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Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia

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Abstract. *Jamili, Yanti NA, Susilowati PE. 2016. Diversity and the role of yeast in spontaneous cocoa bean fermentation from Southeast Sulawesi, Indonesia. Biodiversitas 17: 90-95.* Yeast is one of the microbial group which is role in the process of cocoa spontaneously fermentation. The objective of this study was to determinate and to know the diversity of yeast that role on cocoa bean fermentation. Yeast was isolated by pour plate method from cocoa bean that was naturally fermented by a cocoa farmer in Kolaka District, Southeast Sulawesi using yeast mannitol agar (YMA) media. Yeast was characterized and identified using phenotypic characters based on numeric-phenetic analysis. Yeast isolates applied to cocoa bean to determine its role in cocoa bean fermentation. The result was obtained seven isolates the dominant yeast during cocoa bean fermentation in Kolaka District, Southeast Sulawesi. The result of numerical-phenetic analysis based on phenotypic characters to seven yeast isolates showed that 1 isolates (Klk1) identical with *Candida krusei.* Three isolates (Klk4, Klk5 and Klk7) identical with *Candida tropicalis*, one isolate (Klk2) identical with *Saccharomycopsis fibuligera*, one isolate (Klk3) identical with *Kloeckera* sp. and one isolate (Klk6) identical with *Saccharomyces cerevisiae.* The result also showed that fermentation of cocoa with seeding of yeast inoculums served to increase the quality of cocoa beans than spontaneous fermentation. Therefore, the seven yeast isolates potentially be used as an inoculum to improve the cocoa quality.

Keywords: Cocoa bean, diversity, Southeast Sulawesi, spontaneous fermentation, yeast

INTRODUCTION

Fermentation is one of the important processes to improve the quality of the cocoa bean. However, the majority of cocoa farmers in Indonesia, especially in Southeast Sulawesi is still less interested in doing the fermentation of cocoa beans, since for processing cocoa beans through a fermentation process takes a long time which is five to six days. Fermentation of cocoa beans undertaken by cocoa farmers is a spontaneous fermentation. Spontaneous fermentation is fermentation utilizing natural microorganisms in the environment and proliferate spontaneously because the environment suitable for growth.

Fermentation of cocoa beans involves the role of microorganisms in outlining of polyphenolic compounds, proteins, and sugars contained in cocoa beans through the action of the enzyme produced by such microorganisms so that the fermentation of cocoa beans will undergo physical and chemical changes. The groups of microorganisms involved in the spontaneous fermentation of cocoa are yeast, acetic acid bacteria and lactic acid bacteria (Ardhana and Fleet 2003; Jespersen et al. 2005; Guehi et al. 2010; Pereira et al. 2012). Yeast is one of the microorganisms that play a role in the fermentation of cocoa beans to break down sucrose, glucose, and fructose into ethanol. In addition, the yeast also serves to degrade the pulp with the help of pectinolytic enzymes during fermentation of cocoa beans (Jespersen et al. 2005; Ho et al. 2014).

The diversity of yeasts in the fermentation of cocoa beans has been reported by several researchers earlier. Ardhana and Fleet (2003) found the yeast isolates *Kloeckera apis, Saccharomyces cerevisiae* and *Candida tropicalis* who have contributed in the fermentation of cocoa beans in three plantations in Central Java, Indonesia. Alwi (2009) reported some type of yeast of the genus *Saccharomyces, Candida, Debaryomyces* and *Rhodotorula* that dominate the fermentation of cocoa beans in three districts in Central Sulawesi. Sidarsyah (2005) also was isolate yeast of the genus *Saccharomyces, Endomycopsis* and *Hanseniaspora* of origin cocoa beans Ranomee to plantations in the Southeast Sulawesi. Daniel et al. (2009) also reported a yeast diversity of spontaneous fermentation of cocoa in Ghana is dominated by *Pichia kudriavzevii* (*Issatchenkia orientalis*), *Saccharomyces cerevisiae* and *Hanseniaspora opuntiae*. However, these yeasts, yet unknown role in improving the quality of cocoa beans.

