ANALYSIS OF MICROBIAL COMMUNITIES FROM DIFFERENT JINHUA HAM FACTORIES

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Abstract — Microbes in different aged workshops could play important roles in the flavor formation of Jinhua ham. However, microbial diversity, community structure and age related changes in different workshops are poorly understood. The microbial community structure and diversity in Jinhua ham samples produced in different factories (aged 5, 15 and 30 years) were investigated using the pyrosequencing technique. Results showed that 571,703 high-quality sequences were obtained and located in 242 genera belonging to eighteen phyla. Bacterial diversity decreased with workshop age and microbial community structure was significantly different among three workshops. Three-phase model to characterize the changes of ham microbial communities was observed. GC-MS assays indicated that the hams produced in the old workshop contained the higher concentrations of aldehydes that are important for the ham flavor. This research provides scientific evidence that the higher concentrations of aldehydes are the main factor for the ham flavor formation Jinhua ham.

Key Words — Chinese dry-cured ham, flavor, microbial diversity

I. INTRODUCTION

Previous studies have demonstrated that the unique and broad diversity of flavors in ham is the result of complex reactions including lipid oxidation, maillard reactions and protein degradation. These reactions mainly depend on enzymatic action of microorganisms [1,2]. In the past two decades, the composition of microbial communities and main flora in ham has been widely investigated [3,4]. High quality of ham is attributed to the maturing process of workshop which results in a well-balanced microbial community structure and diversity in the ham to produce special flavors. However, there are few reports concerning the differences in the microbial community structure in the ham that produced in different manufacturing places.

Jinhua ham, a representative of traditional dry-cured meat product from Zhejiang Province in Eastern China, is considered as a high quality product with unique flavor as an outstanding quality parameter and major contribution to consumer acceptance. In the present study, the flavor characteristics of Jinhua ham produced in different-aged workshops and the structure and diversity of the microbial community of these hams were investigated by gas chromatography-mass spectrometry (GC-MS) and the 16S rDNA gene pyrosequencing technique, respectively. The results were expected to provide insights into the intrinsic relations between microbial diversity in long-term batch fermentation workshop and the flavor formation mechanism of Jinhua ham.

II. MATERIALS AND METHODS

Jinhua hams were processed in three workshops following a same traditional technology[1] with same batch of green hams in Zhejiang Provincial Food Company. According to the history of these three workshops, they were distinguished as JN (30 years), JD (15 years) and JM (5 years). At the middle of ripening and the

end of post-ripening, hams were taken as samples for DNA extraction and volatile compounds analysis, respectively.

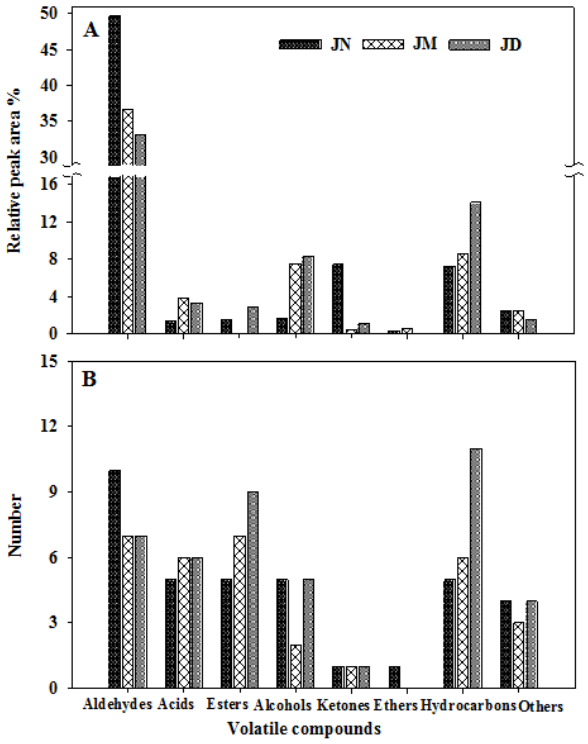
Volatiles compounds analysis was carried out with the method of Lorenzo[5]. The microbial DNA was extracted from the hams with PowerFood Microbial DNA Isolation kit (MO BIO Laboratories, Inc., USA). The V3 hypervariable region of the 16S rDNA was PCR amplified from the genomic DNA using universal primer. The temperature was set as follow: 98 °C:5 min; 25 cycles, 98 °C: 30 s; 58 °C:30 s; 72 °C:30 s; 72 °C:5 min, and V3 amplicon was sequenced by Illumina Miseq at Personal Biotechnology Co., Ltd (Shanghai, China) with the pair-end method.

