DNA Kit, Sigma, USA) according to the manufacturer's instructions, and then dissolved in 200 µl of elute solution[4,5]. All the DNA samples were purified using DNA purification kit (Solarbio, China). DNA solution was estimated by 1.0% agarose gel.

T-RFLP analysis targeting 16S rDNA was performed using the universal bacterial primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') (5' FAM-labelled) and 1492r (5'-GGTTACCTTGTTACGACTT-3') (unlabelled) [3]. Amplification reactions were done by PCR in a 50 µl reaction volume, containing 2µl template DNA and using dNTP(Toyobo, Japan), 1 µl of each primer, and 0.5µl Taq DNA polymerase(Toyobo, Japan). Samples were predenatured at 95 **°C** for 5 min and then subjected to 29 cycles of 60s at 94 **°C**, 30 s at 55 **°C**, and 90 s at 72 **°C**, with a final extension at 72 **°C** for 10 min. Amplicons from three replicate PCR reactions were pooled for each sample and purified with a DNA purification kit (Solarbio, China). About 200 ng of PCR product were digested for 2.5h at 37**°C** in a total volume of 20.0 µl with *Hha*I (Takara, Japan) and then deactivated at 65 **°C** for 20min. Restriction fragments were sent to Shanghai GeneCore BioTechnologies Co.,Ltd in China for test.

The similarity of communities could be estimated by visual comparison and by identification with T-RFLP analysis program. One terminal restriction fragment (T-RF) was an operational taxonomic unit (OTU). Shannon diversity H' and shannon diversity E' were analyzed using BIO-DAP program (http://nhsbig.inhs.uiuc.edu/ wes /populations. html)[6].

III. RESULTS AND DISCUSSION

The dynamics of the number of unique T-RFs are shown in Fig1 and Fig2. The similarity and difference of communities could be estimated by visual comparison. About 7~16 unique T-RFs (OTU) were identified for each sample and there were some differences among them. However, in all samples, most T-RF sizes focused on 200-300bp and 550-600bp. And there were several equal size unique T-RFs in the six samples. The bacterial diversity of samples produced in the same factory with different storage time was much closer than those produced in different factories. Also, with storage time extending, some peaks became stronger, while some became weaker or disappeared. That indicated that bacterial diversity varied with factories and storage time. Changes caused by factories were larger than that caused by storage time. Predominant bacteria existed great similarities in six samples, which would be the key factor for producers to control. Further study, such as application of clone libraries, is needed to define the predominant species.

Shannon diversity H` and shannon diversity E are showed in Table1. They were also different with different producers and storage time. The value of H` on 6 day is higher than that on 4 day except group C. So, the bacterial diversity probably got richer as storage time going. No significant difference was found among E value. That indicated that there was no significant difference on species abundance.

Table1. Analysis of microbial diversity based on T-RFLP profiles

Samples	A-4d	B-4d	C-4d	A-6d	B-6d	C-6d
OTU	12	9	16	15	12	7
H	2.13	1.86	2.23	2.27	2.31	1.43
E	0.86	0.85	0.81	0.84	0.93	0.73