The involvement of yeast in the fermentation process of cocoa beans contributes to determining the quality of cocoa beans product. However, not much is reviewing the role of yeast that is obtained from the fermentation of cocoa beans spontaneously to quality cocoa beans (Ardhana and Fleet 2003;Alwi 2009;Daniel et al. 2009;Pereira et al. 2012;Mahazar, et al. 2015). Therefore, the exploration of the role of yeast in the fermentation of cocoa beans needs to be done.

MATERIALS AND METHODS

Isolation of yeast from the fermentation of cocoa bean

The source of yeast isolates was obtained from samples of cocoa beans that have been fermented spontaneously by farmers in Kolaka District, Southeast Sulawesi, Indonesia. Isolation of yeast was done by pour plate method using yeast mannitol media agar (YMA) (Alwi 2009). A total of10g sample suspended in 10 mL of 0.85% NaCl solution and made serial dilutions. One mL of the suspension was inoculated into a sterile petridish and was added YMA media. Furthermore, the petridish was incubated at a temperature of 37°Cfor 48 hours. Purified colonies were grown on YMA media. Pure culture yeast obtained, was inoculated into the slant YMA media and was stored at 4°C.

Characterization and identification of yeast isolate

Characterization and identification of yeast isolates are based on phenotypic characters which include the characters of the colony and cell morphology, physiological and biochemical. Characterization of yeast isolates carried out based on the guidelines used Kirsop et al. (1984).Identification of yeasts was done based on numeric-phenetic analysis using the program Multi-variate statistical package (MVSP) version 3.1 to determine similarity between strains. Similarity value determined using the Simple Matching Coefficient (SSM) method and the classification is done using algorithms UPGMA (Unweighted Pair Group Method with arithmetic Averages).

Application of yeast isolates on fermentation of cocoa bean

Fermentation of cocoa beans is done on a laboratory scale using fresh cocoa bean varieties Forastero. Cocoa pod obtained from cocoa plantations in Kolaka District, Southeast Sulawesi. Cocoa pod skin surface is chemically sterilized using a solution of detergent and phenol while cocoa beans that will be fermented sterilized by U.V. radiation overnight (Jamili et al. 2014).

A total of 1kg of cocoa beans was fermented in a plastic box. Yeast inoculum was grown for 24 hours at Potato dextrose broth (PDB) +1% extract of cocoa pulp. A total of 10% (v/w) of inoculum containing 10^6 CFU/mL were inoculated in cocoa beans. Fermented cocoa beans without yeast inoculum (spontaneous fermentation) are performed as a comparison. Cocoa fermentation was done at room temperature for five days and sampling at the beginning of fermentation (0), 3 and 5 days of fermentation to measure parameters of quality cocoa beans in accordance with national standards of Indonesia (SNI 2008), which include the levels of unfermented beans and fat content. The method of measuring the levels of unfermented beans according to those described in SNI (2008) and measuring the fat content is done with the Soxhlet method (AOAC 2005).

RESULTS AND DISCUSSION

Yeast isolates from fermented cocoa bean

Yeast isolates were found in cocoa beans fermented naturally/spontaneously for three days by cocoa farmers in the village of Konaweha, Samaturu sub-district Kolaka District, Southeast Sulawesi was as much as seven isolates. Some yeast isolates obtained from the fermentation of cocoa beans are listed in Table1.The result in Table 1 showed that yeast was obtained from cocoa bean fermentation at the first day was as much as four isolates, whereas at the second day is two isolates and the third day only one isolate. These results showed that the amount of yeast isolates in the cocoa fermentation at 1st day (± 24 hours) higher than the yeast isolates were obtained at 2nd and 3rd day of fermentation. This indicates that the yeast acts on the 1st day of fermentation of cocoa bean so that the type more than on the 2nd and 3rd days. The results are consistent with research conducted by Ardhana and Fleet (2003) which states that the yeast at most the number and the type found in 24-36 hours after fermentation of cocoa because of their role in the process of degradation the various types of sugar that contained in the cocoa bean pulp.

