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Microbiological and Chemical Properties of Kefir Made of Bali Cattle Milk

Ketut Suriasih[1,*] Wayan Redi Aryanta[2] Gede Mahardika[1] Nyoman Mantik Astawa[3]

1. Faculty of Animal Husbandry, Udayana University ,PO box 80237, Bali, Indonesia.
2. Faculty of Agricultural Technology, Udayana University, PO box 80237, Bali, Indonesia.
3. Faculty of Veterinary Science, Udayana University, PO box 80237, Bali, Indonesia.

* E-mail of the corresponding author ketutsuriasih@ymail.com

Abstract

Information regarding to microbiological and chemical characteristics, and incubation time is crucial in developing kefir prepared using Bali cattle milk. This study was intended to investigate microbiological and chemical properties of the kefir prepared of Bali Cattle milk and Indonesian kefir grains after 24, 48 and 72 hours incubation periods. A completely randomized design, with 3 treatments, and 9 replicates were undertaken. Kefir samples were taken at the end of incubation period for determination of total lactic acid bacterial and yeast counts, pH, titratable acidity, lactose percentage and protein content. The result of this research showed that the total lactic acid bacterial counts were 10^8 – 10^9 cfu/ml, while yeast counts were ranging from 10^5 – 10^6 cfu/ml, no coliform and Escherichia coli were detected in any kefir samples in this research. Identification of the lactic acid bacteria and yeast revealed that the *Lactobacillus paracasei* ssp. *paracasei 1* was the predominant species found in the kefir samples, followed by *Lactobacillus brevis* and the yeast *Candida famata*. Chemical analysis of the kefir samples showed that, protein, lactose, titratable acidity and pH of the kefir samples were in the range of 5.68 - 6.26%, 3.98 - 4.67%, 0.89 - 1.73% and 3.38 - 4.35, respectively. The result also indicated that the incubation period significantly affected the microbial counts and chemical composition of Bali Cattle milk kefir.

Keywords: Kefir, Bali cattle milk, Lactic acid bacteria, Yeast, Protein, Lactose

1. Introduction

Kefir is a fermented milk beverage which has a slightly acidic taste and some effervescence due to lactic acid, carbon dioxide and a minute (<2%) concentration of alcohol resulting from the action of microorganisms present in kefir grains used to cultured the milk. Kefir grains are small, gelatinous, yellowish in color, and appear as small clamps of irregularly shaped cauliflower. The kefir grain is believed originated from the region of Caucasian mountains (Farnworth, 2005). The grains contain a mixture of complex microflora such as lactic acid bacteria, yeast and sometimes acetic acid bacteria which are lodge by a polysaccharide matrix calls “kefiran”. These microorganisms, called probiotics (Farnworth and Mainville, 2003; Powell, 2006), which consist of lactobacilli, such as *Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus kefir,* and *Lactobacillus casei*; lactococci including *Lactococcus lactis* sbsp. *lactis, Lactococcus lactis* sbsp. *cremoris, Streptococcus salivarius* sbsp. *thermophilus, Leuconostos mesenteroides, Leuconostoc cremoris* and a variety of yeast such as *Candida, Kluyveromyces* and *Saccharomyces* sp. (Oberman and Libudzisz, 1998).

Microbial activity in kefir fermentation is a symbiotic metabolic activity of a number of bacteria and yeast species, which degrade milk constituents to lactic acid, acetic acid, ethanol, carbon dioxide and other flavor compound such as acetaldehyde result in a distinctive flavor of the kefir beverage (Farnworth and Mainville, 2003). The beverage also contains easily digestible proteins and essential amino acids that help the body healing and maintenance functions (Otles and Cagindi, 2003)