After pyrosequencing, all readings were screened and filtered using QIIME 1.6.0 software. Operational taxonomic units (OTUs) were picked only if they had similar values of 97% or higher. All described analyses were performed using version 1.32.1 of MOTHUR software package[6]. Subsequent taxonomic affiliations were then obtained using RDP classifier (http://rdp.cme.msu.edu/).

III. RESULTS AND DISCUSSION

Results indicated that the most abundant chemical family in flavor of the final Jinhua ham was aldehydes (Fig. 1). Consistently, studies have shown the dominant volatile compounds in Bayonne, Jinhua, Corsican, Iberian, Parma and Serrano dry-cured hams were aldehydes [7,8]. Meanwhile, it is not surprising that the content of aldehydes increased over age of the workshop which had well-balanced bacteria community in the present study. Similarly, ketones were the more abundant in the ham produced in JN, whereas acids, esters, ethers and hydrocarbons were more abundant in JM and JD. Previous works have been carried out to study the changes in volatile aldehydes and ketones in dry-cured ham and showed that flavor formation by secondary metabolism of microorganisms, especially aminoacid catabolism in which methyl-branched aldehydes and methyl ketones were generated[9,10]. Above facts indicated that appetizing volatile flavor was mainly affected by aldehydes and ketones.

Figure 1. Volatile flavor compounds detected in Jinhua ham samples after post-ripening.

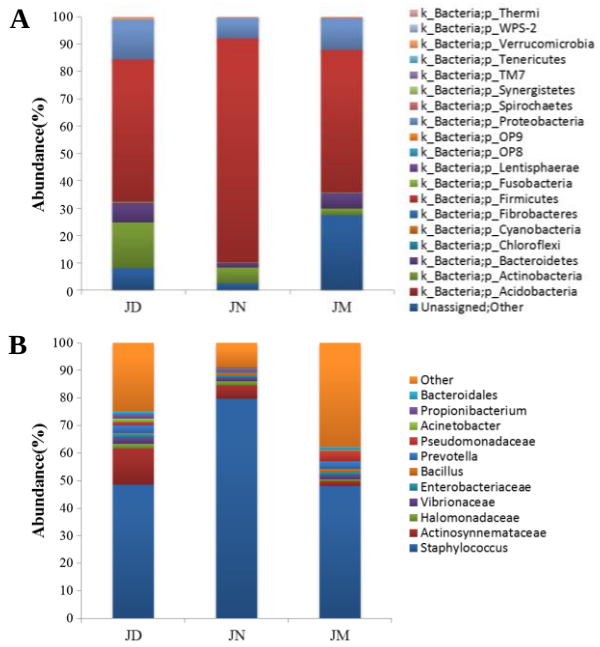


The entire pyrosequencing data set from the three samples contained 614,495 sequences. After filtering, 571,703 high-quality sequences remained with an average read length of 159 bp. The microbiota in Jinhua ham was constituted of nineteen phyla and the vast majority of sequences belong to four major phylas (Fig. 2A). The total bacterial sequences from the three ham samples located in 242 genera. To observe the changes in the ham closely, the 11 most abundant genera that appeared in all three samples were compared. The results showed that after a decade of fermentation acclimation, the distribution differences of bacteria were reduced (Fig. 2B).

Additionally, the clustering analysis led to the division of the 242 genera into 6 prominent categories (Fig. 3). The diversity of prokaryotes decreased with workshop age and sustained advantage and balance in the JN, while those in

