

## THE CHARACTERISTICS YEAST ISOLATED FROM COMMERCIAL KEFIR GRAIN

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### ABSTRACT

The objective of this study was to investigate yeast characteristics macroscopic and microscopic obtained from commercial grain kefir from Surabaya (East Java Province). Isolation based on macroscopic and microscopic morphology. The first research method was activation of kefir grain in 10% reconstitution milk, yeast growth on Potato Dextrose Agar (PDA-Agar) medium, yeast coloration by using Lacto phenol cotton blue. Macroscopic morphological observations were list of the properties of colony morphology including size, shape, texture, color, surface, elevation, and edges. Microscopic morphological observations included cell shape, budding which first conducted preparations with yeast coloring then observed with Microscope [Zeiss Asio Imager A2] using camera [Zeiss AxioCam HRC]. Macroscopic observation of yeast size description colony very small, small, medium, large, colony form is round, the margin is raised, the elevation is entire, the texture is smooth and surface glistening, cream colony color, and yeast smell characteristic. Microscopic observation seen there is cell nucleus, oval, e.i. pseudohypa, budding, Gram-positive, urea test-negative; glucose, lactose, maltose fermentation test were positive, sucrose fermentation test negative, growth test on liquid media growth in surface medium (pellicle), and bottom medium (sediment). Based on the morphological observations in macroscopic and microscopic yeast identified genus *Saccharomyces*.

Keywords: characteristic, kefir grain, *Saccharomyces*, yeast.

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## INTRODUCTION

Kefir is milk fermented by several microbes from lactic acid-producing bacteria (BAL), acetic acid-producing bacteria, and yeast (Winarno and Fernandes, 2007). The role of yeast in kefir is important and it is what distinguishes it from other fermented products. Setyawardani *et al.* (2014) states that characteristic of the resulting kefir is influenced by the microflora composition contained in kefir grain. Brazilian kefir is dominated by lactic acid bacteria (60.5%) and yeast (30.6%) and acetic acid bacteria (8.9%). Surono (2004), suggested that Lactic Acid Bacteria (LAB) and yeast work mutually beneficial. Lactic acid produced by lactic acid bacteria can inhibit the growth of another lactic acid bacteria will be exploited by yeast.

The types of yeast found in kefir and kefir grain successfully isolated. Suriasih *et al.* (2012), that, yeast types *Candida fermata*. The isolates found by Sarikha *et al.* (2015) found 2 species of yeast *Saccharomyces cerevisiae* and *Candida ethanolica*. Simova *et al.* (2002), Witthuhn *et al.*, (2012); Sefidgar *et al.* (2014) found *Saccharomyces cerevisiae* in kefir. Wang *et al.* (2008), that the successful yeast in isolation from Taiwanese kefir is *Kluyveromyces marxianus* and *Saccharomyces unisporus*. Pindato *et al.* (1993) found yeast type *Saccharomyces unisporus*. This studies aims to determine the characteristics of yeast obtained from commercial kefir grain isolation based on macroscopic and microscopic morphology.

## MATERIALS AND METHOD

### **Yeast growth in Potato Dextrose Agar (PDA) media.**

The commercial kefir grain is obtained from Surabaya (East Java Province). The growing mushroom colon was then transferred to a petri dish containing PDA-Agar medium [Merck] by a pouring quadrant streak method, incubated for 24 hours at 37°C, and observed the macroscopic characteristics.

### **Macroscopic morphological observations**

Macroscopic morphological observation was observed colony morphology for isolation and purification, including the size of colony, form, elevation, margin, texture, color, and smell (Kreger-Van Rij, 1987; Widiastutik and Alami (2013).

### **Microscopic morphological observations**

This observation is a yeast cell observation including cell shape, budding which first make preparations with yeast coloring with lactophenol cotton blue then observed with Microscope [Zeiss Asio Imager A2 ] using the camera (Zeiss Axiocam HRC, Germany).

### **Lactophenol Cotton Blue Staining**

Take one dose of mushroom culture put in glass object that has been disinfected with Lactophenol cotton blue, cover with glass deck. Observe under 40× and 100× microscopes (Arumungan *et al.*, 2014).

### **Gram Staining Test**

NaCl drops 0.9% above the glass object, then takes two ounces of yeast colonies, flattens and fixes on bunsen fire, then stains with the sequence Crystal violet, iodine, alcohol, safranin. Wash over running waters then oil emersion drops and observe under a microscope 100×.

### **Urea Test**

Urea test was based on method (Kreger-Van Rij, 1987; Widiastutik and Alami, 2013), yeast inoculation on urea media add to Christensen (Merk) and be observed for seven days, positive if there is a color change.