Generally, kefir is made of cow’s milk, but many types of milk such as goat, ewe, mare, buffalo, camel is also can be used. Bali Cattle is an Indonesian indigenous cattle which yield a high meat quality. Besides, Bali cattle also produce milk about 1.5 - 2.5 litter/head/day. Bali Cattle milk contains around 5.19% protein, 7 - 8% fat, 5.18% lactose, 0.21% calcium, 0.18% phosphor and 18 - 19% total solid (Sukarini, 2000), while other cows milk such as Holstein cows milk, composed of 3.3%, 4.0%, 5.0%, 0.12%, 0.095% and 12.1% protein, fat, lactose, calcium, phosphor and total solid respectively (Campbell and Marshall, 1975). The chemical components of Bali Cattle milk was 50 – 75%

higher than that of the Holstein cows milk. In addition milk have a high nutritive value due to the present of all acids amino acids and the balance of calcium and phosphor ratio (Campbell and Marshall, 1975) Moreover, Hui *et al.* (2007) stated that, in addition to major macronutrients, milk subjected to fermentation is an excellent source of important mineral, namely calcium and phosphorus and B groups vitamins. The low pH in fermented milk, makes the calcium of the fermented milk is readily available as ionic form, which enhanced its absorption, and make it as a good source of calcium for pregnant and postmenopausal women as well as for women suffer from obesity. The phenomena, together with the prime chemical content of Bali cattle milk, bring into a consideration of using it as a potential source of animal protein in rural villages and fermenting it to kefir product will make it more valuable to community healthcare.

There are variations in fermentation period of kefir production in order to obtain higher bactericidal effect again pathogens (Ulusoy *et al.,*2007), to increase the immunostimulator effects (Hong *et al*., 2009), while Motaghi *et al.* (1997) incubate the milk for 72 hours and evaluate the chemical characteristics of the kefir obtained. There is lack of information regarding microbial population of kefir with longer incubation time. There is also no information about chemical composition of kefir prepared using Bali cattle milk. Thus, the objective of this study was to evaluate the microbiological and chemical compositions of Bali cattle milk kefir produced through different incubation periods.

2. Materials and methods

2.1 Kefir grains

Kefir grains were obtained from Faculty of Animal Husbandry, Bogor Institute of Agriculture, Indonesia. The grains were propagated at room temperature (27^o – 28^oC) for 24 h with twice weekly transfers in sterilized milk and kept at 4^oC for short storage.

2.2 Kefir preparation

Bali cattle milk was obtained from farmers around Tabanan Regency, Province of Bali, Indonesia. Milk from the farmer after sieved through cheese cloth was heated to 95^oC for 15 min and then cooled to 25^oC. The heat treated milk was inoculated with 5% (w/v) kefir grains, distributed into sealed glass bottles and incubated at room temperature for 24 , 48 and 72 hours.

2.3 Microbial enumeration

Kefir samples (10 ml) were pipetted aseptically and diluted in 90 ml sterile saline solution (0.85% NaCl) and homogenized in a vortex mixer. Subsequent serial dilutions were prepared and viable numbers of the microorganisms enumerated using a surface spread-plate technique, plating in duplicate. A total of 100 µl of each diluted sample were inoculated on De Man, Rogosa and Sharpe (MRS) (Oxoid CM361) and M17 (CM785) agar for enumeration of lactic acid bacteria; on oxytetracylin glucose yeast extract (OGYE) agar (Oxoid CM545) for yeast. Plates were incubated for 48 hours at 37^oC for lactic acid bacteria and 72 hours at 25^oC for yeast. Colony forming units were quantified from plates showed 30 – 300 colonies. For each type of the medium containing isolated colonies, five to ten well separated colonies were taken at random for identification.

2.4 Identification of isolates

Isolated colonies have been chosen were further purified by successive streaking on the corresponding agar. Isolates of lactic acid bacteria were preserved in sterile MRS broth (Oxoid CM359) supplemented with 30% (v/v) sterile glycerol and stored at -20^oC until further tests, while isolates of yeast were streaked on 4% glucose-yeast–peptone agar slant (Walt and Yarrow, 1987) and stored at 4^oC until tested.

