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CHARACTERISATION OF LACTIC ACID BACTERIA ISOLATED FROM KEFIR MILK MADE FROM DAIRY AND NON-DAIRY SOURCES AND THEIR SENSORY ACCEPTANCE

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Abstract: Kefir is fermented milk obtained by fermenting milk with kefir grains. Chemical composition from dairy and non-dairy sources may affect the growth and characterisation of lactic acid bacteria (LAB). In this study, different sources of milk (cow milk) and non-dairy milk (soymilk and coconut milk) were used as fermentation media for kefir products. The objectives of the study were to isolate and characterise LAB from Kefir drink using dairy and non-dairy milk. LAB were isolated using different cultural methods such as MRS Agar, MRS with 0.8% $CaCO_3$ and M17 Agar. The characteristics of LAB isolates were determined using morphological, biochemical tests and API 50 CHL Kit. Sensory evaluation of the sample of kefir drinks was also determined. Results confirmed that the isolates identified were *Lactobacillus buchneri, Lactobacillus brevis* 1*, Leuconostoc mesenteroide*s, *Lactobacillus acidophilus* 3 and *Lactobacillus plantarum* 1. The *L. buchneri, L. brevis, Leu. mesenteroides* and *L. acidophilus* are heterofermentative bacteria, whereas, *L. plantarum* is homofermentative bacteria. Four LAB isolates have the potential to be used as probiotic strains due to their high resistant to pH and bile salt. The sensory score of these products in the range of 5.00 to 8.00 in nine point hedonic scale. Most of the sensory panelists preferred cow milk ($p < 0.05$) than coconut milk kefir and soy milk kefir in the sensory evaluation in all attributes. Meanwhile, the preference between coconut milk kefir and soy milk kefir was similar ($p>0.05$) in all attributes. Therefore, this study will provide benefits to probiotic manufacturers to produce alternative probiotic drink using dairy and non-dairy milk.

Keywords: Kefir milk, lactic acid bacteria, lactose intolerance, fermented food, probiotic foods, non-dairy milk product.

Introduction

Kefir is a cultured and fermented milk product which is created through the symbiotic fermenaretation of milk by lactic acid bacteria (LAB) and yeasts contained within an exopolysaccharides and protein complex called a kefir grain (Bourrie *et al.,* 2016). Historically, kefir is a traditional drink which is well-known in the Middle East and it is consumed in Turkey. The world "kefir" is said to have originated from the Turkish word "Keyif" which means good feeling (Otles & Cagindi, 2003). Kefir is a fermented milk-based beverage which is cultured with microflora that is encased in the kefir grain. It is a yellowish white beverage with a sourness flavour and is slightly carbonated due to the small quantity production of alcohol. It was originated by shepherds of the North Caucasus region and kefir was described a pleasurable, frothy milk drink (Gaware *et al.,* 2011). Kefir can be made from dairy and non-dairy milk sources and prepared by inoculating with kefir grains containing LAB and yeast that live symbiotically (Gaware *et al.,* 2011).

LAB are rod or cocci shaped bacteria, characterised as Gram-positive, non-motile and non-spore forming bacteria. LAB belong to the Firmicutes phylum, Bacilli class and *Lactobacillus* order, which includes six families. To date, 43 genera have been described (Ruiz-

Rodríguez *et al.,* 2016). Besides, LAB are also characterised by their production of lactic acid as the main product through lactic fermentation. Generally, LAB are industrially used as starter culture and as probiotic, and play a very important role in the production of fermented foods (Ruiz-Rodríguez *et al.,* 2016). Some of LAB strains are also being characterised as probiotics as they can survive under stressful environmental conditions, such as resistance to acidic gastric juice, capacity of adhesion to the gastrointestinal (GI) tract and antagonistic action against pathogens (Rattanachaikunsopon & Phumkhachorn, 2010). LAB have been widely used to improve the preservation, nutritional value, and sensory characteristics in fermented and probiotic foods (Lee *et al*., 2019). Previous studies by Lani *et al.* (2015) have shown that the use of crude bacteriocin of LAB was effective in controlling microbial growth in 'Satar', a ready-to-eat food in Terengganu.