### **Sugar Fermentation Test**

Sugar fermentation test based on method Harley and Prescott (2002); Widiastutik and Alami (2013), the sugar fermentation tests used are glucose [Merck], sucrose [Merck], maltose [Merck], and lactose [Merck]. Observations every day until the seventh day. A positive result if there is a change in color in the media becomes yellowish.

### **Test growth on liquid media**

Test growth on liquid media modification method Nurhayati (2004), yeast isolates are inoculated to Sabouraud Dextrose Broth (SDB) [Oxoid] media. Then the yeast growth in the media observed

characteristic, kefir grain, *Saccharomyces*, yeast.

the growth of yeast by looking at the presence of rings, pellicles on the surface of the media, and sediment on the media base.

## RESULT AND DISCUSSIONS

### Macroscopic Morphological Identification

The isolate of yeast of kefir grain using Potato Dextrose Agar (PDA) medium incubated at 37°C for 24 hours illustrated in figure 1. Macroscopic morphological observations of yeast isolation results can be seen in table 1. The results obtained, that the size of the colony is very small, small, and large. Forms of colonies was grows on PDA media Agar has a round shape, with entire elevation, raised margin, smooth and surface glistening texture, color white cream, has characteristic of smell was the characteristic of yeast.

Table 1. Macroscopic observation results of yeast

Macroscopic Observation	Isolated of Yeast							
	A1	A2	A3	A4	B1	B2	B3	B4
Colony Size	medium	medium	Small	Very smal	large	Medium	small	Large
Colony form	round	round	Round	round	Round	Round	round	Round
Colony edge (margin)	raised	raised	Raised	raised	raised	Raised	raised	Raised
Colony surface (elevation)	entire	entire	Entire	entire	entire	Entire	entire	Entire
Texture	smooth	smooth, Surface glistening	smooth, Surface glistening	smooth	smooth	smooth	smooth	Smooth
Colony color	White Cream	White Cream	White Cream	White Cream	White Cream	White Cream	White Cream	White Cream
Smell	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast	Yeast

Macroscopic observation the size of the colonies varies from very small to large in size. These results show that although the size of the colonies varies but shows the characteristics of colony shapes, colony edges, surfaces, color, and smell shows the same characteristics. Yavad and Tiwari (2016) was studied about observations macroscopic characteristics *Saccharomyces* that found that the yeast was visible smooth surface, circular margin, yellowish color. Widiastutik and Alami (2013), observed the morphological characteristics of yeast colonies based on macroscopic observations and report that the yeast was white to creamy butyrous textures with dull surfaces,

raised elevations. Suriasih *et al.* (2012) describes colored macroscopic morphology of the yeast was cream, whole dull shapes, and convex surface.

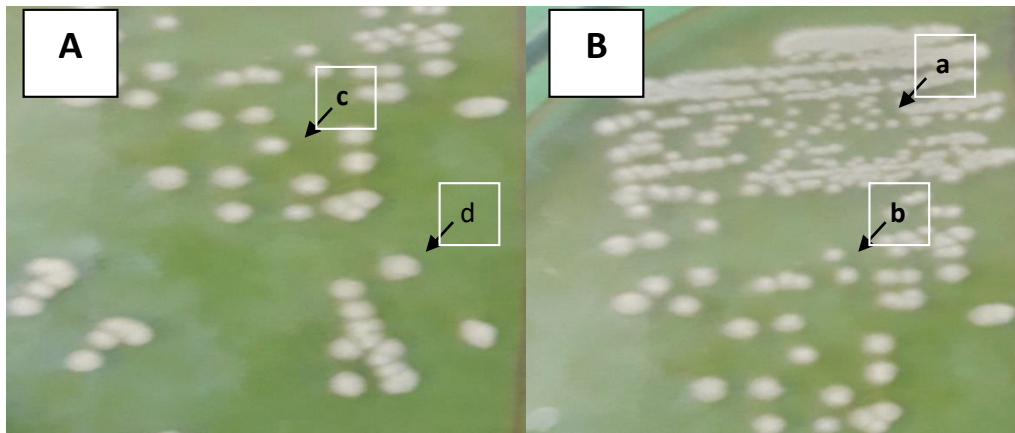


Figure 1. Yeast on PDA-Agar medium (Potato Dextrose Agar).