For identification of lactic acid bacteria to genus level, isolates were microscopically examined for gram stain reaction, cellular morphology, and cellular arrangement (Sneath *et al.,* 1986); catalase activity was tested by spotting colonies with 3% hydrogen peroxide on glass object and production of CO2 from glucose was tested in MRS broth containing Durham tube (Cowan, 1974). Isolates showed Gram-positive and catalase-negative reaction were then identified to species level by subjecting them to growth at 15^oC, 37^oC and 45^oC as well as at pH 2, 3, 4 and at 6.5% NaCl, which were assayed in MRS broth. Except stated, broth were incubated at 37^oC (Holt *et al.,* 1994). Finally the isolates were assayed for carbohydrate fermentattion using API 50 CH (BioMerieux, France).

Isolates of yeast were characterized by colony and cell morphology, type of reproduction, pseudohyphae formation, growth at 37^oC, growth in 50% glucose, hydrolysis of urea, assimilation of nitrate, assimilation and fermentation of

different carbon sources , and yeast identities were verified using the keys of Yeast (Kreger-Van Rij, 1987) and API 20C system.

2.5 Chemical analysis

After incubation for 24, 48 and 72 hours, kefir samples were characterized in relation to their chemical composition. Protein percentage was analyzed according to Kjeldahl Method, AOAC official method 991.22, base on the protein nitrogen content of the kefir samples and then expressed as protein percentage by multiplying the nitrogen content determined by 6.38 (AOAC, 1999). Titratable acidity were determined according to AOAC official method No. 947.05 (AOAC, 1999), pH was measured using a digital pH-meter (ISTEK, Model 720P. Korea). The pH meter was calibrated with standard buffer solutions pH 4.0 and 7.0 before measuring the kefir samples.

Lactose concentration of the kefir samples were measured by HPLC method using Metacarb 87C column (ICI instruments- Australia), a refraction index detector (Shodex RI SE-61) and an integrator CR6A Chromatopac (SHIMADZU, Kyoto). The mobile phase (eluent) is deionized water at a flow rate of 0.5 mL/min. The column is maintained at 90^{o}C. A total of ten µl of samples by sequential treatments with centrifugation at 12,000 rpm for 15 min., and filtering by 0.45 µm (Whatman, Kent, UK) were applied.

2.6 Statistical analysis

Incubation process was carried out in nine replications and duplicate analyses were performed on each replication. Values of different test were expressed as the mean ± standard deviation (x ± SD). The SPSS version 13 program for Windows was applied for the statistical analysis. Significant differences between mean ($P<0.05$) were determined by a one-way ANOVA (Least significant different test)

3. Results and discussion

3.1 Microbial characteristics

The data obtained from microbial enumeration were presented in Table 1. Lactic acid bacteria were more frequently microorganisms group found in any kefir sample, ranging from 10^8 to 10^9 cfu/ml, followed by yeast, which ranging from 10^5 to 10^6 cfu/ml. The lactic acid bacterial counts in kefir incubated for 24 hours were in accordance with Paramita *et al.* (2008) who reported that, the population of lactic acid bacteria in kefir were ranging from $10^9 – 10^{10}$ cfu/ml, but were higher than that reported by Farnworth and Mainville (2003) and Garcia-Fontan *et al.* (2006) of around 10^8 cfu/g. The differences in microbial count were caused by different incubation temperature during kefir preparation. In this experiment, as well as at the study conducted by Paramita *et al.* (2006), kefir preparation was hold at temperature of 28 ± 2^{o}C, while kefir preparation reported by Farnworth and Mainville (2003) and Garcia-Fontan *et al.* (2006) were incubated at temperature of 22 – 25^{o}C. Growth of microorganisms including bacteria were greatly affected by temperature of the environment. Lactic acid bacteria are mesophiles, grow at temperature range of 10 – 45^{o}C, with optimum temperature of 30 – 40^{o}C. Within the optimum temperature range, growth of bacteria will increase as the environmental temperature raise, which result in fast growth rate and so that, greater microbial population. Beyond the optimal temperature, the microorganims will grow better if the environmental temperature was nearer to the optimum temperature (Todar, 2009).