Environmental conditions during cocoa fermentation process such as temperature, also be one cause of the decline in the number of types of yeast during the fermentation process. The result of temperature measurements during spontaneous fermentation of cocoa beans was done by farmers in Kolaka, after one day was 33°C, on the second day was 36,5°C and the third dayreach42°C. The high temperature on the third day of fermentation of cocoa causing some type of yeast can not survive. According to Pereira et al. (2012), the range of optimum temperature for yeast in general is 25°C-35°C. Ardhana and Fleet (2003) also found that there are only a few yeasts were able to survive at high temperatures (>40°C), such as *Saccharomyces cerevisiae* and *Candida tropicalis.*

Identification of yeast isolates

Seven yeast isolates were identified based on similar phenotypic characters with eight reference strains, namely *Saccharomyces cerevisiae, Saccharomyces bayanus, Candida tropicalis, Candida krusei, Candida lambica, Saccharomycopsis fibuligera, Saccharomycopsis fermentant* and *Kloeckera* sp. Eight reference strains of yeasts are selected based on the resemblance of character with yeast isolates were isolated and the species of yeast that are often found in fermented cocoa. Phenotypic characters were analyzed by systematic numeric-phenetic is as much as31 characters (Table 2). Numerical systematic analysis results based on phenotypic characters yeast isolates and reference strains with 3.1 MVSP program are visualized in the form dendrogram listed in Figure1.

Table 1. Result of selection yeast isolates from fermented cocoa bean

Time fermentation of cocoa bean (days)	Number of yeast isolate
1	4
2	2
3	1

Table 2. Phenotypic characters of yeast isolates and reference strains

Characters	yeast isolates and reference strains														
	Klk1	**Klk2**	**Klk3**	**Klk4**	**Klk5**	**Klk6**	**Klk7**	***Candida krusei***	***C. tropicalis***	***C.lambica***	***Saccharomyces cerevisiae***	***S.bayanus***	***Saccharomycopsis fermentant***	***Saccharomycopsis fibuligera***	***Kloeckera* sp.**
Colonies morphology															
Shape															
Circular	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Irregular	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Edge															
Entire	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-
Undulate	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Color															
White	+	+	-	+	-	-	+	+	-	-	-	-	-	+	-
Beige	-	-	+	-	+	+	-	-	+	+	+	+	+	-	+
Elevation															
Low convex	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+
Convex	+	-	-	+	+	+	+	+	+	+	+	+	-	+	-
Structure in															
Opaque	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell morphology															
Shape															
Ovale	-	-	-	-	+	+	-	-	+	-	+	+	-	-	-
Round	+	+	-	+	-	-	+	+	-	+	-	-	+	+	-
Elliptical	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Vegetative reproduction															
Type of budding															
Bipolar	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+
Multipolar	+	-	-	+	+	+	+	+	+	+	+	+	-	-	-
Pseudomycelium	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-
Sexual/asexual spores															
Ascospore	-	+	+	-	-	+	-	-	-	-	+	+	+	+	+
Chlamydospore	+	-	-	+	+	-	+	+	+	+	-	-	-	-	-
Biochemical															
Urea hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Carbohydrate fermentation															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	-	-	+	+	+	+	+	+	-	+	+	-	+	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	-	+	+	+	+	-	+	-	+	+	-	+	-
Assimilation															
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	-	+	+	+	+	+	+	-	+	+	-	+	-
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	+	+	-	+	+	+	+	-	+	-	+	+	-	+	-
Ethanol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Physiological															
Temperature Tolerance (^{o}C)															
37	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
40	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose tolerance of 50%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Note: characters for reference strains according to Kirsop et al. (1984) and NCYC (2016).

Dendrogram based on similarity values listed in Figure 1, shows there are 3 clusters are formed. Cluster I formed four sub-clusters with similarity value of 83.3%, which shows the group consisting of strains of the genus *Candida* that included isolates Klk1, *C. krusei, C. tropicalis, C. lambica*, isolates Klk5, isolates Klk7 and isolates Klk4. Sub cluster 1 consists of isolates Klk1 and *C. krusei* with a similarity value of 86.7%. This indicates that Klk1 isolates identical with *C. krusei*, the only difference being the shape of the colony and the ability to ferment maltose (Table 2).

Sub-cluster 2 consists of *C. tropicalis* and isolates Klk5 with a similarity value of 100%. This indicates that isolates Klk5 is a member of the species of *C. tropicalis* because all the phenotypic characters are compared, are similar. Sub Cluster 3 consists isolates Klk4 and isolates Klk7 has a similarity value of 100%, but the two isolates has similarities with *C. tropicalis* with the similarity value of 93.3%. This indicates that the two isolates were identical to *C. tropicalis*, the only difference from the structure of colony morphology and shape of the cells (Table 2). Sub cluster 4 only consists of *C. lambica* and it is joined with all strains in the cluster I with 83% similarity values.

