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Identification of Microorganism from Ragi for Bioethanol Production by API Kit

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Abstract: Ragi is one of the fermentation starters in food fermentation. Domestic ragi from Sarawak (for tapé and tuak) and Pahang (tapé), which are made from mixtures of rice floor, spices and water or sugarcane juice, naturally contains filamentous fungi, bacteria and yeasts. This research is aim to identify the microorganisms in the domestic ragi by using API kit after screening and isolating the microbes by selected media (YPD and Sabaroud). From the results, *Cryptococcus humicola* and *Candida glabrata* are identified in the Sarawak (tapé) sample, while *Cryptococcus humicola*, *Saccharomyces cerevisiae*, *Candida glabrata* and *Rhodotorula glutinis* were identified in the Sarawak (tuak) sample and for Pahang (tapé) sample, *Cryptococcus humicola*, *Saccharomyces cerevisiae* and *Candida guilliermondii* was identified by using both API kit (20C AUX and ID 32C).

Key words: API, identification, ragi, tapé

INTRODUCTION

Tapé is a traditional fermented food in Asia (Malaysia, Indonesia, Philippine and Vietnam) made from starch and ragi, the microbial starter. Ragi was made from a mixture from rice floor, spices and water or sugarcane juice and naturally contains filamentous fungi, bacteria and yeasts (Kuriyama *et al*., 1997). Ragi tapé is used as inoculums in a production method where liquefaction and alcoholic fermentation occur simultaneously without any effort to control the fermentation. Suitable yeasts play an important roles in ethanol production thus the research on yeasts identification selection are critical in bioethanol industry enhancement and improvement of other industries productivities. Even though the development of yeasts research is progressing, but the information of local yeasts strain are still limited in Malaysia. This research is aimed identify the specific strain of microorganism in ragi which commonly used for fermentation of food.

MATERIALS AND METHODS

Materials: For this study, domestic ragi is donated by Mr. Jong, N.S. The starter ragi is generally uses to make tuak, an alcoholic beverage brewed traditionally in Sarawak, Malaysia from fermentation with sago, banana and any local ingredients. Its will be started on the selected medium namely YPD and Sabaroud agar.

Tapé preparation: In this study, the procedure to made tapé is the same as the procedure for brem (Balinese rice wine) production. A mixture of black and white glutinous rice (100 and 300 g, respectively was washed and soaked in tap water for 4 h. After rinsing the mixed rice, it was steamed for 30 min until soft and sticky. Steamed rice was added with 400 mL hot water (about 95°C) then soaked for 30 min followed by another steaming for 30 min. After cooling, it was inoculated with 0.3% (w/w) powdered ragi (ragi tuak from Sarawak, ragi tapé from Pahang and ragi tapé from Sarawak). The inoculated rice (about 450 g) was incubated in a plastic container that has pores at the bottom to allow the liquid to drip and accumulate in the second container. After about 110 h of solid-state fermentation, the fermented rice was pressed to obtain the tape juice (liquid portion) using sterile nylon filter (Kuriyama *et al*., 1997).

Isolation of microorganism: Microorganisms are isolated from tapé juice and were grown on two types of media which is YPD and Sabaroud agar and in two conditions (aerobic and anaerobic). All these samples were incubated in 37°C for 48 h. After 48 h, the microorganism was inoculated on another new media until the single colony was defined.

Identification by API kit: In this study, the API 20C AUX and ID 32C systems were used for identification of microorganism. The API 20C AUX system (bioMérieux,

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Marcy l'Etoile, France), a commercial kit for the evaluation of the assimilation of selected multiple carbon sources. This test is conducted according to the manufacturer's instructions. About 135 µL samples from API medium displaced to each strips and incubate for 24 to 48 h in 29±2°C. Numerical profiles were constructed from the reaction patterns and were used to obtain identifications software program (Djien, 1972).

RESULTS AND DISCUSSION

From the experiment, for Sarawak (tapé) yeasts only define from anaerobic culture. This is because of the contaminant dominant the ragi (fermentation starter) and the ragi is the mix culture. From the study, aerobic sample show cream colony colour, have a convex shape and shining. For anaerobic sample, only one sample did not show any growing but grow with two colonies after 5 days fermentation. One of the colonies has a white in colour, smooth, dense and convex shape and the other is like mycelium in shape with furry like fungi. Under microscope 100X show an oval shape cell. Tested by using both API kit (ID 32C and 20C AUX), the result showed *Cryptococcus humicola*, *C. laurentii*, *C. albidus*, *Candida glabrata*, *C. guilliermondii*, *C. sake*, *C. silvicola*, *C. famata*, *C. albicans*, *C. pelliculosa*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Stephanoascus ciferii*, *Rhodotorula glutinis* and *Trichosporon mucoides* were defined from all samples. From the study, most of the samples of ragi contained of *Cryptococcus humicola*, *Candida glabrata*, *Saccharomyces cerevisiae* and *Stephanoascus ciferii*. But only sample from Sarawak (tuak) and Pahang (tapé) contained *Rhodotorula glutinis* and *Candida guilliermondii*, respectively.

