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Diversity of Yeast Species Identified during Spontaneous Shallow Box Fermentation of Cocoa Beans in Malaysia

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Abstract

The chocolate flavour of cocoa beans is influenced by cocoa fermentation which is closely associated with the involvement of microorganisms such as yeasts. Research was carried out to identify types of yeast species involved in the cocoa fermentation during 48 hours fermentation periods. Samples of cocoa beans were obtained from two research stations which were Malaysian Cocoa Board, Hilir Perak, Perak and Samarahan, Sarawak, Malaysia. A total of 53 pure cultures of yeasts were obtained from the cacao beans of these two locations. Of these, 16 yeast species were identified based on morphology characteristic and molecular technique. Only six yeast species known as *Candida xylopsoci, Wickerhamomyces anomalus, Pichia kudriavzevii, Saccharomyces cerevisiae, Wickerhamomyces onychis* and *Geotrichum candidum* were found in cacao samples of Perak. However, 14 yeast species were found in samples of Samarahan which were *Candida quercitrusa, Candida xylopsoci, Candida jaroonii, Rhodotorula mucilaginosa, Pichia kudriavzevii, Candida tropicalis, Saccharomyces cerevisiae, Hanseniapora thailandica, Hanseniaspora opuntiae, Haneniaspora* sp, *Saturnispora diversa, Candida ethanolica, Pichia kluyveri / Pichia fermentas* and *Wickerhamomyces anomalus*. Of these, *Pichia* and *Candida* species were the dominant yeast species found in Perak. While, *Candida* species was the dominant yeast species found in Samarahan. The results indicated that different yeast species could be found in cocoa bean of different locations during fermentation.

Keywords*: Cocoa; Fermentation; Isolation; Identification; Yeast species*

1. Introduction

Fermentation in cocoa is an important process as no chocolate flavour in cocoa bean can be developed without a good fermentation process (Lopez, 1986). Often, the process of cocoa bean fermentation has microorganisms such as yeasts, lactic acid bacteria and acetic acid bacteria (Heide *et al.,* 2009). The microbial diversity in cocoa fermentation is different according to location and process factors (Heide *et al.,* 2009). Of the microorganisms involve in cocoa bean fermentation, yeast plays a fundamental role as a pioneer organism. Yeast has been found dominating the cocoa fermentation for the first 24 hours of the microbial succession (Schwan, 1998). The predominance and importance of yeast in cocoa fermentation is well recognized (Graham & Hugh, 2007). A research on the assessment of the microbial community of cocoa bean heap fermentations in Ghana involved 91 yeast isolates which were detected by PCR fingerprinting with the primer M13 (Heide *et al.,* 2009). They found 16 clusters of yeast which showed a large degree of variability in strain variation. *Hanseniaspora opuntiae* was found tolerating to low pH and preferably to grow at the earlier phase of fermentation (Heide *et al.,* 2009).

Nielsen (2006) researched on the microbiology of a number of Ghanaian cocoa fermentations by tray, small heap and large heap fermentation. Yeast was found as the dominating microorganisms followed by lactic acid bacteria which dominates after 12 to 24 hours of fermentation and remained predominant throughout the fermentations with acetic acid bacteria (AAB) in the mid phase of fermentations. *Bacillus* spp. was only detected during heap fermentations which reached high number during the later stages of fermentation. His research showed *Hanseniaspora guilliermondii* was the predominant yeast during the initial phase and *Pichia membranifaciens* was found during the later phases of fermentation (Nielson, 2006). Other yeast species which were *Issatchenkia orientalis, Candida zemplinina, Saccharomyces cerevisiae* and three undescribed yeast species were also isolated from the cocoa fermentation. Martelli and Dittmar (1960), reported that in the cocoa beans of the Forastero type from Brazil, only *Saccharomyces rosei, Hansenula anomala* and *Pichia fermentans* fermenting the sugars of cocoa mucilage pulps, while unknown species *Saccharomyces* as the agent responsible for the alcoholic phase of cocoa fermentation.
Although Malaysia is a cocoa producing country, there is not much information on yeast involvement in cocoa fermentation has been published. There are many unknown yeast species are yet to be identified and expected present in Malaysia cocoa. The objective of this research is to isolate and identify different types of yeast species involved in cocoa fermentation during the 48 hours fermentation.

