Isolation of Yeasts from Grapes for Rice Wine Starter Culture Preparation

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ABSTRACT Rice wine is an alcoholic drink produced by fermentation of glutinous rice. It is a famous traditional drink in East Malaysia. The starter culture origins, which consisted of a yeast mixture determines the wine taste and alcohol content percentage. Most of the rice wine producers relied on the yeast starter culture sold in the market or from the leftover stock from the previous rice wine preparation which may not have proper quality control. Very often the content and composition of microbes in the yeast starter culture are unknown. Hence, rice wine produced is not consistent. Sometimes it is tasty and sweet, and sometimes it is sour, and this can be an issue if one wishes to market it as a product. Therefore, there is a need to formulate good quality yeast starter cultures to address the issues of product consistency and product quality. There is a long history of using grape yeasts to ferment grapes for wine production. Nevertheless, information on the fermentation of glutinous rice or starch using grape yeasts is sparse. Hence, the objectives of this project were to isolate yeast present during the grape must spontaneous fermentation, for the formulation of starter cultures. Different types of growth medium such as Yeast Potato Dextrose (YPD) and potato dextrose agar media were used to isolate the yeast. Fifteen yeast isolates, GY1 to GY15 were successfully isolated and purified. The fifteen isolates were combined and freeze-dried to form the starter culture for batch fermentation of glutinous rice. The colony-forming units (cfu) of the starter culture were 1 x10^5 which formed a good starter culture.

KEYWORDS: Rice wine, yeast, grapes, starter culture, rice.
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INTRODUCTION

In Asia, many countries produce rice wine such as Japan (Sake), China (Jiu), and Malaysia (Tuak and Tapai). In Japan, the tradition of making rice wine is based on the starter culture used in saccharification, in which sugar (glucose) is converted to ethanol in submerged fermentation by yeast (Ueda & Teramoto, 1995). In China, the rice wine from fully fermented and filtered rice or millet beverage has an alcoholic content of 10-15% (w/w). They used fungi to carry out special saccharification of rice (Chen *et al.*, 1999; McGovern *et al.*, 2004). Meanwhile, in Malaysia rice wine is produced by fermentation of glutinous rice. It is a famous traditional drink especially during the harvest festival celebration among the local ethnic groups in East Malaysia (Chiang *et al.*, 2006). Rice wines are mainly homemade by communities from various regions. Some are made at an industrial scale by smallholders and each rice wine made has its unique taste and aromas. The source of the starter culture (sasad) is important as it determines the taste, aroma, and alcohol contents of the end-product. Starter culture consisted mainly of yeasts (Chiang *et al.*, 2006). Most traditional rice wine producers purchased starter cultures from the market or used starter cultures from a previous rice wine fermentation. These starter cultures may not always have proper quality controls. Sometimes some of the starter cultures are contaminated by other organisms. Hence, the quality of rice wine produced is not consistent. The rice wine quality produced in using such a starter culture is not consistent and may taste sweet or sour. This can be an issue if one wishes to market it as a product. Occasionally, it may contain a high amount of hazardous alcohol such as methanol (Ohimain, 2016). This occurs due to several reasons and one main concern is the starter culture that is contaminated by microbes that produce methanol as one of the end products. Therefore, there is a need to look for

a good quality starter culture and analyze it as there is an urgency to address the two issues of product consistency and product quality.

In western countries, winemaking from grapes fermentation also has a long history. Grape instead of rice is used as the source of sugars and carbohydrates for fermentation. Many of the yeasts used as starter cultures for the fermentation of grapes originated from those present naturally on the skin of grapes. Yeast, *Saccharomyces cerevisiae* which is the main agent in alcoholic fermentation and grows in low populations and fermenting in grapes cluster as a winery (Martini, 1993). The wine produced from properly prepared grape yeasts is upgraded to industrial scales for a long time. Interestingly, there is very little information concerning the use of yeasts from grape skin as a starter culture for glutinous rice fermentation to make tapai or tuak. This is something very interesting to investigate, that is on whether the grape yeast will be able to produce good quality rice wine. Therefore, this project was initiated as the first step to prepare a starter culture from grape yeast for that purpose. The objectives of this project are: (1) to isolate yeasts from spontaneously fermented grapes, (2) To use them to prepare the starter culture for rice wine fermentation.

METHODOLOGY

Isolation of yeast

Imported red and green grapes were purchase from local supermarkets from around Kota Kinabalu, Sabah, and used for yeast isolation. The grapes were crushed inside a sterilized plastic bag, and the grape must be allowed to spontaneously ferment for two weeks. The grape must be serially diluted up to 10^{-6} and used for yeast isolation using the Yeast Potato Dextrose (YPD) and potato dextrose agar media. The agar plates were incubated at 30°C.

Yeast Identification

The yeast cells were morphologically visualized under an Olympus light microscope under a 100X magnification.

Freeze-drying of Yeast Starter Culture

The fifteen yeast isolates were grown to mid-log phase in 50 ml YPD medium. Ten ml of each yeast culture were mixed in a 250 ml sterilized Erlenmeyer flask. The freeze-drying was performed according to the procedures described by Gadd *et al.* (1987).

