International Food Research Journal 19(2): 775-777 (2012)

Short Technical Communication
Yeast identification from domestic ragi for food fermentation by PCR method

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Abstract: In this study polymerase chain reaction (PCR) was used to identify yeast in domestic ragi obtained from two local markets in Sarawak and Pahang. These ragi are normally used as a dry starter in food fermentation (tapai) for Pahang (ST2) and Sarawak (ST3) and tuak (ST1) which is an alcoholic drink in Sarawak. Universal primer, NL1 and NL4 were used as a primer in this study to amplify D1/D2 fragment. Based on the result from the sequencing and after the BLAST search of the nucleotide sequences, the strain was confirmed as *Candida glabrata* (FN424108.) partial 26S rRNA gene, strain IMUFRJ 51955 for ST1, *Saccharomyces cerevisiae* (EU285514.1) isolate 35 26S ribosomal RNA gene, partial sequence for ST2 sample and *Candida glabrata* (FN393990.1*)* partial 26S rRNA gene, strain MUCL 51244 for ST3. All these strains were found in domestic ragi used for food fermentation.

Keywords: Y**easts identification, PCR, domestic ragi**

Introduction

Ragi was used in the food fermentation as a dry starter for inoculation (Sujaya *et al.*, 2004). It is commonly use in the traditional fermentation food in Asia for tapai (fermented rice) and tuak (alcoholic drink). Sujaya *et al.* (2002) reported that ragi contains filamentous fungi, yeasts and bacteria. From the early study, three domestic ragis from 2 different locations (Pahang and Sarawak) were identified using methods ie API Kit and Biolog Identification System. Using these two methods, the strain identified as *Candida glabrata* for both test (API Kit and Biolog) for tuak sample (ST1), *Saccharomyces cerevisiae* (API Kit) and *Eremothecium ashbyi* (Biolog) for Pahang, ST2 and *Candida glabrata* (API Kit) and *Candida montana* (Biolog) for Sarawak, ST3. The usage of commercial kits may result in inaccurate identification since some of these kits databases are normally referred only to clinical yeasts (Noorhisham and Siti Hajar, 2010). In this study, we use of PCR to identify the species to ensure the strains observed from the previous methods using API kits and Biolog system.

Materials and Methods

Strains and growth condition

Samples were screened and isolated from domestic ragi obtained from 2 locations in Malaysia ie (Pahang and Sarawak, resulted in three isolated strains namely ST1 (Sarawak tuak), ST2 (Pahang) and ST3 (Sarawak tapai) on YPD agar. These strains were incubated in 37ºC for 20 h in YPD broth for DNA extraction.

Primer

In this study primer NS1 (5'-GTA GTCATATGCTTGTCTC-3') and F18SB1 (5'-CTTGTTACGACTTTTACTTCCTC-3') (primer pair a) proposed by Kuriyama *et al.* (1997) and universal primer NL1 (forward primer)(5'-GCATATCAATAAGCGGAGGA AAAG-3') and NL4 (reverse primer) (5'-GGTCCGTGTTTCAAGACGG-3') (primer pair b) were used to amplify the rDNA region NTS 2 (Ribosomal Non Transcribed Spacer 2) (Maoura *et al*., 2005) producing an amplified band of 680 bp.

DNA extraction

The procedures of DNA extraction are according to the manufacturer's procedure (Promega Corp., Madison, WI, USA). 1 ml young cultures of yeast (approximately 20 h age) was transferred in a 1.5 ml tube and centrifuged at 13000 to 16000 g for 2 min. The supernatant was discarded and the cell pallet was suspended in 293 µL of 50 mM EDTA. 7.5 µL (20 mg/ml) of lyticase were added and mixed gently. Then, the samples was incubated in 37ºC for 30 to 60 min and cooled to room temperature. The samples were centrifuged at 13000 to 16000 g for 2 min after discarding the supernatant. 300 µL of nuclei lysis were added to the samples followed by 100 µL of protein precipitation solution, vortexed vigorously and incubated in ice for 5 min. After that, the mixtures were centrifuged at 13000 to 16000 g for 3 min and the supernatant were transferred into a clean tube with addition of 300 µL isopropanol. The samples were mixed by inversion and centrifuged at 13000

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to 16000 g for 2 min. Then, the decanted supernatant was added with 300 µL of 70% ethanol. The samples were again centrifuged at 13000 to 16000 g for 2 min and the supernatant were discarded. Ethanol were then aspirated using air to dry the pallet for 30 min. 50 µL of rehydration solution and 1.5 µL of RNase were added and the solution was incubated at 37ºC for 15 min. The samples were then rehydrated 65ºC for 1 h or at 4ºC overnight.

PCR conditions

PCR were conducted in a 50 µL reaction volume in a thin-walled 0.2 ml tubes. Briefly 1 µL of DNA template (1 ng quantified with spectrophotometer) was added to a 49 µL master reaction mixture containing 5 µL of 10x Dream Taq™ Green Buffer (contains KCL, $(NH_4)_2SO_4$ and includes 20 mM of $MgCl_2$), 37.75 µL of sterile distilled water, 5 µL of 2 mM dNTPs mix, 1 µL of NL1 and NL4 primers, and 0.25 µL of DNA polymerase (Dream Taq™). Thermal cycling parameters were initial denaturation at 95ºC for 2 min, followed by 35 cycles consisting of denaturation at 95ºC for 30 s, annealing at 56.75 ºC for 1 min and extension at 72ºC for 1 min. A final extension at 72ºC for 10 min followed. Each set of PCRs included a control reaction without template DNA.