Cluster II consists of isolates Klk3 and *Kloeckera* sp. with a similarity value of 100% (Figure 1). This indicates that isolates Klk3 is a member of the species *Kloeckera*. Characters of Klk3isolatesthat can ferment glucose, but it can not ferment sucrose, lactose and maltose according to the key characters of the species *Kloeckera* (Kirsop et al. 1984).

Cluster III formed three sub-clusters that show the group comprising the genus *Saccharomyces* and *Saccharomycopsis* (Figure 1). Sub-cluster 1 consisted of isolates Klk6 and *S. cerevisiae* with a similarity value of 100% and *S. bayanus* joined by second strains with a similarity value of 96.7% (Figure 1). This indicates that isolates Klk6 is a member of the species *S. cerevisiae*. Characters of Klk6 isolates that reproduce by budding multipolar and not forming pseudomycelium, are consistent with the key character of the species *S. cerevisiae* (Kirsop et al. 1984), thus strengthening the identity of the isolates Klk6 is a member of that species. Sub-cluster 2 consists of isolates Klk2, *Sachharomycopsis fibuligera* and *Saccharomycopsis fermentant*. Klk2 isolates and *S. fibuligera* have a similarity value of 93.3%. Sub Cluster 3 consist of *S. fermentant* joining both sub clusters 1 and 2 with a similarity value of 74%. Isolates Klk2 possibilities are members of the species *S. fibuligera* because isolates Klk2 reproduce by budding bipolar and does not form pseudomiselium and the character similar with the character *S. fibuligera* (Kirsop et al. 1984). These three main clusters are then joined with a similarity value of 58.3%.

The diversity of yeast were obtained in this study showed similar results with the diversity of yeast from fermented cocoa beans in East Java, Indonesia plantation found by Ardhana and Fleet(2003), there are of the species *Kloeckera*, *S. cerevisiae* and *C. tropicalis*. Species of yeast that is found by Alwi (2009) from the cocoa bean origin Central Sulawesi, namely from the genera *Saccharomyces*, *Candida*, *Debaryomyces*, and *Hanseniaspora*. In addition, in some countries such as Brazil, Ghana and Malaysia was found yeast of the genus *Saccharomyces* and *Candida* were dominant in the cocoa fermentation (Schwan and Wheals 2004; Daniel et al. 2009). This indicates that the yeast of the genus *Saccharomyces* and *Candida* are indigenous yeasts in the cocoa fermentation.

The role of yeast isolates on the cocoa fermentation

The role of the seven yeast isolates to improve the quality of cocoa beans in accordance with the standards of quality based on cut test results to determine of unfermented cocoa beans levels and fat content. Characteristics of the quality of cocoa beans are fermented using yeast isolates listed in Table 3.