RESULTS AND DISCUSSION

Fifteen isolates were purified, and they are designated as GY1 to GY15. They grew well at 30°C in YPD medium and formed creamy white colonies (Figure 1). Their morphologies under the light microscope are consistent with those of the *S. cerevisiae.* The results show the natural diversity of grape yeasts consisted mainly of *S. cerevisiae or Saccharomyces*-like yeasts. This is in agreement with the findings by Romano *et al.* (2003).

The fifteen *S. cerevisiae* from grape must fermentation were successfully used to prepare a starter culture that is suitable for the fermentation of glutinous rice to make wine. The freeze-dried yeasts were viable and had cfu of 1×10^5 and it was free from other microorganisms. They are ready to be mixed with glutinous rice for the fermentation process. Besides, these yeasts may be sporulated, and the tetrad is dissected and separated into monosporic clones. Each spore can be tested on the wine fermentation process and the resulted wine may have a variety of tastes and aromas (Romano *et al.*,

2003). Since *S. cerevisiae* has made a significant contribution to the grape wine industry (Bartle *et. al.*, 2019), it is likely that the grape yeasts will also produce good quality wine from the fermentation of glutinous rice. This is because, yeasts from grapes are reported to be a significant contributor to the flavor of wine and are used extensively in industrial-scale winemaking (Romano *et al.*, 2003; Bartle *et al.*, 2019).

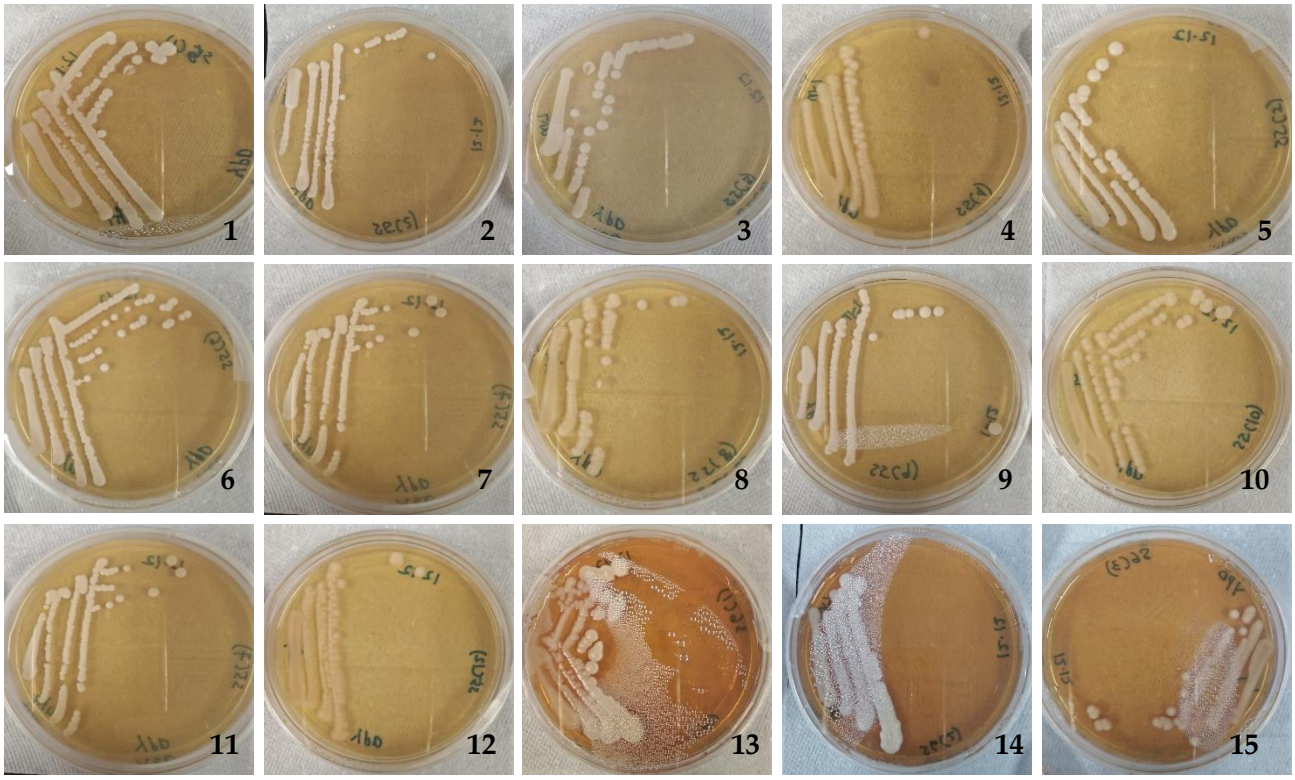

Figure 1. Fifteen yeast isolates from grape musts were isolated using YPB agar medium. They were GY1 to GY15 represented by 1 to 15 respectively.

CONCLUSION

The starter culture formulated in this work ensures that there were no other unwanted microorganisms or contaminants in the starter culture. In addition, *S. cerevisiae* is recognized as a GRAS (Generally Regarded as Safe) yeasts that are widely used in the food industries. Hence, the formulated starter culture fulfilled the safety requirements. This is a crucial step to produce a quality-controlled starter culture and to avoid the production of a hazardous substance such as methanol in the wine (Ohimain, 2016).

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