Description: (A) c. medium yeast colony, d. large yeast colonies, (B) PDA media. a. very small size yeast colonies, b. small yeast colony

### Microscopic Morphological of Yeast Identification

The results of microscopic morphology from yeast isolation can be seen in Table 2. The Results indicated that all isolates have an oval-shaped cell shape, multilateral sprays characteristics, with clearly pseudohypha and budding that was illustrates in Figure 2. Biochemical fermentations was carbohydrate fermentation for glucose, maltose and lactose tests but it does not have ability to ferment sucrose. Urea test indicated that all isolates were unable to hydrolyze urea. The results of the Gram staining test showed that all isolates were purple or included Gram Positive (Figure 3). Based on the growth test results, yeast growth is on the surface and there is sediment at the bottom (Figure 4).

Table 2. Microscopic observation results of yeast

Isolate code	Cell form	germination pattern	Sexual reproduction	Sugar fermentation test				Urea test	Gram staining test	Growth test on liquid media
				Glukosa	Sukrosa	Laktosa	Maltosa			
A1	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
A2	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
A3	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment

characteristic, kefir grain, *Saccharomyces*, yeast.

A4	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
B1	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
B2	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
B3	Oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment
B4	oval	Multilateral	Budding	+	-	+	+	-	Gram positive	The are pellicle and sediment

In Table 2 describes the yeast microscopic of yeast was shape of an oval cell, with multilateral sprouting patterns that have pseudohypha, and budding sexual reproduction. Yeast morphology observations can be seen in Figure 2. Based on the observation of yeast morphology found in kefir grain (Figure 2) seen there are two types of cell shape visible. The codes (a) and (b) show the shape of a round mushroom and have one cell nucleus and also see yeast form buds in the codes (c) and (d). Based on the morphological results of these types of yeast including *Saccharomyces mushroom* species. Widiastutik and Alami (2013) states that the genus *Saccharomyces* has cell-shaped round, elliptical or cylindrical, forming pseudohypha but not for hyphae. Asexual reproduction with multilateral and sexual encounters with ascospores (1-4 or more per askus).

The sugar fermentation test results of each isolate code were able to ferment glucose, lactose, and maltose, but were unable to ferment sucrose. The urea test results are negative, and the gram test includes Gram-positive (Figure 3). Yavav and Tiwari (2016) describes that *Saccharomyces cerevisiae* in the urea test is negative and the cell color is purple (Gram positive). Growth in liquid media can be seen with rings and sediment in the isolated section. This indicates that isolates have the ability to grow aerobically which is characterized by the presence of pellicles on the surface and the presence of sediment at the bottom of media was anaerobic growth.