2. Materials and methods

2.1 Cocoa fermentation

Cocoa shallow box fermentation method was used in this research. It is the standard practice in Malaysian Cocoa Board. The cocoa fermentation process was carried out for five days. The turning process of cocoa beans was done at 72 hours of cocoa fermentation. Mixed clones of cocoa pods were used for the cocoa fermentation. Four batches sampling of cacao fermentation were carried out to isolate and identify yeast species. One sampling was conducted in Malaysian Cocoa Board, Hilir Perak, Perak on 7th June 2011 while other three batches of samplings were conducted in Malaysian Cocoa Board Samarahan, Sarawak on 5th November 2012, 25th January 2013 and 25th February 2013.

Sample collections were done according to method by Bariah (2011). Samples were collected at 0, 6, 24 and 48 hours after fermentation. Three positions were selected as shown in Figure 1. Samplings were done by randomly collecting cocoa beans at the top, centre and bottom of the cocoa shallow box for each position. All of the collected samples were kept in polybag and sealed, labelled and stored in refrigerator at -20˚C for laboratory and microbial analysis. At each sampling time, the yeast species were isolated and identified.

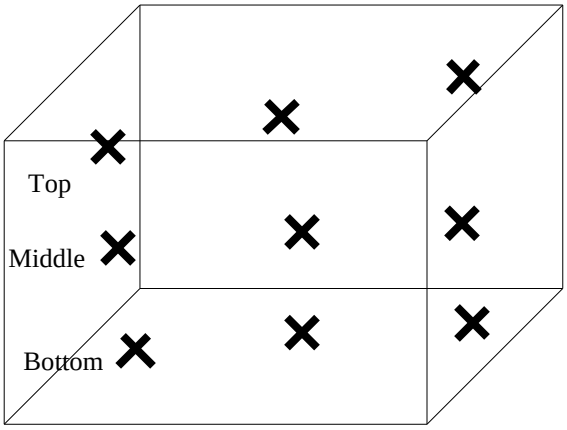

Figure 1. Collection of cocoa beans samples at three positions in shallow box collected: top, middle and bottom parts

2.2 Isolation of yeast species

Yeast isolates were obtained from the collected samples by bean swab methods as suggested by Bariah (2011). Yeast extract media was used for yeast isolation. The media consisted of glucose 50 g (GLUCOLIN), yeast extract 3.0 g (USB Corporation Cleveland, USA), KH_2PO_4 0.1 g (Hamburg Chemicals), NaCl 0.1 g (Nen Tech Ltd, UK), $CaCl_2$ 0.013 g (Fluka Chemika), distilled water 1 litre and agar 15 g (QReC Chemical Co Ltd). Media was adjusted to pH 5.5 before autoclaved (TOMY SX-300 High Pressure Steam Sterillize) at 121˚C for 15 minutes. The bean swab method was carried out where the cocoa bean taken at 0, 6, 24, and 48 hours after fermentation was respectively swabbed on the yeast extract media and incubated at room temperature for yeast growth.

2.3 Identification of yeast

2.3 a Yeast identification by morphology descriptions

The morphology of the yeast isolates was described based on its colour, shape, margin and elevation. Yeast isolates were selected for pure culture preparation and identification by molecular technique. Besides, yeast isolates were preserved in glycerol 25% (Hamburg Chemicals) as stock culture.