Fermentation process in milk also makes the milk easier to digest, especially for the lactose intolerant, as well as it also increases the shelf-life of the milk product. During fermentation, amino acids and peptides were present due to the microbial digestion**,** similar to the base ingredient which does not create difficulty in consumption. Fermented milk products have lower lactose levels as compared with milk which have higher lactose levels. The lactose present in milk is hydrolysed by microbial beta-galactosidase during fermentation to produce galactose and glucose, making fermented milk products useful for lactose intolerance (McKevith & Shortt, 2003).

Kefir and yogurt are the oldest fermented milk and are popular until today. Non-dairy probiotic foods were produced and manufactured due to the high demand from consumers for alternatives to dairy probiotic foods (Grabato *et al.,* 2010). The problem with intolerance and allergy, desire for vegetarian alternatives have further increased the demand in non-dairy probiotic foods. Soybean which is the important legume that is commonly prepared in the Asian diet are characterised with high protein content. Soy milk is commonly used as a substitute for dairy milk by the lactose intolerant that are unable to digest a significant amount of lactose, which is the major milk sugar in dairy milk. The consumption and availability of cow milk has decreased whereas the consumption and the availability of non-dairy milk is increasing due to the fact that cow milk is more expensive than non-dairy milk (Hass *et al.,* 2019). Today, soy milk which is also a non-dairy milk and its derivatives have attracted the attention of consumers and researchers all around the world, due to its high protein content. Kefir can be made from dairy and non-dairy milk sources and prepared by inoculating with kefir grains which contained LAB and yeast that live symbiotically. According to Magalhães *et al*. (2011), LAB were the predominant group (60.5%) in Brazilian kefir beverage, followed by yeasts (30.6%) and acetic acid bacteria (8.9%).

Today, numerous studies have been carried out on fermented foods. However, there are limited studies on the kefir milk, especially the prevalence of LAB strains that are isolated from kefir milks that are produced in Malaysia which are made from different milk substrates. Modern consumers are increasingly conscious and interested in their personal health and expect the food consumed to be healthy and capable of preventing illness. According to Mattila-Sandholm *et al.* (2002), the probiotic yogurt market is well established, but the key growth sector recently has been the probiotic drinks. Hence, more natural alternatives and effort are required to develop probiotic drinks outside the dairy sector. India has become the largest producer of milk and having the greatest advantage in the probiotic field along with its booming economy, as Indian probiotic drink is evolving at a steady pace with conditions set for tremendous growth in the near future (Raja & Arunachalam, 2011). Therefore, the objectives of this study were to identify the LAB from different kefir milks and determine the sensory evaluation of the kefir products made from cow milk, coconut milk and soy milk.

Materials and Methods

Sources of Milk Samples

Cow milk, coconut milk and soy milk were purchased from the same suppliers throughout this research. The suppliers of milk samples were Dairy Industry Service Centre, Bukit Payong, Kuala Terengganu (cow milk), Santan Segar Gong Badak, Kuala Terengganu (fresh coconut milk) and China Town, Kuala Terengganu (homemade soy milk). Kefir grains were purchased from My Kefir World (@ mykefirworld), Cheras, Kuala Lumpur.

Preparation of Kefir Milk Production

Cow milk, coconut milk and soy milk were fermented traditionally and aseptically with kefir grains for the production of kefir milk as described by Otles and Cagindi (2003). Raw cow milk, coconut milk and soy milk were boiled in three different glass jars and cooled to 20-25ºC and inoculated with 5% kefir grain purchased from supplier, My Kefir World. After a period of fermentation at 18-24 h at 20-25ºC, the grains were separated from the milk by filtering with a sieve and dried at room temperature (25ºC ± 2). Kefir milks in the sterile jar (500 ml) were then kept in the chiller at 4°C for storage purpose.

Isolation of LAB from Different Kefir Milks

The isolation of LAB from different milk substrates of kefir milks were performed by sequences of serial dilution and incubation at 30°C for 24 to 48 hours in anaerobic conditions. Appropriate serial dilutions with 0.85% saline water were carried out to ensure colonies grown on the plates were not overgrowth. The final dilution was then spread plated on three selective media, which were MRS, M17 and MRS with 0.8% $CaCO_3$ Agar. The presumptive of LAB isolates were selectively tested for characterisation tests. LAB isolates with Gram-positive characteristic and catalase negative were conserved in slanted agar at 4°C.

