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Isolation and identification of histamine-producing Enterobacteriaceae from Qu fermentation starter for Chinese rice wine brewing

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ABSTRACT

Histamine (HIS) producers in fermented wines are generally believed to be lactic acid bacteria (LAB), and other microorganisms have received little or no attention. In this work, HIS-producing bacteria were isolated from Qu fermentation starter for Chinese rice wine brewing by decarboxylase medium, and their identity was confirmed by RP-HPLC and PCR. Surprisingly, the histidine decarboxylase gene (*hdc*) was present in only 2 out of 26 isolates. All 26 isolates were genotyped using the randomly amplified polymorphic DNA (RAPD)-PCR assay, which revealed the presence of 21 biotypes. Single type isolates were identified via 16S rRNA sequence analysis, in some cases coupled with partial sequencing of the *rpoB* or *dnaJ* gene. All isolates belonged to the Enterobacteriaceae, and included *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Citrobacter amalonaticus* and *Cronobacter sakazakii*. All these strains were capable of producing > 3.5 mg/L of HIS in TS medium without ethanol, but did not grow in TS medium with 8% ethanol. Small-scale Chinese rice wine fermentation revealed that HIS contents exhibited the same trend as the LAB and ethanol no matter what kinds of Qu were used. However, in the early stages of fermentation (from day 2 to day 4), the HIS contents had a stronger correlation with Enterobacteriaceae (0.943) than with LAB (0.369) when the Qu fermented samples are analyzed as a whole. Moreover, the lowest HIS content was measured in Xiao Qu (Q) fermented sample at the end of fermentation, which suggests that the formation of HIS in the early stages of fermentation has a decisive effect on HIS content in the final product. Our results demonstrate that Enterobacteriaceae from Qu are an important cause for HIS formation in Chinese rice wine. Consequently, selecting Qu with a low content of Enterobacteriaceae contaminants and inhibiting the growth of Enterobacteriaceae in the early stages of fermentation are useful approaches for preventing excessive amounts of HIS formation in Chinese rice wine brewing.

1. Introduction

Chinese rice wine is one of the most ancient alcoholic beverages in the world, along with grape wine and beer, and it plays an important role in Chinese history, cultural heritage and people's daily life (Wang and Yi, 2001; Zhou, 1996). The brewing process of Chinese rice wine (Fig. 1) is mainly divided into two parts: the first step encompasses the pretreatment of the raw materials, which includes rice soaking and steaming; the second step is the fermentation process, which refers to the fermentation of steamed rice using Qu fermentation starter and yeast. Usually, steeping the rice takes 3–5 days, and the fermentation requires 20–25 days. After fermentation, the rice wine mash is filtered through a press and the filtered clear liquid is heated with steam, yielding the fresh rice wine. Usually, the fresh rice wine is aged for more than one year in sealed pottery jars (Mo et al., 2010; Zhou, 1996).

Chinese rice wine has been popular in China for thousands of years due to its unique aroma and flavor, as well as high medicinal value and nutritional benefits (Yu et al., 2012; Zhou, 1996). However, in recent years, high levels of biogenic amines (BAs) have been found in some Chinese rice wines, and their safety has been questioned (Zhang et al., 2017). The most common BAs found in Chinese rice wine are histamine (HIS), tyramine, cadaverine, phenylethylamine, spermidine and putrescine (Lu et al., 2007; Zhong et al., 2012). Among these, HIS has the highest toxicity (Dadáková et al., 2009). Although there are no regulations governing the HIS content in alcoholic beverages, 2–10 mg/L of HIS have been suggested as limits recommended by some countries in order to minimize HIS toxicological effects in wines (Soufleros et al., 2007).

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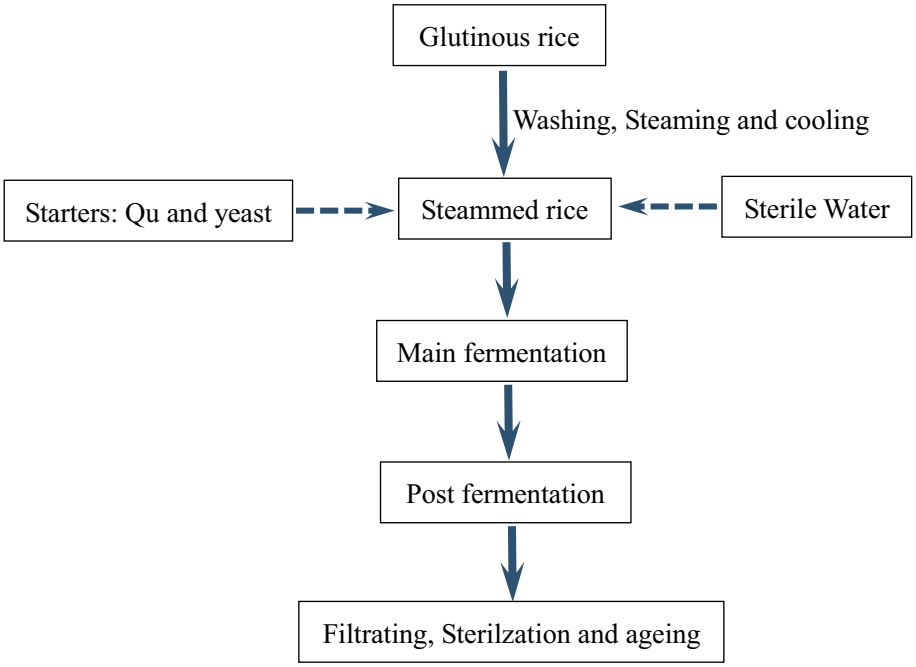