2.3 b Yeast identification by molecular technique

Colonies of yeast from the pure yeast colony after three days of growth at 25˚C were picked by using sterilized tooth picks and placed into the Eppendolf tube filled with 200 µl of lysis buffer solution. Sample in lysis buffer solution was spun maximum at 13000rpm for 10 minutes to precipitate the pellet. The tube was heated in water bath (JSWB- 11T) at 70 ˚C for 3 minutes and later was frozen by using liquid nitrogen. This thawing process was repeated once and tubes was vortex and spun (Eppendorf Centrifuge 5430) for 10 minutes at room temperature at 13000rpm. v/v of phenol (R&M Chemicals): chloroform (J. T. Baker): isoamyalcohol (aMRESCO) (25:24:1) was added into the tube. Tubes was centrifuged (Eppendorf Centrifuge 5430) for 10 minutes at 13000rpm. The new aqueous phase was then transferred to a new tube and labelled. v/v of chloroform (J. T. Baker): isoamyalcohol (aMRESCO) (24:1) was added into the tube, centrifuged at 13000rpm for 10minutes. A new aqueous phase was again transferred to a new tube and labelled. 0.1 v/v 5M sodium acetate (Bendosen) (pH 5.2) and 2 volume of absolute cold ethanol (R & M Chemicals) was added into the new aqueous phase. The tube was incubated at -20˚C for overnight. Then, centrifuged (Eppendorf Centrifuge 5430) again at 13000rpm for 10 minutes. A pellet was precipitated at bottom of the tube. Supernatant was thrown away and pellet was let dried under the air flow laminar for 10 minutes. After the pellet was dried, 50 µl of Tris-EDTA (TE) buffer was added. The tube was incubated at -20˚C and gel electrophoresis was carried out.

The extracted DNA was then amplified by Polymerase Chain Reaction (PCR). Primers ITS 1 (1st base, Singapore) and ITS 4 (1st base, Singapore) were used for DNA amplification. A LabCycler (SENSQUEST Labcycler) heated lid thermocycler was used to amplify DNA; 50µl reactions were prepared by adding 1.5 µl of

20µM ITS1 and ITS4 each primer mixture (1st base, Singapore), 4.0 µl of DNA sample, 4.0 µl of 2mM $MgCl_2$ (Promega), 0.2µl of 2mM of dNTP (Promega), 0.25 µl of 5u/ml Taq DNA polymerase (Promega), 10.0 µl of 5x PCR buffer (Promega) and 28.55 µl of pure sterile water. All amplification reactions were hot started at 94°C for 4 minutes followed by 29 cycles of denaturation at 94°C, 2 minutes annealing at 55°C, 2 minutes primer extension at 72°C, 2 minutes and a final extension for 10 minutes at 72°C. The cooling step is 10°C for infinity.

Gel electrophoresis was conducted in 1% agarose gel (Vivantis, USA) dissolved in 1X TAE buffer (Promega, USA). The PCR products were sent to 1st Base Company for purification and sequencing. The sequencing information obtained was edited and aligned by using Chromas Pro then analysed by Basic Local Alignment Search Tool (BLAST) through the National Centre for Biotechnology Information (NCBI) to identify the closest species of yeast to the yeast isolates.

3. Results

3.1 Isolation of yeast

Yeast species was isolated and identified for cocoa bean fermentations conducted in Samarahan and Perak. A total of 16 different types of yeast species were identified from 57 yeast isolates from the two locations. Each batch of cocoa bean fermentation carried different combinations of yeast species.

Table 1 shows that 11 yeast species were recorded at 0 hours fermentation period from the two locations which were *Candida quercitrusa*, *Candida xylopsoci, Rhodotorula mucilaginosa, Pichia kudriavzevii, Wickerhamomyces anomaluss, Saturnispora diversa, Hanseniaspora* species and *Geotrichum candidum*. *P. kudriavzevii* and *W. anomalus* were the most frequent species found at 0 hours. Subsequently, 12 yeast species were identified at 6 hours; eight and five yeast species were found at 24 and 48 hours respectively from the two locations.

A total of five yeast species were identified from 12 yeast isolates in batch 1 of Samarahan which were *C. quercitrusa, C. xylopsoci, Candida jaroonii, R. mucilaginosa* and *P. kudriavzevii.* In addition to that, five yeast species was obtained from 12 yeast isolates in batch 2 of Samarahan which were *W. anomalus, P. kudriavzevii, Candida tropicalis, Hanseniaspora opuntiae* and *Saccharomyces cerevisiae*. Subsequently, seven yeast species were discovered from ten yeast isolates in batch 3 of Samarahan which were *Hanseniapora thailandica, H. opuntiae, Hanseniapora* sp, *Saturnispora diversa, Candida ethanolica, Pichia kluyveri / Pichia fermentas* and *W. anomalus.* Besides, six yeast species had discovered from 19 yeast isolates in batch of Perak which were *C. xylopsoci, W. anomalus, S. cerevisiae, P. kudriavzevii, Whickerhamomyces onychis* and *Geotrichum candidum* The occurrence of various yeast species identified from 0 hours to 48 hours in cocoa fermentation are tabulated in Table 1.