Morphological Characterisation of LAB

Morphology of the pure cultures of LAB isolates were carried out using Gram staining, endospore staining and scanning electron microscopy (SEM) methods. A single colony of LAB isolates from agar plates was selected to perform all these methods. In Gram staining method, the smear of well-isolated LAB colony with one drop of distilled water was fixed on glass slide with heat. The heat-fixed smear was stained with crystal violet staining reagent for one minute and rinsed with running tap water. The smear was flooded with Gram iodine solution for one minute and rinsed off using 95% alcohol which acts as decolourising agent. Lastly, safranin solution was used as counterstain.

The slides were observed using light microscope under 1000 x magnification power. Bluish-purple colour represents Gram-positive LAB isolates. In endospore staining, slides with heat-fixed smear were placed over steaming water bath. Malachite green was applied for five minutes and rinsed off with water. Counter stain (safranin) was then added for one minute and rinsed with water again. Next, the slide was then observed using light microscope under 1000 x magnification power. This staining was used for further confirmation for the absence of endospore in all *Lactobacillus* strains. Reddish colour represents non-spore forming LAB isolates.

SEM was carried out at the Institute of Oceanography and Environment (INOS), UMT by modifying the procedures that have been used in previous studies due to insufficient specific chemicals (Pyar & Kok-Khiang, 2014). Bacterial cell pellets were collected after centrifugation for five minutes at 3000 rpm. The cell pellets were fixed with 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer for two to four hours and followed by rinsing three times in 0.1 M sodium cacodylate buffer at pH 7.2 for 15 minutes intervals. Bacterial cell pellets were dehydrated by ethanol in a series of ascending gradation (30%, 50%, 75%, 95% and 100%) for 10 minutes intervals. The dried cell pellets were mounted on specimen stubs and coated with

gold. Samples were observed using Scanning Electron Microscope at 10 to 25 kV.

Biochemical Characterisation of LAB

Biochemical characteristics of the pure cultures of LAB isolates were carried out through catalase, oxidase, and motility tests. A well-isolated and single colony of each LAB isolates was picked using wooden applicator stick and placed on a microscope slide. A drop of 3% hydrogen peroxide (H_2O_2) was dropped on the isolated colony (Goyal, 2012; Hasali *et al.,* 2015). The production of gas bubbles was recorded as catalase positive and vice versa. Oxidase test was performed using filter paper method. Tetramethyl-p-phenylenediamine was used as oxidase reagent. A few drops of oxidase reagent were dropped on filter paper and well-isolated LAB colony was rubbed on the treated filter paper. The oxidised oxidase reagent changed from colourless to dark blue colour within 15 seconds, indicating oxidase positive. Motility test was performed to identify the ability of bacteria to move due to the presence of flagella. SIM (Sulphide Indole Motility) medium was used perform motility test. Cells are stab-inoculated in the centre of the medium to a depth of half an inch.

Physiological Characterisation of LAB

The physiological characteristics of LAB isolates from kefir milks were assessed by the growth of LAB isolates at different conditions affected by temperatures, pH, salinity, and bile salt concentrations, together with carbohydrate fermentation test. MRS broth tubes were inoculated with active LAB isolates and incubated for 24 hours at the following temperatures of 10°C, 37°C and 45°C. Meanwhile, sterilised MRS broth with different pH (4.4 and 9.6) was prepared by adjusting the pH of MRS broth with 0.1 N NaOH and 0.1 N HCl solutions. Some MRS broth tubes were prepared by incorporating 1%, 2%, 3%, 4%, 5% and 6% (w/v) sodium chloride (NaCl). Other tubes with MRS broth were prepared by adding 0.3%, 0.5% and 1.0% (w/v) bile salt (Oxoid, United Kingdom) into each tubes respectively. The sterilised MRS broth were inoculated with active LAB isolates and incubated for 24 hours at 37°C. Growth of LAB isolates in each condition was determined by turbidity and measured by using spectrophotometer at 600 nm by obtaining the optical density (Rhaiem *et al.,* 2016). Phenol red broth was used as the medium in this test. Then, 1% of different sugar substrates, glucose, lactose, mannitol and sucrose were added into each phenol red broth tubes. Durham tubes were added into each phenol red tubes for the purpose of gas bubbles detection. Sterilised phenol red broth tubes were inoculated with active LAB isolates and incubated for 24 hours at 37°C. Changes of phenol red broth from red to yellow indicates positive reaction (Rhaiem *et al.,* 2016).