Incubation periods significantly affected the lactic acid bacteria counts of kefir samples (Table 1). Increasing incubation periods from 24 to 48 and 72 hours, respectively reduced the number of lactic acid bacteria of 2.1% and 3.44 % ($P<0.05$). The reductions appeared due to the drop in pH of kefir samples (Table 2). During kefir preparation, lactic acid bacteria will convert lactose of the milk to obtain energy for their maintenance and growth, and released metabolites, primarily lactic acid, result in a decrease in the pH of the environment (kefir). Longer incubation time, means more time available for the lactic acid bacteria to metabolized the lactose of the milk, and so that more lactic acid produced, which contributed to much lower pH surrounding the lactic acid bacteria. The dropped in pH of kefir samples below the optimum level, affect the intracellulair pH of the lactic acid bacteria, which inhibit the enzyme activity, ion transport and nutrient uptake, and so that retard the growth and then the counts of the lactic acid bacteria. According to Kandler and Weiss (1986), growth of lactic acid bacteria were better in neutral pH, and they grow optimally at pH 5 to 9. Moreover, Kashket (1987), Hutkins and Nannen (1993) found that, reduction of extracellular pH below the optimal level is harmful to the bacteria, cause acidification of cytoplasmic pH, below a threshold value and subsequent inhibition of cellular function, which result in slower metabolism and growth of the bacteria, and lower the lactic acid bacterial counts.

The yeast counts (10^5 – 10^6 cfu/ml) of kefir samples were higher than that reported by Oner *et al.* (inpress) who reported that, the yeast counts of the kefir samples prepared using kefir grains were about 10^4 to 10^5cfu/g. The higher counts of yeast in this study were caused by higher incubation temperature (28 ± 2°C), compared to 22°C in the study of Oner *et al.* (in-press). Higher temperature (within the optimum range) will increase metabolic activity of the yeast which result in faster growth rate and higher population of the yeast. This is in accordance with Jay (1992) and Todar (2009), who stated that yeast can grow at temperature range of psychrotroph and mesophile (< 7° – 45°C), with the optimum temperature range from 20° – 30°C. Within the optimum temperature range, growth of yeast will increase, in parallel with the increase in environmental temperature due to the increase in metabolism and enzyme activity. Lengthening incubation periods from 24 to 48 and 72 hours significantly reduced the yeast counts of 2.9 and 5.1% ($P<0.05$) respectively. The reductions appeared due to the drop in the pH of the kefir samples. In general, yeast is an acidophilic organism and, so that, grow better under acidic condition. The optimal pH for growth of yeast can vary from pH 4 to 6 (Narendranath and Power, 2005). Deviation of the surrounding pH below optimal level, as such, in kefir samples incubated for 48 and 72 hours, in which the pH drop were 0.18 and 0.62 unit (or 0.83 and 0.44 unit from pH of kefir sample incubated for 24 hour), cause difficulties in enzymes activity, disturb yeast metabolism, and so that, the yeast cell will not be able to grow normally, result in the drop of the yeast counts.

3.1.2 Identification of microbial isolates of Bali cattle milk kefir

A total of 115 isolates were obtained from Bali cattle milk kefir samples with different incubation periods (Tables 2). Among the isolates, 79 (68.7%) were lactic acid bacteria and 36 (31.3%) were yeast. Identification to genus level showed that all the isolates of lactic acid bacteria were gram positive, catalase negative, rod in shaped, no spore formation, grew at 15°C , 37°C but not at 45°C; fifteen (19%) of the isolates produced CO_2 from glucose and 64 (81%) isolates did not produce CO_2 from glucose. After these tests, ten representative isolates were chosen from those 79 isolates, and then identified to species level using API 50 CH strip and API 50 CHL medium. Result showed that 64 (81%) isolates of the lactic acid bacteria were *Lactobacillus paracasei* ssp. *paracasei 1*, and the rest (15 isolates) were Lactobacillus brevis (Tables 3 and 4). This is in line with Karna *et al.* (2007) and Simova *et al.* (2002) who found that, *Lactobacillus paracasei* ssp. *paracasei* is a probiotic strain occur in dairy products such as yakult and kefir. Furthermore, Magalhaes *et al.* (2011) found that, *Lactobacillus paracasei* predominate the lactic acid bacterial population and contributed to 35.74% of the total lactic acid bacteria in Brazilian kefir. In addition to *Lactobacillus paracasei*, Simova *et al.,* (2002), Frengova *et al.* (2002) and Bosch *et al.* (2008) also found *Lactobacillus brevis* in kefir and kefir grains. Occurrence of *Lactobacillus paracasei* and *Lactobacillus brevis* in different sources of kefir were also reported by Farnworth (2005).