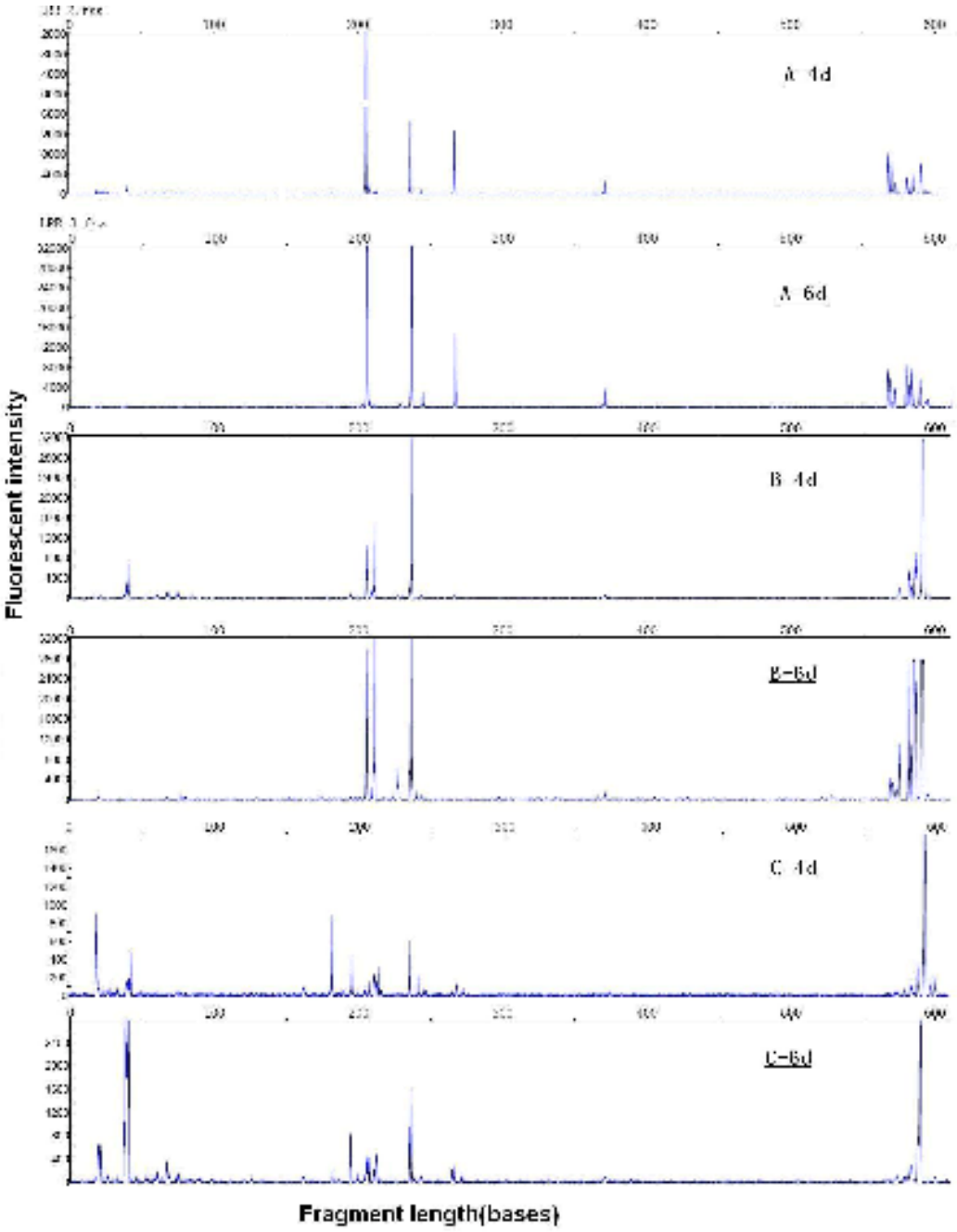

Fig2. T-RFLP profiles of *HhaI*-digested 16S rDNA genes amplified from the bacteria of samples from different factories stored for 4 days and 6 days.

IV. CONCLUSION

Bacterial diversity in Chinese bone and chicken strings during storage varied with different brands and storage time. Changes caused by factories or brands were much more than storage time. Products from different factories have different bacterial community, and the diversity probably gets richer with storage time extending. But, predominant bacteria existed great similarities in all samples. So, producers can control the bacterial community of products by control predominant bacteria.

REFERENCES:

[1] Annelies Justè, Bart Lievens, Michael Klingeberg, Chris W. Michiels, Terence L. Marsh and Kris A. Willems(2008), Predominance of Tetragenococcus halophilus as the cause of sugar thick juice degradation. Food Microbiology, 25(2), 413-421.

[2] Jay, J.M., Vilai, J.P., & Hughes, M.E.(2003). Profile and activity of the bacterial biota of ground beef held from freshness to spoilage at 5-7**°C**. Food Microbiology, 81,105-111.

[3] Justè, A., Thomma, B.P.H.J., Lievens, B.(2008). Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiology, 25 (6), 745-761.

[4] Li, M.Y., Zhou, G.H., Xu, X.L. & Zhu, W.Y.(2006). Changes of bacterial diversity and main flora in chilled pork during storage using PCR-DGGE. Food Microbiology, 23(7), 607-611.

[5] Russo, F., Ercolini,D., Mauriello, G. and Villani, F.(2006). Behaviour of Brochothrix thermosphacta in presence of other meat spoilage microbial groups. Food Microbiology, 23(8), 797-802.

[6] Yuan, S. Q., Xue, Y. F., Wang, W. D., Ma, Y. H.(2007). Microbial diversity in Shengli petroleum reservoirs analyzed by T-RFLP. Acta Microbiologica Sinic of China, 47 (2) :290-294.