Phenotypic Identification of LAB Isolates Using API 50 CHL Kit

Pure colonies of overnight cultures of LAB isolates which were freshly grown on MRS plates at 37°C for 24 hours were suspended in API 50 CH medium (Biomerieux, France). The suspension of each isolate was transferred into each of the 50 wells of the API 50 CH strips that contained different carbohydrates. After that, all wells were covered with sterilised mineral oil to make them anaerobic and incubated at 37°C for 24 and 48 hours because some strains of LAB require longer time to complete carbohydrate fermentation (API 50 CHL Manual Kit, Biomerieux, France). Fermentation is revealed by a colour change in the tube, caused by the anaerobic production of acid and detected by the pH indicator present in the chosen medium. The results were analysed with API Web (Hasali *et al.,* 2015).

Sensory Evaluation of Kefir Milk Samples

The sensory evaluation was carried out to determine the preference of kefir milk with different substrates. Sensory attributes evaluated were appearance, colour, odour, taste, sourness and overall acceptance. Sample preparation for

kefir milks was performed by transferring of 25 ml of kefir milk into a small plastic cup container with lid. The containers were coded with three random numbers and arranged according to permutations. All samples were evaluated by 30 volunteer panelists after 24 hours of the production of kefir milks. The scoring used nine point hedonic scale from one (dislike extremely) to nine (like extremely). The data from sensory evaluation was subjected to One Way ANOVA using IBM SPSS (Version 20).

Statistical Analysis

Each data of physicochemical properties, sensory acceptance and LAB count were subjected to One-way Analysis of Variance (ANOVA) using IBM SPP Version 20.

Results and Discussion

Isolation and Enumeration of LAB

Results found that LAB were successfully isolated from kefir milks which were made from different milk substrates using standard microbiological procedures. The LAB counts from each sample of kefir milks were enumerated and calculated as shown in Table 1. However, there was no significant difference of LAB count (CFU/ml) between each sample kefir milks ($p < 0.05$). As compared with each kefir milk sample, coconut milk kefir had the highest count with $\log_{10}$ 9.11 CFU/ml on MRS Agar, $\log_{10}$ 9.12 on MRS Agar added with 0.8% $CaCO_3$ and $\log_{10}$ 9.07 CFU/ml on M17 Agar, followed with cow milk kefir and soy milk kefir respectively.

Table 1: LAB counts ($\log_{10}$ CFU/ml) from sample kefir milks

	Mean ± SD ($\log_{10}$ CFU/ml)		
Sample of Kefir Milks	**MRS Agar**	**MRS Agar + 0.8% $CaCO_3$**	**M17 Agar**
Coconut Milk Kefir	9.11 0.27 [a]	9.12 0.17 [a]	9.07 0.20 [a]
Cow Milk Kefir	9.080.27 [a]	9.02 0.27 [a]	8.56 0.78 [a]
Soy Milk Kefir	9.03 0.36 [a]	9.01 0.34 [a]	8.99 0.58 [a]

*Means with similar superscript indicates non-statistically significant difference (n=3)