Figure 1 to 3 showed the yeasts colonies from the different sources of ragi (fermentation starter) and yeasts microscopic under microscope (40 magnification). Samples from Sarawak (tapé and tuak), are not much different in microscopic which had an oval in shape but sample from Pahang (tapé) have a long shape.

From the results in Table 1, shows that the results give good identification by using API ID 32C and 20C AUX. Both API kit (20C AUX and ID 32C) can identified yeasts from all samples. The results showed that *Cryptococcus humicola* was identified from all samples by using API kit whether by using API 20C AUX or ID 32C. Ramani *et al.* (1998) make comparison the abilities of yeasts identification for 123 common and 120 of rare clinical yeast isolates by using API 20C and ID 32C proves that API 20C facilitated correct identification of 97% common and 88% rare isolates while for ID 32C facilitated correct identification of 92% common and 85% rare isolates.

Table 1: The test results by using API kit (ID 32C and 20C AUX)

Types of samples	API test	
	ID 32C	20C AUX
Sarawak (tapé)	*Cryptococcus humicola* *Candida glabrata*	*Candida glabrata* *Cryptococcus humicola* *Stephanoascus ciferrii* *Candida famata*
Sarawak (tuak)	*Cryptococcus humicola* *Candida glabrata* *Candida sake* *Candida silvicola* *Saccharomyces cerevisiae* *Rhodotorula glutinis*	*Cryptococcus laurentii* *Candida glabrata* *Candida albicans* 2 *Candida pelliculosa* *Candida tropicalis* *Trichosporon asahii* *Saccharomyces cerevisiae* *Cryptococcus humicola* *Rhodotorula glutinis*
Pahang (tapé)	*Cryptococcus humicola* *Candida guilliermondii* *Saccharomyces cerevisiae*	*Candida guilliermondii* *Trichosporon mucoides* *Cryptococcus humicola* *Saccharomyces cerevisiae* *Cryptococcus albidus* *Stephanoascus ciferrii*

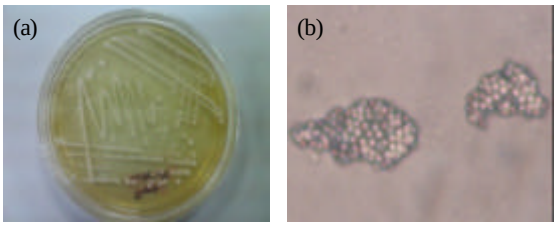

Fig. 1: (a) Sample from Sarawak (tapé), (b) tapé sample under microscope (40x magnification)

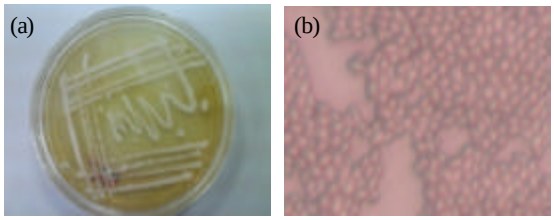

Fig. 2: (a) Sample from Sarawak (tuak), (b) Tuak sample under microscope (40 x magnification)

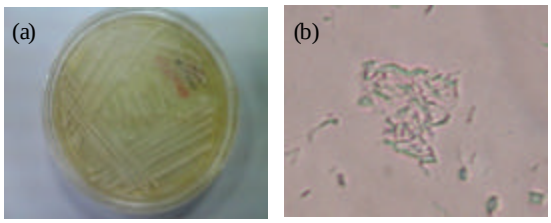

Fig. 3: (a) Sample from Pahang (tapé), (b) Pahang (tape) sample under microscope (40x magnification)

This API kit is for clinical yeasts identification so, it not too suitable for industrial yeasts identification. Because of this cause, microorganisms in the sample give

unacceptable profile. Many profiles unacceptable may be of that yeasts species not in the data index or that species not yeasts but other microorganisms like fungi or bacteria. Doubtful and low discrimination profiles may be of the some tested characteristics are not match with the expectation species in the data index.

From the study, *Saccharomyces cerevisiae* was identified in two samples (Sarawak (tuak) and Pahang (tapé)) and it was known as numerous predominant in alcoholic fermented beverages (Barnett *et al*., 2000; Battcock and Ali, 1993) while *Candida glabrata* is the lower ethanol producing capacity (Dung *et al*., 2007). These species are known with their capabilities in producing ethanol. The DNA study about these samples is in the progress to be confirmed these identifications.

CONCLUSION

From the study, *Cryptococcus humicola* and *Candida glabrata* are identified in the Sarawak (tapé) sample, while *Cryptococcus humicola*, *Saccharomyces cerevisiae*, *Candida glabrata* and *Rhodotorula glutinis* were identified in the Sarawak (tuak) sample and for Pahang (tapé) sample, *Cryptococcus humicola*, *Saccharomyces cerevisiae* and *Candida guilliermondii* was identified by using both API kit (20C AUX and ID 32C).

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