Fig. 1. Schematic outline of the manufacturing process of traditional Chinese rice wine.

HIS stems from the decarboxylation of the natural amino acid histidine catalyzed by a specific enzyme - the histidine decarboxylase (HDC). Two distinct classes of HDC have been documented. The first class, found in eukaryotes and Gram-negative bacteria, requires pyridoxal phosphate as a cofactor, and the other class from Gram-positive bacteria uses a covalently bound pyruvoyl moiety as a prosthetic group (Landete et al., 2008). In wine production, it is usually considered that HIS is produced by Gram-positive lactic acid bacteria (LAB), which are capable of proliferating in the harsh wine environment, i.e. low pH (ca. 3.5), high alcohol content (14% *v*/v), high concentration of SO_2 (50–80 mg/L) and low temperature (18–20 °C) (Versari et al., 1999). In other fermented beverages, such as cider, LAB have also been reported to be responsible for HIS production (Garai et al., 2007).

The brewing process of Chinese rice wine is different from that of fruit wines or beer, and is to some extent an open and spontaneous microbiological process (Lv et al., 2016). In the saccharification stage, the Qu fermentation starter - a mixture of microorganisms and enzymes, is mixed with the steamed rice under open, nonsterile conditions (Zhou, 1996). While the amylase- and protease-producing fungi in Qu can grow better under these conditions, some facultatively anaerobic bacteria can also grow and reproduce, and these bacteria can cause concerns surrounding the accumulation of HIS in Chinese rice wines. However, bacteria other than LAB that can potentially also be HIS-producers in Chinese rice wine have received little or no attention.

Therefore, the purpose of this study was to investigate whether there are other bacteria besides LAB that are responsible for HIS formation in Chinese rice wine. To this end, suspected HIS-producing strains were isolated from four kinds of Qu fermentation starter using the selective decarboxylase medium, after which their ability to produce HIS and the presence of the histidine decarboxylase gene (*hdc*) were respectively analyzed by RP-HPLC and PCR. All the HIS-producing strains were genotyped using the randomly amplified polymorphic DNA (RAPD)-PCR assay, and all single type isolates were identified via 16S rRNA sequence analysis, in some cases coupled with partial sequencing of the *rpoB* or *dnaJ* gene. Then, the survival of these strains in TS medium with different ethanol contents and their HIS production capacity were also evaluated. Finally, small-scale Chinese rice wine fermentation were performed with four kinds of Qu to evaluate the real contribution of Enterobacteriaceae to HIS formation. The knowledge we gained may contribute to a better understanding of HIS formation in Chinese rice wine, and we propose Qu as a potential target for the implementation of a control strategy to prevent spoilage and HIS production.

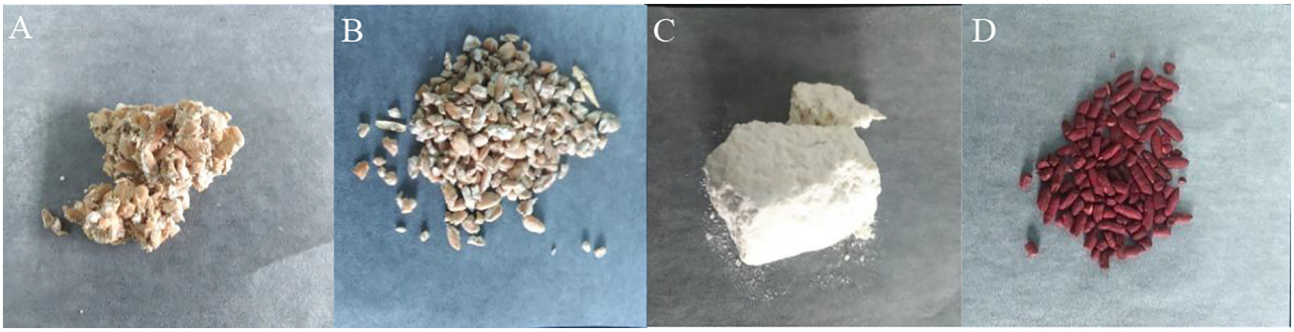

Fig. 2. Four samples of Chinese rice wine Qu fermentation starter. (A) M. (B) L. (C) Q. (D) H.