With respect to the yeast isolates, all the isolated colonies were white, circular, entire, convex, grow at 37°C, cells were ellipsoidal, elongate, occurring in single, no formation of pseudomycelium, nitrate not assimilated, no growth in vitamin free medium, and no growth in 50% glucose-yeast extraxct agar. Glucose, galactose, maltose were fermented, but not lactose. Assimilation of carbon compound with reference to API 20 C revealed that these isolates were *Candida famata* (Table 5.),

Occurrence of *Candida famata* in kefir in this study was in accordance with Johnson and Erasun (2011) who stated that representative yeast frequently found in fermented milk products including kefir were *Candida kefyr, Candida krusei, Candida famata, Debaryomyces hansenii, Saccharomyces cerevisiae, Kluyveromyces marxianus* and *Kluyveromyces lactis*. Yeast play an important role in preparation of kefir, by providing essential growth nutrients such as amino acids and vitamins to the lactic acid bacteria occurred in kefir, secrete ethanol and produce CO_2, and released metabolites that contribute to the flavour and mouthfeel of kefir (Farnworth, 2005). With respect to *Candida famata,* Meyer *et al.* (1987) reported that, *Candida famata* was lactose-negative fermenting yeast, but could assimilate D/L- lactose. Moreover, Farnworth and Mainville (2003) explained that, yeast species use lactic acid released by lactic acid bacteria, as energy sources for growth, and thus raise the pH environment, and stimulate further growth of the lactic acid bacteria. This symbiotic metabolic activity of yeast and lactic acid bacteria in kefir maintain the stability of the product (Motaghi *et al.,* (1997).

3.2 Chemical Composition of kefir samples

Chemical composition of kefir depends on many factors such as kind of milk and technological conditions (Sady e*t al.,* 2007). Chemical composition of pasteurised Bali cattle milk used for kefir preparation composed of 5.50 ± 0.26% protein, 5.48 ± 0.16% lactose, 6.06 ± 0.14% fat. Technological process in kefir preparation will changes some

of these chemical components.

Chemical properties of kefir samples are given in Table 2. The data showed that, pH, titratable acidity, protein and lactose content were significantly affected by incubation periods ($P< 0.05$). Lengthening the incubation periods from 24 to 48 and 72 hours, significantly decreased the pH of kefir samples of 11.83% and 20.42%, respectively. pH of the kefir samples were a reflection of organic acids (primarily lactic acid) accumulation, yield from lactose metabolism by lactic acid bacteria grew in the kefir. The lower pH of the kefir samples incubated for 48 and 72 hours compared to that incubated for 24 hours appeared due to accumulation of lactic acid yield during the first and the second 24 hours fermentation (48 hours incubation period) and during the first, second and third 24 hours fermentation (72 hours incubation period). The result was in line with Motaghi *et al.* (1997) who reported that, kefir produced through 24 hours incubation period had the highest pH, followed by kefir produced through 48 and 72 hours incubation periods.

Lactose content of kefir samples incubated for 48 and 72 hours were significantly lower than that of the kefir samples incubated for 24 hours. Increasing incubation period from 24 to 48 and 72 hours, significantly reduced the lactose content of the kefir samples of 10.92% and 4.32% ($P<0.05$), respectively. The decrease in lactose percentage due to longer incubation periods are supported by the finding of Garcia-Fontan *et al.* (2006) and Purnomo and Muslimin (2012) who reported that, lactose content of kefir, decreased 0.70% and 0.07%, respectively during the first 24 hours fermentation. Lower lactose percentage in kefir prepared through longer incubation periods was in accordance with Motaghi *et al*. (1997) who found that, the reduction in sugar content of kefir was observed as a function of incubation time. The phenomena was caused by more lactose fermented by microorganisms occurred in kefir preparation, as more time available for fermentation activity, due to longer incubation period. In addition, lactose is a carbohydrate component of milk and, as such, the primary energy sources for growth of microorganisms occur in milk, especially for lactose fermenting microorganisms. According to Ismaiel *et al.* (2011), lactose was found as the most effective carbohydrate for growth of kefir grains microorganisms in kefir preparation.