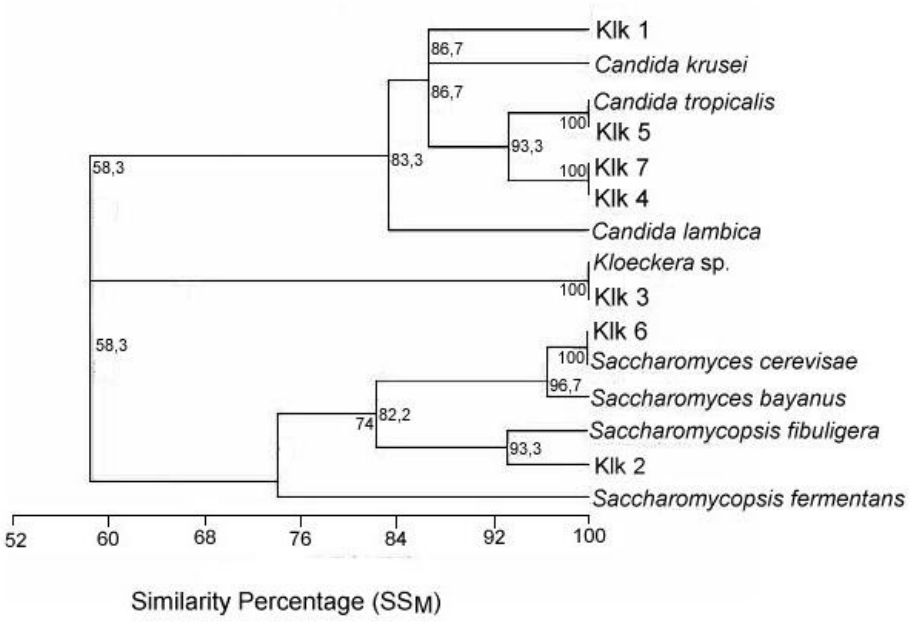

Figure 1. The dendrogram showing the phenetic relationship between 7 local yeast isolates and 8representatives of the yeasts species based on *simple matching coefficient* (SS_M) analysis and *unweighted pair-group method with arithmetic average* (UPGMA) algorithm based on phenotypic characters.

Table3showsthat the seven yeast isolates role in improving the quality of cocoa beans. Based on the levels of unfermented beans (Table 3) showed that the fermentation process, either fermented with the addition of yeast inoculum and spontaneous fermentation (without the addition of inoculum) can reduce levels of unfermented beans to 5-0% after five days of fermentation. This indicates that the fermentation process can improve the quality of cocoa beans up to the quality I (max. 3%) and quality II (max.8%) accordance with National Standard of Indonesia (SNI 2008). The addition of several inoculum yeasts (Klk3, Klk4 and Klk6) even produce cocoa beans that fulfilling the quality standards II within three days of fermentation and after five days of fermentation has fulfilled the quality standard I, while cocoa beans are fermented naturally only fulfilling quality standards II after five days of fermentation. Therefore, the local yeast isolates an important role in improving the quality of cocoa beans. It indicated that yeast inoculum in the fermentation can improve the quality of cocoa bean with reduced unfermented beans and enhanced fermented beans (entirely brown in color). This result consistent with research was done by Ho et al. (2014) was found beans fermented with yeast growth were fully brown in color and gave chocolate with typical characters which were clearly preferred by sensory panels. Therefore, yeast growth and activity were essential for cocoa bean fermentation and the development of chocolate characteristics.

The addition of yeast inoculum in the fermentation of cocoa beans can also increase the fat content of cocoa so that fulfilling the quality standards in accordance with SNI (2009), i.e. ≥ 48%. Table 3 shows that the addition of some of inoculum yeasts (Klk2, Klk4, Klk5, Klk6, and Klk7) in fermentation, able to produce cocoa beans with a fat content that fulfilling the quality SNI (2009) within 3 days of fermentation whereas spontaneous fermentation (without addition inoculum) has a fat content of less than standard quality up to the fifth day of fermentation. Joel et al. (2013) stated that the higher the percentage of fat content, hence the higher also quality of cocoa beans because fat is the most expensive component of cocoa beans. High-fat content in cocoa beans causes the cocoa is not easy rancid because the fat of cocoa contains polyphenols that act as antioxidants which can prevent rancidity and good for human health (Hii et al. 2009). Fat Cocoa contains one molecule bound triglycerides, oleic acid, palmitic and stearic cause cocoa easy to melt and give a distinctive flavor to the chocolate so that cocoa can be used in the manufacture of sweets or confectionery (Towaha et al. 2012).

Based on the observations result on cocoa beans were obtained after the fermentation using yeast inoculum (Table 3), showed that the addition of yeast inoculum role in the process of cocoa fermentation and can improve the quality of cocoa beans. The addition of yeast inoculums specially isolates Klk6 can also shorten the fermentation time from 5 days to 3dayswith the quality of cocoa beans fulfilling the SNI (2009).

Table 3. The characteristics of quality cocoa bean were fermented using yeast isolates for five days

Isolate code	Fermentation time (day)	Characteristics			
		Unfermented bean (%)		Fat content (%)	
		Sample	Quality standard[1]	Sample	Quality standard[2]
Klk1	0	80		43.18	
	3	10		45.93	
	5	5		50.55	
Klk2	0	80		44.30	
	3	15		48.70	
	5	0		50.05	
Klk3	0	85		44.46	
	3	5		46.07	
	5	0		52.67	
Klk4	0	85	**I: max. 3%**	44.67	
	3	7		48.04	
	5	3	**II: max. 8%**	50.71	**≥ 48**
Klk5	0	85		45.03	
	3	10	**III: max. 20%**	48.35	
	5	3		53.03	
Klk6	0	80		46.16	
	3	5		50.35	
	5	0		53.03	
Klk7	0	75		44.63	
	3	10		49.60	
	5	5		51.24	
Kontrol[3]	0	80		42.18	
	3	10		45.77	
	5	5		45.73	