Purification of PCR products

The amplified D1/D2 fragment was purified with the Wizard Plus SV Minipreps (Promega Corp., Madison, WI, USA) according to manufacturer's protocol.

DNA sequencing and analysis

Samples were sent to the First BASE Laboratories Sdn. Bhd. for the DNA sequencing. A BLAST search of the nucleotide sequences was conducted through the National Center for Biotechnology Information homepage *(http://www.ncbi.nlm.nih.gov)*.

Results and Discussions

In this study, we tested two types of primers (NS1-F18SB1 and NL1-NL4) and two brand of master mixed (Promega® and Fermentas). Table 1 shows the results of the primer and master mixed used, indicating that universal primers, NL1 and NL4 were found to be suitable. All the amplified DNA fragments were then sent to the First Base Laboratories Sdn. Bhd. for sequencing. After the BLAST search of the nucleotide sequences, the results shows that the isolates were *Candida glabrata* (FN424108.1) partial 26S rRNA gene, strain IMUFRJ 51955 for ST1, *Saccharomyces cerevisiae* (EU285514.1) isolate 35 26S ribosomal RNA gene, partial sequence for ST2 sample and *Candida glabrata* (FN393990.1*)* partial 26S rRNA gene, strain MUCL 51244 for ST3 sample after the blasting. The percentages of the identification were 99% of *C. glabrata* for ST1 and 97% for ST3, while 99% identification of *S. cerevisiae* for ST2. From our early identification study, similar result was observed with API kit and biolog test for ST1 sample but different result for ST2 and ST3 in biolog test. For ST2 and ST3 samples, the result of identification is the same between API kit and PCR but different result in Biolog test. The summary of those results are depicted in Table 2 and Figure 1 which shows the scanning electron microscope of the yeasts samples on YPD agar.

Table 1. Concentration of PCR product and the BLAST Results

Sample	Primer and Master mixed	con. (ng/µL)	Result
ST1	a*	36.0	-
	b*	63.5	*C. glabrata*
ST2	a*	190.5	-
	b*	144.6	*S. cerevisiae*
ST3	a*	17.6	-
	b*	117.1	*C. glabrata*

*a: master mixed from Promega and NS1-F18SB1 primer, b: master mixed from Promega and NL1-NL4 primer, -: unidentified

Table 2. Yeast identification by using different methods

Samples	Identification		
	API	*Biolog*	*26S rRNA*
ST1	*C. glabrata* (99.1%)	*C. glabrata* (99.7%)	*C. glabrata* (99%)
ST2	*S. cerevisiae* (99.9%)	*E. ashbyi* (95%)	*S. cerevisiae* (97%)
ST3	*C. glabrata* (91.5%)	*C. Montana* (85.9%)	*C. glabrata* (99%)

* C.: Candida, S.: Saccharomyces and E.: Eremothecium

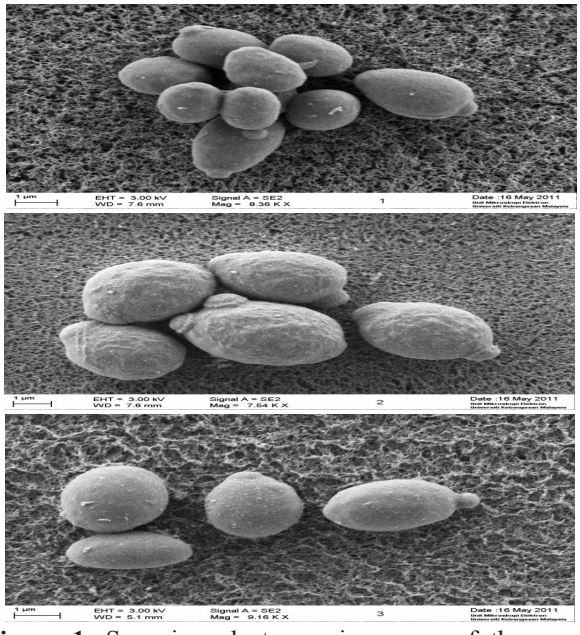

Figure 1. Scanning electron microscope of the yeasts samples under YPD agar. a: ST1 sample (*Candida glabrata*), b: ST2 sample (*Saccharomyces cerevisiae*) and c: ST3 sample (*Candida glabrata*)

In 2004, Sujaya *et al.* identified that *Saccharomyces cerevisiae, Candida glabrata, Pichia*

anomala and *Issatchenkia orientalis* were found to be inside the brem (Balinese rice wine) using different types of ragi tapai. It was identified on the basis of their 18S rDNA sequences. Thus, our results seemed to agree with these observations since both *Saccharomyces cerevisiae* and *Candida glabrata* were also found in the ragi tapai used in this study too. It can be concluded that *Saccharomyces cerevisiae* and *Candida glabrata* are present in Malaysia domestic's ragi that were used for food fermentation.

Acknowledgement

The authors duly acknowledged financial assistance from the Universiti Kebangsaan Malaysia (UKM) via Research University Grant (UKM-GUP-BTT-07-25-165).

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