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Bacterial community dynamics during the traditional brewing of *Wuyi* Hong Qu glutinous rice wine as determined by culture-independent methods

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ABSTRACT

Wuyi Hong Qu (black-skin-red-koji) glutinous rice wine, as one of the most typical representatives of Hong Qu glutinous rice wine in Fujian province of China, is brewed under non-sterile and uncontrolled fermentation condition based on empirical knowledge, causing uncontrollability of fermentation process and instability of the final quality. The objective of this study was to investigate the bacterial dynamics during the traditional fermentation of *Wuyi* Hong Qu glutinous rice wine using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) and 16S ribosomal RNA (rRNA) gene clone libraries analysis.

The DGGE profile indicated that the dominant bacterial species in the traditional wine fermentation starters were *Pediococcus pentosaceus*, *Pediococcus acidilactici* and *Bacillus* sp. (including *Bacillus aryabhattai* or *Bacillus megaterium* and *Bacillus amyloliquefaciens*). Bacterial dynamic obtained from the PCR-DGGE revealed the presence of *Bacillus* sp. and LAB (including *Lactobacillus plantarum* group, *Lactobacillus brevis*, *P. acidilactici* and *P. pentosaceus*) during the traditional fermentation process, but they varied in different brewing phases. The relative proportions of some bacterial species (such as *Bacillus* sp., *P. acidilactici*, *L. brevis* and *P. pentosaceus*) detected at early fermentation stage decreased as the fermentation progressed. While *L. plantarum* group was consistently detected with high light band intensity throughout the fermentation process. 16S rRNA gene clone libraries revealed that the two different molecular biological methods gave similar results, but clone library analysis was more representative of the bacterial community to some extent. For example, *Leuconostoc mesenteroides* was detected by 16S rRNA gene clone library but not discovered by bacterial DGGE profile throughout the whole fermentation process. Therefore, the combined approach of nested PCR-DGGE and 16S rRNA gene clone libraries would give a more comprehensive profile of the bacterial dynamics than either alone. Finally, species-specific multiplex PCR was also performed to confirm the *L. plantarum* group. Result showed that only *L. plantarum* can be detected from the total bacterial DNA extracted from samples of different fermentation phases.

This is the first report to reveal the dynamics of bacterial species involved in *Wuyi* Hong Qu glutinous rice wine brewing process using culture-independent methods. It might be useful to control wine production systems and improve wine quality.

1. Introduction

Chinese yellow rice wine, one of the three most famous brewed wines (yellow wine, grape wine and beer) in the world, plays an important role in Chinese culture and people's daily life (Wang & Yi, 2001). Based on the raw materials and wine starters used, Chinese yellow rice wine can be categorized into three major groups: Hong Qu glutinous rice wine (*Monascus purpureus* rice wine produced mainly in Fujian province), wheat-yeast rice wine (represented by Shaoxing rice wine produced in Zhejiang province) and millet yellow wine in north China (represented by Jimo old wine produced in Shandong province). Hong Qu glutinous rice wine, brewed from glutinous rice with the addition of two traditional wine fermentation starters — Hong Qu (hóng qū) (also called Chinese red yeast rice) and Yao Qu (yào qū), is a typical representatives of Chinese traditional fermented

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foods and widely consumed in southeast of China and even Southeast Asia.

Wuyi Hong Qu (black-skin-red-koji), as one of the most typical representatives of Hong Qu, is a mixed culture of *Monascus* sp. with *Aspergillus niger*, and usually used for rice wine brewing in Fujian province of China. *Wuyi* Hong Qu glutinous rice wine is brewed under non-sterile and uncontrolled fermentation conditions based on empirical knowledge, causing uncontrollability of fermentation process and instability of the wine quality. Thus, it is important to monitor the microbial dynamic changes during traditional fermentation processes in order to provide both high quality and safe products for consumers.

Traditionally, rice wine is brewed from glutinous rice with the addition of wine fermentation starter. Rice wine starters were considered to contain various types of microorganisms such as filamentous fungi, yeasts, and bacteria. A few studies have been conducted to describe the microbial diversity of rice wine fermentation starters (Jeyaram, Mohendro Singh, Capece, & Romano, 2008; Lv, Huang, Zhang, Rao, & Ni, 2012; Lv, Weng, Zhang, Rao, & Ni, 2012; Thanh, Maile, & Tuan, 2008) and the microorganisms that are responsible for the rice wine fermentation (Han, Lei, Li, Lu, & Zhao, 2009; Li et al., 2011; Zhang, Chang, & Zhong, 2008). These studies helped us to understand how a fermentation product was made and the relationship among the microbial diversity, dynamic changes and special characteristics. Of which, bacteria play a prominent role in determining the chemical composition of wine and their effects on flavor development are of primary importance (Li et al., 2011; Li & Ren, 2005; Sumby, Grbin, & Jiranek, 2010; Wee, Reddy, & Ryu, 2008; Zheng et al., 2012). In general, bacteria can be divided into two groups: functional bacteria (strains that produce enzymes, prebiotics and some typical flavor compounds) and spoilage bacteria or pathogenic bacteria (Dung, Rombouts, & Nout, 2007; Xie et al., 2007). The study on bacterial flora of rice wine fermentation has gained interest since they contribute to the overall quality of the fermented product. Besides, spoilage bacteria and pathogenic bacteria would be detected so as to avoid damaging from these harmful bacteria, keep stable quality of rice wine and decrease unfavorable effect on the health of consumers. So knowledge of the bacterial composition during rice wine traditional fermentation is a prerequisite for the development of defined starter cultures and the control of wine brewing.