Note: [1]SNI (2008); [2]SNI (2009); [3]Fermentation without yeast inoculum

In conclusion, diversity of seven yeast isolates from cocoa beans fermented spontaneously in Kolaka District, Southeast Sulawesi is quite high, which is identical to the five species of yeast, namely *Candida krusei, Candida tropicalis, Saccharomycopsis fibuligera, Kloeckera* sp. and *Saccharomyces cerevisiae*. The seven yeasts isolates role in the process of cocoa beans fermentation and can improve the quality of cocoa beans in a shorter time compared with spontaneous fermentation.

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REFERENCES

Alwi M. 2009. Characterization of yeast lived in the cocoa fruit in Southeast Sulawesi. Biocelebes 3 (1): 51-58. [Indonesian]

AOAC. 2005. Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg, MD.

Ardhana MM, Fleet HG. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. Intl J Food Microbiol 86 (1): 87-99.

Daniel HM, Vrancken G, Takrama JF, Camu N, De Vos P, De Vuyst L. 2009. Yeast diversity of Ghanaian cocoa bean heap fermentations. FEMS yeast Res 9: 774-783.

Guehi TS, Koffi KPB, Dabonne S. 2010. Spontaneous cocoa bean heap fermentation: Influence of the duration and turning on the quality of raw cocoa. World Acad Sci Eng Technol 46: 118-123.

Hii CL, Law CL, Suzannah S, Misnawi, Cloke M. 2009. Polyphenols in cocoa (*Theobroma cacao* L.). Asian J Food Agro-ind 2: 702-722.

Ho VTT, Zhao J, Fleet G. 2014. Yeast are essential for cocoa bean fermentation. Intl J Food Microbiol 174: 72-87.

Jamili, Yanti NA, Susilowati PE. 2014. Enhancement of cocoa quality by the indigenous yeast *Candida tropicalis* KLK4 through cocoa bean fermentation. J Adv Biotechnol 4 (1): 327-335.

Jespersen L, Nielsen DS, Hønholt S, Jakobsen M. 2005. Occurrence and diversity of yeasts involved in fermentation of West African cocoa beans. FEMS Yeast Res 5: 441-453.

Joel N, Pius B, Deborah A, Chris U. 2013. Production and quality evaluation of cocoa products (plain cocoa powder and chocolate).AmerJ Food Nutr 3 (1): 31-38.

Kirsop B, Painting K, Henry J. 1984. Yeast: Their Identification. Thailand Institute of Scientific and Technology Research, Bangkok.

Mahazar NH, Sufian NF, Meor Hussin AS, Norhayati H, Mathawan M, Rukayadi Y. 2015. *Candida* sp. as a starter culture for cocoa (*Theobroma cacao* L.) beans fermentation. Intl Food Res J 22 (5): 1783-1787.

NCYC [National Collection Yeast Cultures]. 2016. The National Collection yeast Cultures. Institute of Food Research, UK. http://www.ncyc.co.uk. [January 1, 2016]

Pereira GVM, Miguel MGCP, Ramos CL, Schwan RF. 2012. Microbiological and physicochemical characterization of small-scale cocoa fermentations and screening of yeast and bacteria strains to develop a defined starter culture. Appl Environ Microbiol 78: 5395-5405.

SchwanRF, WhealsAE. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr 44 (4): 205-221.

Sidarsyah. 2005. Characterization of Microorganisms in the Cocoa Beans (*Theobroma cacao* L.). [Hon. Thesis]. Halu Oleo University, Kendari. [Indonesian]

SNI [Indonesian National Standard]. 2008. Cocoa Beans 2323, 2008. [Indonesian]

SNI [Indonesian National Standard]. 2009. Mass Cocoa Beans 3749, 2009. [Indonesian]

Towaha J, Anggraini DA, Rubiyo. 2012. Performance of cocoa beans quality and their derivative products at various levels of fermentation: A case study in Tabanan, Bali. Pelita Perkebunan 28 (3): 166-183. [Indonesian]