According to Gülel (2014), probiotic product should have a minimum concentration of 10^6 CFU/ml or CFU/g to provide therapeutic effect. The LAB counts from three samples of kefir milks recorded high concentrations of CFU/ml varied from 10^8 to 10^9 CFU/ml, suggesting that all milks used exceeded the minimum concentration of probiotic products. Moreover, the total presumptive LAB counts found in soy milk were almost similar compared with the report of LAB counts in soy milk kefir in the previous year (Dadkhah *et al.,* 2011). Previous research has shown that kefir made from cows' milk using a commercial starter culture *Lactococcus* sp. predominated during the first 48 h of fermentation, approximately 8.0 $\log_{10}$ CFU/g; *Lactobacillus* sp. became the predominant species after 48 h, approximately 8.5 $\log_{10}$ CFU/g (García Fontán *et al.,* 2006). Dadkhah *et al.* (2011) reported that soy milk kefir produced using 3% kefir grains had the highest *Lactobacilli* sp. levels (9.64 ± 0.03 $\log_{10}$ CFU/ml) and *Lactococci* sp. (9.48 ± 0.08 $\log_{10}$ CFU/ml). The finding is in agreement with findings in other studies that reported *Lactobacillus* was present in cow milk and soy kefir milk, however, there was no published data on detection of *Lactobacilli* or *Lactococci* in coconut milk kefir. Therefore, this study is the first to report on the presence of *Lactobacilli* in coconut milk kefir.

Morphological Characterisation of LAB

All LAB isolates successfully grew on three different types of kefir milk samples. Cell morphology is important to determine the morphological characteristics of LAB and their species genera. Through SEM, the results show that kefir milks were dominated by rod shaped LAB cultures of LAB 001 as *L. buchneri*, LAB

002 as *L. brevis*, LAB 004 as *L. acidophilus* and LAB 005 as *L. plantarum*, whereas the cocci shaped of LAB 003 as *Leu. mesenteroides*. According to Elzeini *et al.* (2017), the shape of a bacterial cell influences many aspects of its life, including nutrient access, motility, chemotaxis and resistance to predation. Five presumptive LAB isolates were morphologically investigated using SEM. The cells of each presumptive LAB isolates were photographed and representative images were shown in Figure 1.

All the LAB isolates were Gram-positive

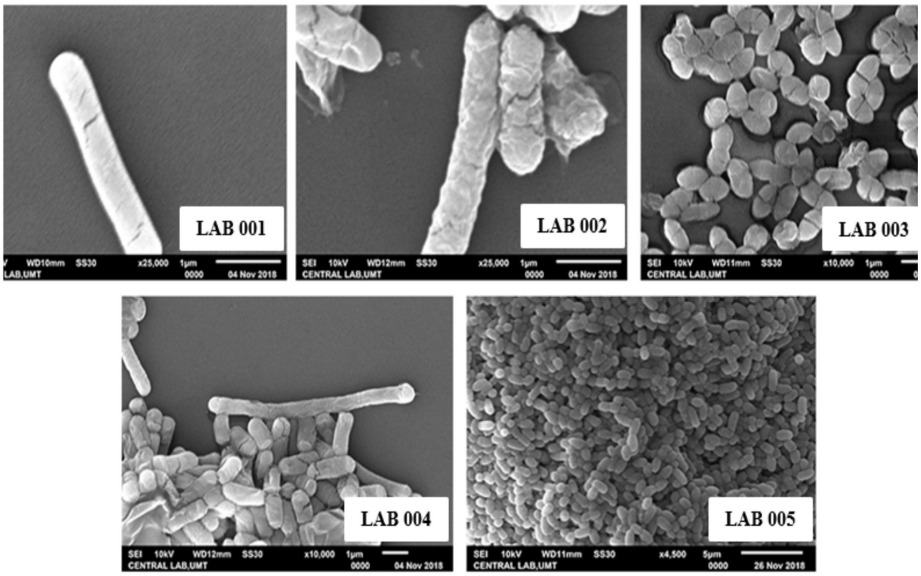


Figure 1: SEM observation on LAB isolates (LAB 001, LAB 002, LAB 004 and LAB 005 were rod shaped and LAB 003 was cocci shaped)

and non-spore forming bacteria. Gram positive LAB stained dark blue due to the presence of thick layer of peptidoglycan within the cell wall. According to Chapot-Chartier and Kulakauskas (2014), LAB consist of a thick peptidoglycan sacculus that surrounds the cytoplasmic membrane and decorated with teichoic acids, polysaccharides and proteins. The thick peptidoglycan layer protects the dehydrating effect of decolourising agent (95% alcohol) extracts the crystal violet iodine complex (CV-I) from the cell walls. Generally, LAB do not contain bacterial endospores which are metabolically inactive and highly resistant to unfavourable environmental conditions. According to Oktari *et al.* (2017), malachite green and safranin were able to work well in bacteria due to the alkaline (chromophore component positively charged), while the bacterial cytoplasm is basophilic so there was an attraction between the components chromophore in a stain with bacterial cells, which resulted in the bacteria being able to absorb the stains well.