Culture-independent methods based on molecular biology techniques (Prieto, Jara, Mas, & Romero, 2007) have advanced significantly and provided a rapid, high-resolution description of microbial communities by targeting ribosomal genes. Among these techniques, PCR-DGGE as a culture-independent method has been widely applied to study microbial diversity both quickly and economically (Abe et al., 2008; Kittelmann & Janssen, 2011; Nielsen et al., 2007). It would speed up the development of starter cultures, and the identification of major microorganisms that play major roles in flavor development or result in the spoilage of traditional fermented foods. The construction of ribosomal RNA gene clone libraries, which is a powerful approach to identify community members and measure relative abundance (Filteau, Lagacé, LaPointe, & Roy, 2010; Kittelmann & Janssen, 2011; Koyanagi, Sakamoto, Takeuchi, Ohkuma, & Izumi, 2010), has been frequently used to analyze the species composition of fermented samples. The microbial variations in several types of food, such as kimchi (Chang et al., 2008), traditional fermented mustard (Chao, Wu, Watanabe, & Tsai, 2009) and Vietnamese alcohol starter (Thanh et al., 2008), have recently been examined using rRNA gene sequences analysis. However, only a small number of microbiological studies have performed to examine the process of traditional rice wine fermentation until now (Han et al., 2009; Wang, Cheng, Zhang, & Lin, 2008). To date, culture-independent approach has not been employed to investigate the bacterial dynamics during the traditional brewing of *Wuyi* Hong Qu glutinous rice wine yet.

The purpose of the present study was to investigate the structure and dynamic changes of the bacterial community during traditional fermentation process of *Wuyi* Hong Qu glutinous rice wine (Fig. 1). Nested PCR-DGGE was firstly applied to investigate the bacterial community existing in the fermentation process. Meanwhile, three 16S rRNA gene clone libraries were constructed to relative quantitatively study the bacterial structure.

2. Materials and methods

2.1. The traditional brewing of Hong Qu glutinous rice wine

Wine fermentation starters [*Wuyi* Hong Qu (black-skin-red-koji) and Yao Qu] used in this study were purchased from the wine factories in Fujian province of China. A flowchart describing the Hong Qu glutinous rice wine process is outlined in Fig. 1. To initiate the brewing, 2.0 kg of glutinous rice was washed and soaked in water for 4–8 h at room temperature, and then steamed for 1 h at 100 °C. After the steamed rice cooled to room temperature, it was mixed with the fermentation starter powder (200 g, of which Yao Qu 100 g and *Wuyi* Hong Qu 100 g) and transferred together to a traditional Chinese wine jar. Finally, sterilized water was added up to 3 L and started the wine fermentation at 15–20 °C for 46 days. The traditional brewing using the same starters (*Wuyi* Hong Qu and Yao Qu) was conducted through two independent experiments.

2.2. Sample collection

10 g of wine mash samples from each fermentation jar were aseptically collected at different phases of wine fermentation (1, 2, 3, 5, 7, 10, 20 and 46 days), transferred to sterilized bottles and thoroughly mixed, and finally stored at −20 °C before further analysis.

2.3. Total DNA extraction

Before extraction, the wine mash samples were washed with sterile water twice to eliminate the influences of substances such as ethanol. Total bacterial DNA was extracted from samples at different times of fermentation using Universal Genomic DNA Extraction Kit Ver: 3.0 (Takara, Dalian, China) after glass bead

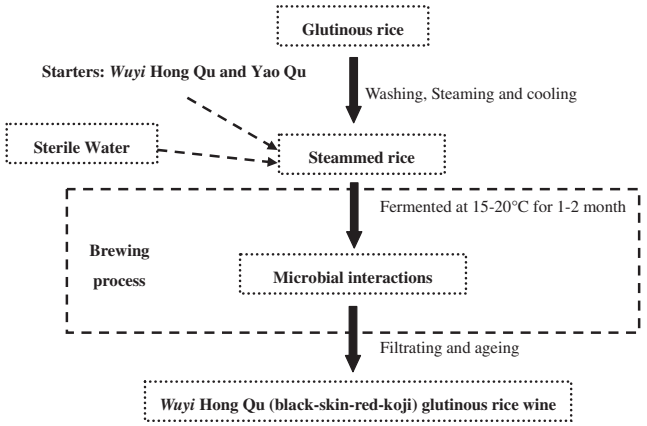

Fig. 1. Flow-chart for the traditional brewing of *Wuyi* Hong Qu (black-skin-red-koji) glutinous rice wine.