Table 1. Yeast species identified at 0 hours to 48 hours fermentation periods of cocoa fermentation in Samarahan and Perak

Yeast species	Locations	Fermentation period (Hours)			
		0	6	24	48
Candida quercitrusa	Batch 1 Samarahan	+	+	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	-	-
Candida xylopsoci	Batch 1 Samarahan	+	+	+	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	+	+
Candida jaroonii	Batch 1 Samarahan	-	+	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	-	-
Rhodotorula mucilaginosa	Batch 1 Samarahan	+	+	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	-	-
Pichia kudriavzevii	Batch 1 Samarahan	+	-	-	-
	Batch 2 Samarahan	+	+	+	+
	Batch 3 Samarahan	-	-	-	-

	Perak	-	+	+	-
Wickerhamomyces anomalus	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	+	-	-	-
	Batch 3 Samarahan	+	-	-	-
	Perak	+	+	-	+
Candida tropicalis	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	+	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	-	-
Hanseniaspora opuntiae	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	+	-	-
	Batch 3 Samarahan	-	+	-	-
	Perak	-	-	-	-
Saccharomyces cerevisiae	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	+
	Batch 3 Samarahan	-	-	-	-
	Perak	-	+	-	-
Hanseniapora thailandica	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	+	-	-
	Perak	-	-	-	-
Saturnispora diversa	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	+	-	+	-
	Perak	-	-	-	-
Candida ethanolica	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	+	-
	Perak	-	-	-	-
Pichia kluyveri / Pichia fermentas	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	+
	Perak	-	-	-	-
Hanseniaspora sp	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	+	-	+	-
	Perak	-	-	-	-
Wickerhamomyces onychis	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	-	-	+	-
Geotrichum candidum	Batch 1 Samarahan	-	-	-	-
	Batch 2 Samarahan	-	-	-	-
	Batch 3 Samarahan	-	-	-	-
	Perak	+	-	-	-
Total yeast species		11	12	8	5

3.2 Identification of yeast

3.2a Yeast identification by morphology descriptions

Based on the morphological characteristic, most of the yeast species were cream or white in colour, circular in shape, entired in margin and raised in elevation. *Hanseniapora* genus was discovered in light brown colour, oval shape, entired margin and raised elevation. On the other hand, *Rhodotorula* genus was found in pink colour,

irregular shape, undulate margin and flat. After a series of subculturing in this research, *Rhodotorula mucilaginosa* became white in colour. It was difficult to distinguish *Candida* genus, *Wickerhamomyces* genus, *Pichia* genus and *Saccharomyces* genus solely based on morphological description as they were cream or white colour, usually circular in shape, entire in margin and raised in elevation. Therefore, molecular work was needed in order to identify their identities. The morphological characteristics for batch 1, 2, 3 in Samarahan and batch in Perak are shown in Table 2.

Table 2. Summary for description of yeast species identified from cocoa fermentation in Samarahan and Perak

Yeast isolates	Description of yeast colony			
	Colour	Shape	Margin	Elevation
Candida jaroonii	Cream	Oval	Entire	Raised
Candida quercitrusa	Cream	Circular	Entire	Raised
Candida quercitrusa	Cream	Circular	Filiform	Flat
Candida xylopsoci	White	Circular	Filiform	Raised
Candida xylopsoci	Cream	Circular	Entire	Raised
Rhodotorula mucilaginosa	Pink	Irregular	Undulate	Flat
Pichia kudriavzevii	White	Circular	Filiform	Flat
Pichia kudriavzevii	White	Irregular	Undulate	Flat
Wickerhamomyces anomalus	White	Circular	Entire	Flat
Wickerhamomyces anomalus	White	Circular	Entire	Raised
Hanseniaspora opuntia	Brown	Oval	Entire	Raised
Candida tropicalis	White	Oval	Entire	Raised
Saccharomyces cerevisiae	Creamy white	Oval	Entire	Raised
Saturnispora diversa	White	Circular	Lobate	Flat
Candida ethanolica	Cream	Circular	Filiform	Raised
Hanseniaspora thailandica	Brown	Oval	Entire	Raised
Pichia kluyveri	Brown	Oval	Entire	Raised
Hanseniaspora sp	Brown	Oval	Entire	Raised
Wickerhamomyces onychis	Cream	Circular	Entire	Raised
Geotrichum candidum	White	Filamentous	Filiform	Flat