With respect to protein content, it was found that lengthening the incubation period from 24 to 48 and 72 hours, significantly increased the protein content of the kefir samples. Significant increase was also occurred when the incubation period was lengthened from 48 to 72 hours. The increase in protein content due to longer incubation period was in accordance with Magalhaes *et al*. (2011), who reported that the protein content of kefir determined after 24 hours fermentation was higher than that before fermentation, due to the increase of microbial biomass, which also reveals that protein content of kefir samples consist of protein originated from the milk used for kefir preparation and biomass of the microbe grow in the kefir samples. It could be deducted that, protein content of 48 and 72 hours kefir samples come from protein of the milk and accumulation of biomass from microbe grow during 48 and 72 hours incubation periods. This accumulation caused the protein content of the kefir samples incubated for 48 and 72 hours were higher than that incubated for 24 hours.

4. Conclusions

Kefir prepared through 24 hours fermentation showed the highest microbial population, lactose content and pH, but the lowest titratable acidity and protein concentration. Lengthening the incubation period from 24 to 72 hours decreased the lactic acid bacteria and yeast counts to about 1 log. There were two species of lactic acid bacteria found in kefir samples in this study, namely, *Lactobacillus paracasei* sbsp. *paracasei 1* and *Lactobacillus brevis*, and only one species of yeast, namely *Candida famata* was identified from the kefir samples in this study. Those microorganisms were found in any kefir samples through out the three incubation periods. The increased in incubation from 24 to 48 and 72 hours, significantly raised the protein and titratable acidity concentration of the kefir samples of around 0.31 to 0.58% and 0.39 to 0.84% respectively, but significantly decreased the pH and concentration of lactose of the kefir samples of around 0.53 to 0.97 unit and 0.51 to 0.69% respectively.

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Table 1. Microbial counts of Bali cattle milk kefir samples from different incubation periods (log cfu/ml) *

Culture Medium/Microorganisms group	Incubation periods (h)		
	24	48	72
MRS/ Lactic acid bacteria	9.51 ± 0.18[a**]	9.31 ± 0.09[b]	8.99 ± 0.20[c]
M17/ Lactic acid bacteria	9.49 ± 0.08[a]	9.39 ± 0.06[b]	9.28 ± 0.15[c]
OGYE/ yeast	6.44 ± 0.15[a]	6.25 ± 0.19[b]	5.93 ± 0.17[c]

* Means of nine replications ± standard deviation

** Means with different superscript in the same line showed significant differences (P< 0.05)

Table 2. Average chemical composition of Bali cattle milk kefir with different incubation period *

Components	Incubation periods (hours)		
	24	48	72
Protein (%)	5.68 ± 0.27[b]**	5.99 ± 0.13[b]	6.26 ± 0.27[c]
Lactose (%)	4.67 ± 0.20a	4.16 ± 0.13[b]	3.60 ± 0.23[c]
pH	4.35 ± 0.08[a]	3.82 ± 0.06[b]	3.38 ± 0.10[c]
Titratable acidity (%)	0.89 ± 0.03[a]	1.28 ± 0.04[b]	1.73 ± 0.02[c]

* Means of nine replications ± standard deviation

** Means with different superscript in the same line showed significant differences (P< 0.05)

Table 3. Morphological Properties of lactic aci bacteria species isolated from kefir made of Bali cattle milk compared to standard description of Sneath et al. (1984)