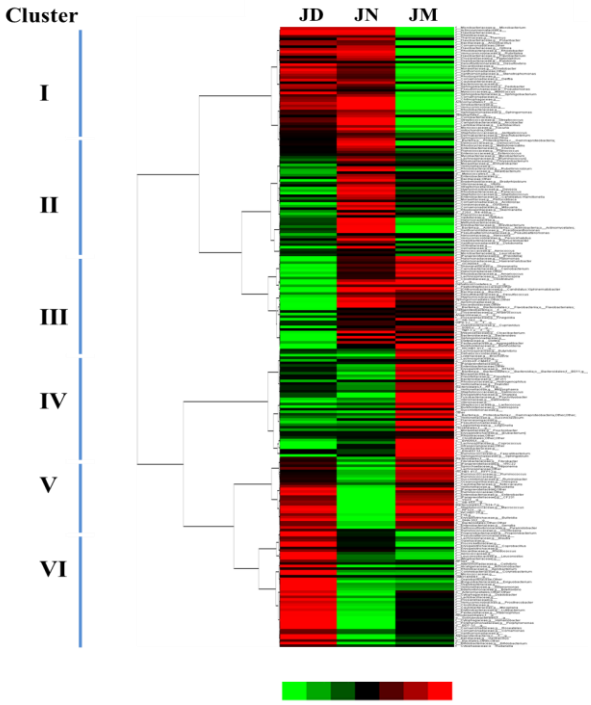
the JD were in the transition state.

Figure 2. Changes in abundance of bacterial phyla based on 16S rDNA sequencing. A: phyla-based; B: family-based. In the legend, “k” stands for kingdom and “p” for phyla.



Three distinct phases were separated by the changes of workshop microbial communities. Phase A was the initial period and it was high in diversity and species richness which likely resulted from biogeochemical environment similar to the surrounding air environment. At this phase, the abundance of genera belonging to clusters III, IV and V was higher (Fig. 3). Additionally, there were so many unassigned bacteria in phase A but decreased in phase B (Fig. 2). Phase B was a transitional period and its community structure dramatically changed and significant decreased in prokaryotic diversity. This could be due to the fact that the microbial community was optimized and adapted at very different environmental conditions (e.g., temperature and humidity) created by the Jinhua ham process in the workshop. The abundance of genera belonging to clusters I in Phase B was consistent with Phase C. Phase C was the relative mature period of the ham microbial community. Microbial diversity was stable in this phase and was significantly lower than in the young workshop.

Figure 3. Heat map of the genera in JD, JN and JM ham. The heat map plot depicts the relative percentage of each genus (variables clustering on the Y-axis) within each sample (X-axis clustering). The values of genera based on the log2 transformed relative abundance were performed using the Gene Cluster 3.0 software. The results were visualized using the JAVA TREEVIEW software. The relative values for the genera are depicted by color intensity.

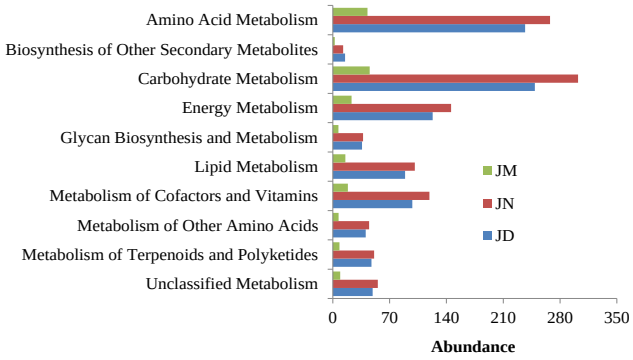


The specific microbial distribution in the ham may be resulted in periodic fermentation and enrichment for more than 30 years without interruption. Moreover, mutual collaborations and interactions among different bacteria species led to a well-balanced bacteria community in the biogeochemical environment resulting in ham flavor improvement.

In the present study, in order to effectively analyze the difference in the ham, OTUs involved in metabolism that appeared in Jinhua hams was compared. The abundance of amino acid metabolism-related and carbohydrate metabolism-related OTUs were significantly higher than others (Fig. 4). Specifically, the entire metabolism-related OTUs increased with the extension of age of the workshop indicating that the long-term batch fermenting benefits the

Jinhua ham flavor formation for well-balanced bacteria community with strong amino acid and carbohydrate metabolism.

Figure 4. Functional genes related to metabolism in Jinhua ham. The functions of OTUs were assigned according to KEGG.



IV. CONCLUSION

In the present study, GC-MS assays indicated that remarkable difference among different Jinhua ham concerning number of volatiles compounds and their relative contents. Meanwhile, the concentrations of aldehydes and ketones might play a pivotal role in increasing the appetizing volatile flavor of ham produced in the old factory. Bacterial diversity and OTUs involved in the metabolism were significantly different among three factories. Three-phase model were separated by the changes of ham factory microbial communities. Moreover, microflora became well-balanced in older factory for produce aromatic Jinhua ham. These results in this study could support the practical experience that old workshop produces Jinhua ham with better flavor.

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