3.2 b Yeast identification by molecular technique

Homology search through BLAST Search Engines (http://blast.ncbi.nlm.nih.gov/) was used to find all the possible sequences that similar to the sequences obtained. *C. xylopsoci* (NR_077074.1) and *W. anomalus* (GQ376075.1) were the most commonly identified from the yeast isolates collected. The identification of dominant yeast species during cocoa fermentation was categorized as shown in Table 3.

Table 3. Identification of dominant yeast species during cocoa fermentation by sequencing

Samarahan Batch 3	93	193/208	KT175190	*Saturnispora diversa*
	93	542/584	KT175198	*Wickerhamomyces anomalus*
	94	413/439	KT175192	*Candida ethanolica*
	81	115/142	KT175193	*Saturnispora diversa*
	87	638/731	KT175194	*Hanseniaspora thailandica*
	99	443/444	KT175195	*Pichia kluyveri*
	97	685/704	KT175183	*Hanseniaspora opuntiae*
	98	705/719	KT175196	*Hanseniaspora species*
	-	-	-	*Hanseniaspora species*
	-	-	-	*Hanseniaspora species*
Perak	99	470/471	KT175174	*Candida xylopsoci*
	100	471/471	KT175174	*Candida xylopsoci*
	98	609/620	KT175197	*Wickerhamomyces onychis*
	99	511/518	FM178339.1	*Candida xylopsoci*
	99	577/579	KT175201	*Wickerhamomyces anomalus*
	90	207/229	NC_001144.5	*Saccharomyces cerevisiae*
	98	473/481	KT175182	*Pichia kudriavzevii*
	99	129/130	KT175182	*Pichia kudriavzevii*
	99	467/471	KT175174	*Candida xylopsoci*
	99	578/579	KT175198	*Wickerhamomyces anomalus*
	94	546/583	KT175198	*Wickerhamomyces anomalus*
	95	558/587	KT175198	*Wickerhamomyces anomalus*
	99	617/619	KT175201	*Wickerhamomyces anomalus*
	99	293/297	KT175200	*Geotrichum candidum*
	99	616/619	KT175201	*Wickerhamomyces anomalus*

4. Discussions and Conclusions

H. opuntiae was closed to *Hanseniaspora guilliermondii* and *Hanseniaspora uvarum* (Cadez *et al.*, 2003). It was suggested that *H. opuntiae* had misidentified as *H. guilliermondii* (Daniel *et al.*, 2009). The predominant yeast genera that found in this research were *Candida*, *Pichia*, *Rhodotorula*, *Wickerhamomyces*, *Hanseniaspora* and *Geotrichum* genus. They were found at 0 hours and 6 hours during cocoa fermentation. Nevertheless, a diversity of yeast species that had reported as primary colonizers yeast genera of cocoa fermentation were *Saccharomyces*, *Hanseniaspora* (anamorph Kloeckera) and *Pichia* (Pereira *et al.*, 2012). In this study, *S. cerevisiae* was only discovered in batch 2 of Samarahan at 48 hours and in batch of Perak at 6 hours.

Microbial succession occurred during the cocoa fermentation as shown in Table 1 could be related with the changes in the microenvironment such as changes in nutrient availability, pH, temperature, presence and concentration of organic acids and oxygen concentration (Jespersen *et al.*, 2004). *P. kluyveri* was identified at 48 hours in third batch fermentation of Samarahan which none of the other types of yeast species was obtained at that fermentation period. This may due to the fact that *P. kluyveri* could produce mycocins which inhibited other yeast growth such as *S. cerevisiae* and *C.* species (Jespersen *et al.*, 2004). In this experiment, it was found that *C.* species was the most dominant yeast species as several *C.* species which were *C. jaroonii*, *C. quercitrusa*, *C. xylopsoci*, *S. diversa*, *C. ethonolica* and *C. tropicalis* were identified. The second most dominant yeast species was *Hanseniaspora* species and *Pichia* species followed by *Wickherhamomyces* species and other types of yeast species which were *Saccharomyces* species, *Galactomycetes* species and *Rhodotorula* species.