Properties		Strain 1 (4 isolates)	Strain 2 (3 isolates)	Strain 3 (3 isolates)
Colonies		Round, smooth, white, convex	Cream, raised, dull, triangular	Round, smooth, white, convex
Cells		rods, with square ends, pairs and chains	rods, square ends, pairs and chains	rods, square ends, pairs and chains
Gram reaction		+	+	+
Catalase		-	-	-
Spore formation		-	-	-
Gas from glucose		-	-	+
Growth at:	15°C	+	+	+
	37°C	+	+	+
	45°C	-	-	-
Growth at pH :	2	+	+	+
	3	+	+	+
	4	+	+	+
Growth at 6.5% NaCl				
Growth at 0.2 mM NaDeoxy Cholate		+	+	+

Table 4. Sugar Fermentation of lactic acid bacteria species isolated from kefir made of Bali cattle using API 50 CH

Compounds	Strain 1(4 isolates)	Strain 2(3 isolates)	Strain 3 (3 isolates)
Glycerol	-	-	-
Erythritol	-	-	-
D-arabinose	-	-	-
L- arabinose	-	-	+
D- ribose	+	+	+
D-Xylose	-	-	+
L-Xylose	-	-	-
D-adonitol	-	-	-
Methyl-ßD-xylopranoside	-		-
D-galactose	+	+	+
D-glucose	+	+	+
D-fructose	+	+	+
D-mannose	+	+	+
L-sorbose	-	-	-
L-rhamnose	-	-	-
Dulcitol	+	+	+
Inositol	+	+	-
D-Mannitol	+	+	+
D-Sorbitol	+	+	+
Methyl-αD-mannopyranoside	-	-	-
Methyl-αD-glucopyranoside	+	+	-
N-acetyl glucosamine	+	+	+
Amygdalin	+	+	+
Arbutin	+	+	+
Esculin ferric citrate	+	w	+
Salicin	+	+	+
D-cellobiose	+	+	+
D-maltose	+	+	+
D-lactose	+	+	+
D-meliobiose	-	-	-
D-saccharose	+	+	+
D-trehalose	+	+	+
Inulin	+	+	+
D-melezitose	+	+	+
D-raffinose	-	-	-
Amidon (starch)	-	-	-

Glycogen	-	-	-
Xylitol	-	-	-
Gentibiose	+	+	+
D-turanose	+	+	+
D-lyxose	-	-	-
D-tagatose	+	+	+
D-fucose	-	_	-
L-fucose	-	_	-
D-arabitol	-	_	-
L-arabitol	-	_	-
Potassium gluconate	+	+	+
Potassium 2-keto gluconate	-	_	-
Potassium 5-ketogluconate	-	_	-
Identification by API 50 CH kit	*Lactobacillus paracasei 1*	*Lactobacillus paracasei 1*	*Lactobacillus brevis*
Closest relative			
Identity (%)	99.9%	99.9%	99.9%

Note: + = positive reaction; - = negative reaction; w = weak reaction;

Table 5. Morphological and chemical properties of yeast isolated from Bali cattle milk kefir.

Properties	Strain 1 (3isolates)	Strain 2 (3 isolates)
Colony	White to cream, convex, entire dull,	White to cream, convex, entire dull,
Cells	Unicellular, elip, ovoid, to long	Unicellular, elip, ovoid, to long
Reproduction	Multilateral budding	Multilateral budding
Growth in malt	Turbidity and sediment	Turbidity and sediment
Pseudomycelium	absent	absent
Growth at 37^{o}C	+	+
Assimilation of nitrate	-	-
Fermentation of:		
Glucose	+	+
Lactose	-	-
Maltose	-	v
Galactose	-	
Sucrose	+	+
Assimilation of :		

D-glucose	+	+
Glycerol	+	+
2 keto D-gluconate	+	+
L-Arabinose	+	+
D-Xylose	+	+
Adonitol	+	+
Xylitol	+	+
D-Galactose	+	+
Inositol	-	-
D-sorbitol	+	+
Methyl-α-D-glucopyranoside	+	+
N-Acetyl-glucosamine	+	+
D-Cellobiose	+	+
D-Lactose	+	+
D-Maltose	+	+
Sucrose	+	+
D-trehalose	+	-
D-melibiose	+	+
D-raffinose	+	+
Pseudohyphae	-	-
Identification by API 20C kit	*Candida famata*	*Candida famata*
Closest relatives Identity (%)	99.9%	98.2%