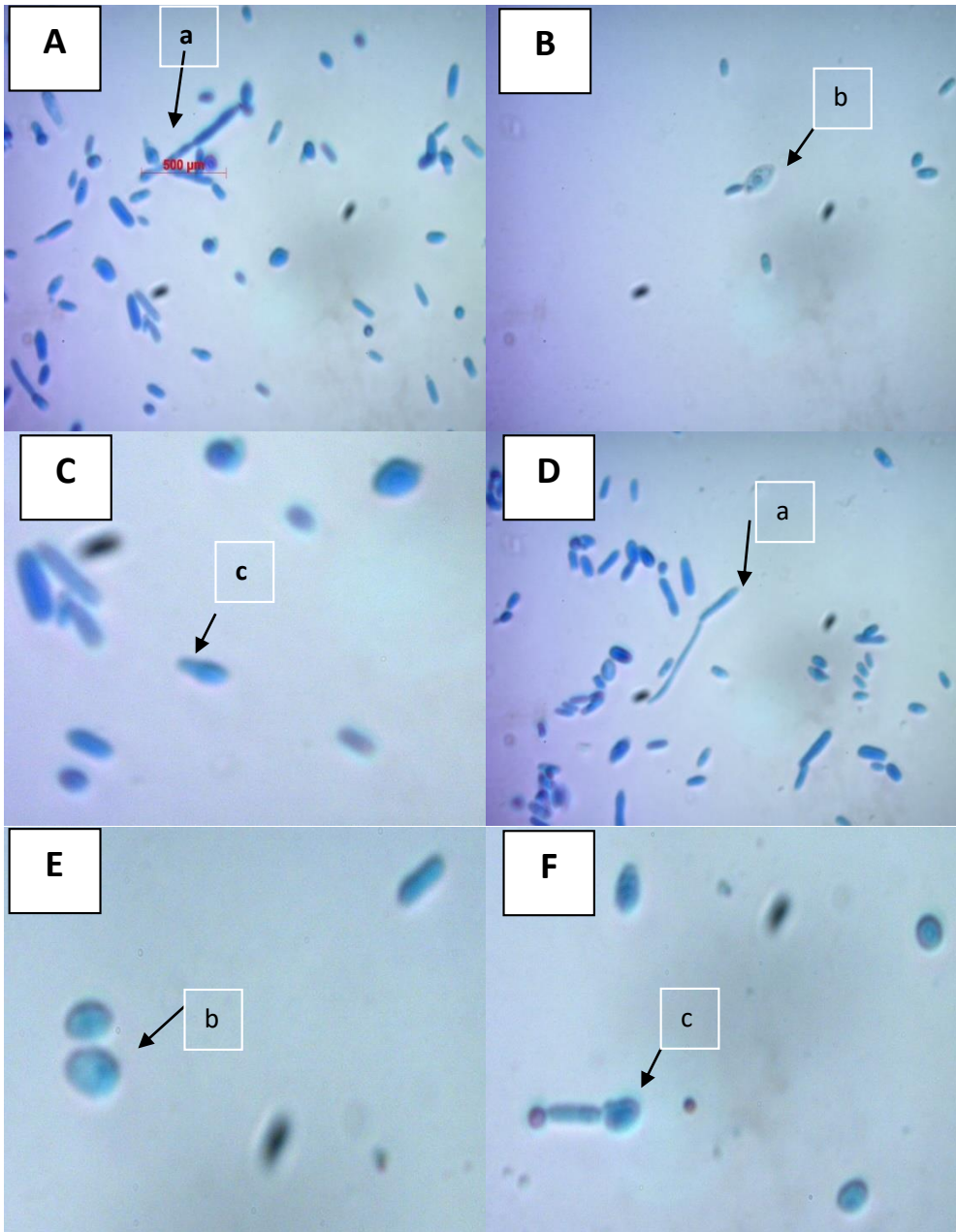


Figure 2. The observed morphology of yeast on kefir grain with lactophenol cotton blue stain:  
a) Pseudohypha, a cell nucleus, c) budding)

characteristic, kefir grain, *Saccharomyces*, yeast.