The 16 different types of yeast species identified in this research had variation compared to previous study done. According to Jespersen *et al.*, (2004), *C. krusei* anamorph *P. kudriavzevii* (Carlotti *et al.*, 1996; Kurtzman *et al.*, 1980) was the dominant species followed by *Pichia membranifaciens*, *P. kluyveri*, *H. guilliermondii*, *Trichosporon asahii*, *S. cerevisiae* and *Rhodotorula glutinis* identified in Ghana, West Africa. Furthermore, the principal species found in Indonesia by Ardhana and Fleet (2003) were *Penicillium citrinum*, an unidentified basidiomycete, *Kleockera apis*, *S. cerevisiae*, *C. tropicalis*, *Lactobacillus cellobiosus*, *Lactobacillus plantarum* and *Acetobacter pasteurianus* followed by *Bacillus pumilus* and *Bacillus licheniformis*. *S. cerevisiae* and *C. tropicalis* were the common yeast species matched with the yeast species found in this research.

Besides, *S. cerevisiae* and *C. tropicalis* were reported to have significant growth at higher temperature

Locality	Homology to original Gen Bank sequence (%)	Identities	Gen Bank accession number	Yeast species name closest Gen Bank Fit
Samarahan Batch 1	99	615/618	KT175172	*Candida jaroonii*
	99	599/607	KT175173	*Candida quercitrusa*
	99	510/511	KT175174	*Candida xylopsoci*
	98	606/617	KT175173	*Candida quercitrusa*
	99	607/615	KT175173	*Candida quercitrusa*
	98	603/613	KT175173	*Candida quercitrusa*
	99	510/511	KT175174	*Candida xylopsoci*
	99	470/471	KT175174	*Candida xylopsoci*
	100	527/527	KT175176	*Rhodotorula mucilaginosa*
	100	539/539	KT175177	*Rhodotorula mucilaginosa*
	99	532/539	KT175175	*Candida quercitrusa*
	-	-	-	*Pichia kudriavzevii*
Samarahan Batch 2	97	598/615	KT175179	*Wickerhamomyces anomalus*
	99	618/619	KT175180	*Wickerhamomyces anomalus*
	99	617/618	KT175180	*Wickerhamomyces anomalus*
	100	597/597	KT175191	*Wickerhamomyces anomalus*
	100	618/619	KT175180	*Wickerhamomyces anomalus*
	99	510/512	KT175182	*Pichia kudriavzevii*
	99	663/664	KT175183	*Hanseniaspora opuntiae*
	100	526/526	KT175184	*Candida tropicalis*
	99	509/512	KT175182	*Pichia kudriavzevii*
	88	419/475	KT175185	*Issatchenkia orientalis/ Pichia kudriavzevii*
	99	509/512	KT175186	*Pichia kudriavzevii*
	100	510/510	KT175187	*Pichia kudriavzevii*
	95	478/505	KT175182	*Pichia kudriavzevii*
	99	504/509	KT175187	*Pichia kudriavzevii*
	99	838/840	KT175188	*Saccharomyces cerevisiae*
	99	820/827	KT175189	*Saccharomyces cerevisiae*

of 40°C and small portions demonstrating weak growth at 47 °C to 50 °C (Ardhana & Fleet, 2003). In this research, *C. tropicalis* was identified at 6 hours fermentation period at average temperature 29.87°C whereas *S. cerevisiae* was identified at 48 hours fermentation period at average temperature of 38°C which were different than previous reported.

As a summary, the result in present study showed that cocoa fermentation was a diverse process depending on localities, seasonal variations, batch sizes and other external factors such as climate and handling method. The variations were revealed by the yeast species succession and the combination of predominant yeast species involved in cocoa fermentation at Perak and Samarahan, Malaysia. These variations could influence the quality of cocoa beans from the aspect of organoleptic quality.

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