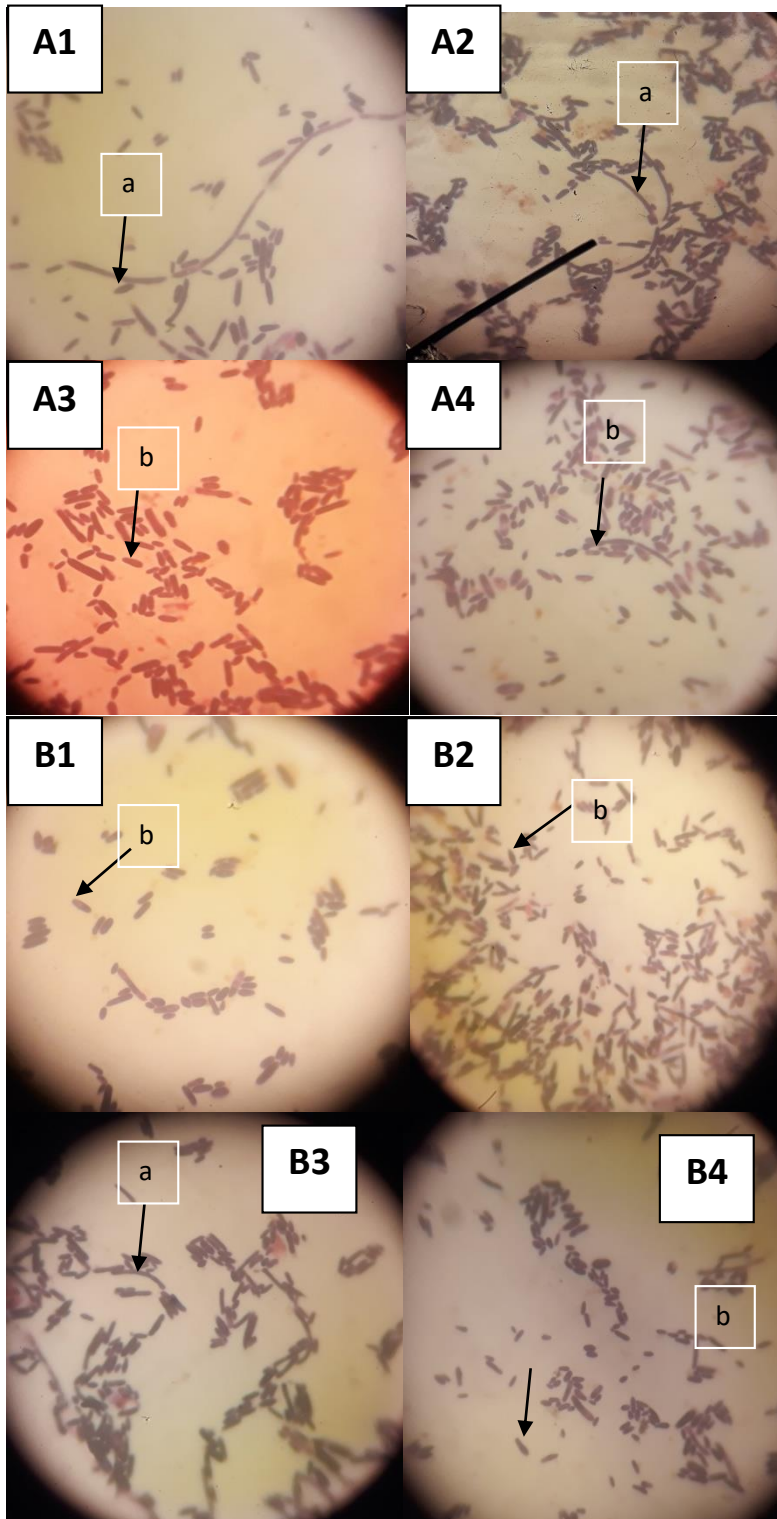


Figure 2. The observed of yeast on kefir grain with gram stain a) Pseudohypha, b) budding



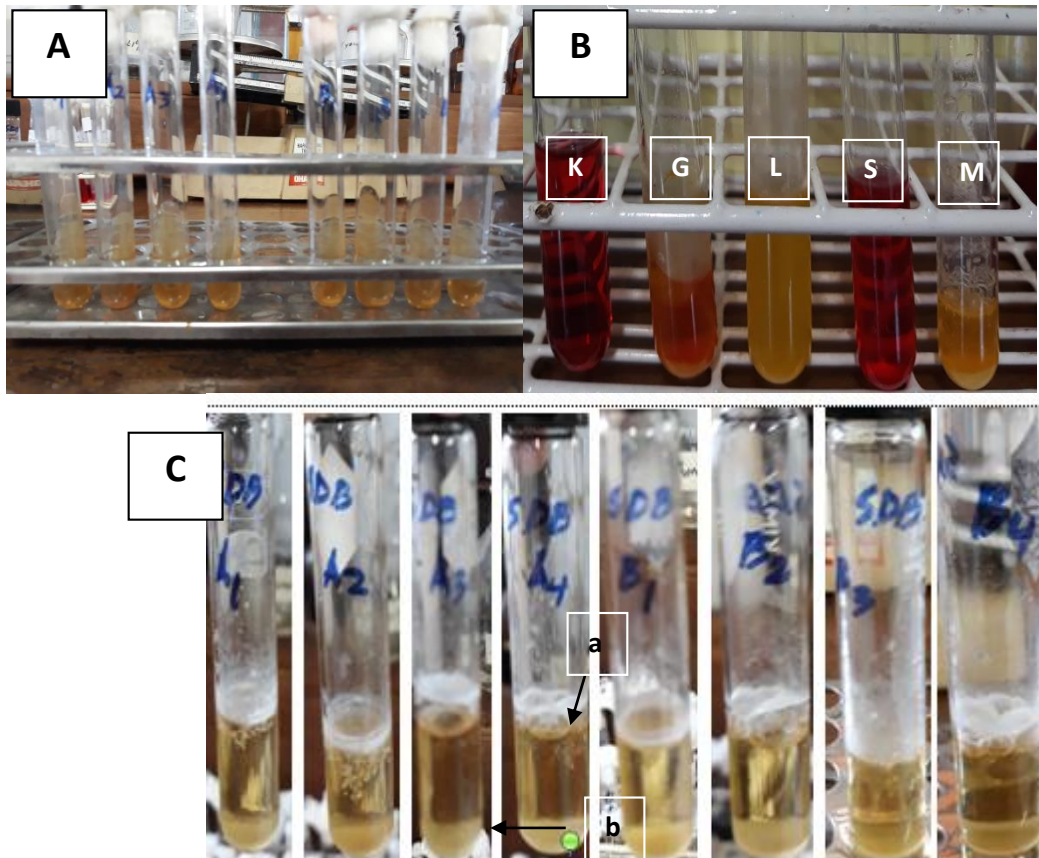


Figure 4. Results of biochemical observations (description of A = urea test, B = sugar fermentation test, C = growth test on liquid media, K = control, G = glucose, L = lactose, S = sucrose, M = maltose, a = pellicle , b = sediment)

## CONCLUSIONS

Based on the macroscopic and microscopic morphological observations that yeast types were identified that were isolated from commercial grain kefir obtained yeast genus *Saccharomyces sp.*

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