Phenotypic Characterisation of LAB Using Biochemical Tests and API 50 CHL Kit

From Table 2, all LAB isolates from sample kefir milks of coconut milk kefir, cow milk kefir and soy milk kefir were confirmed as oxidase negative, catalase negative and non-motility by a series of biochemical characterisation tests. The negative result by LAB isolates from kefir milks directly indicated that the absence of enzyme cytochrome c oxidase in LAB isolates which react with oxygen to form a coloured end product. This is supported by Ouoba *et al.* (2009), where the isolated LAB from African traditional alkaline-fermented foods were characterised as oxidase negative. The catalase

enzyme recomposited the hydrogen peroxide to water and oxygen. Some bacteria rely on defence mechanisms which protect them from bactericidal effects of hydrogen peroxide through the production of catalase (Pine *et al.,* 1984). Sefidgar *et al.* (2014) reported that LAB isolated from Iranian kefir drink was also catalase negative. Results of motility test showed all LAB isolates were categorised as non-motile bacteria.

Table 2: Identification and characteristics of LAB from sample kefir milks

Cultures of LAB	Identification Using API 50 CHL	Morphological Characterisations		Biochemical Characterisations	
	Identification ID and Similarity (%)	Morphology	Endospore Staining	Oxidase Test	Motility Test
LAB 001	*L. buchneri (*82.50%)	Gram positive rod	-	-	-
LAB 002	*L. brevis* 1 (93.20%)	Gram positive rod	-	-	-
LAB 003	*Leu. mesenteroides* ssp. *mesenteroides* (99.90%)	Gram positive cocci	-	-	-
LAB 004	*L. acidophilus* 3 (67.50%)	Gram positive rod	-	-	-
LAB 005	*L. plantarum* 1 (99.80%)	Gram positive short rod	-	-	-

*(+) indicates the positive result, (-) indicates the negative result

Using API 50 CHL Kit, this study confirmed that among five LAB isolates which were randomly taken from all three samples of kefir milks, four of them belong to *Lactobacillus* sp. and one isolate belongs to *Leuconostoc* sp. as shown in Table 2. LAB 002, LAB 003 and LAB 005 showed high similarity to *Lactobacillus brevis* 1 (93.2%), *Leuconostoc mesenteroides* ssp. *mesenteroides* (99.9%) and *Lactobacillus plantarum* 1 (99.8%). The presence of viable and potential probiotic *Lactobacilli* in kefir milks showed that LAB plays an important role in probiotic drink. Maekawa *et al.* (2014) proved that probiotic *L. brevis* can inhibit periodontitis through modulatory effects on the host response and the periodontal microbiota.

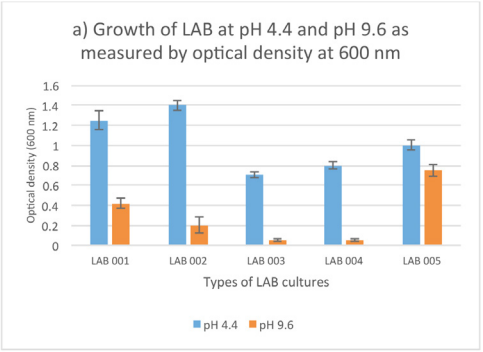
Physiological Characterisation of LAB

Physiological characteristics of five presumptive LAB isolates were determined by temperature, pH, salt, bile and salt tolerance as well as carbohydrate fermentation tests. From Table 3, it is shown that the selected LAB isolates were acid-producing bacteria, due to their reaction with $CaCO_3$. Acid-producing LAB isolates produce lactic acid which then react with $CaCO_3$ to produce calcium lactate as the end product and form a clear zone that surrounded the colonies of LAB isolates.

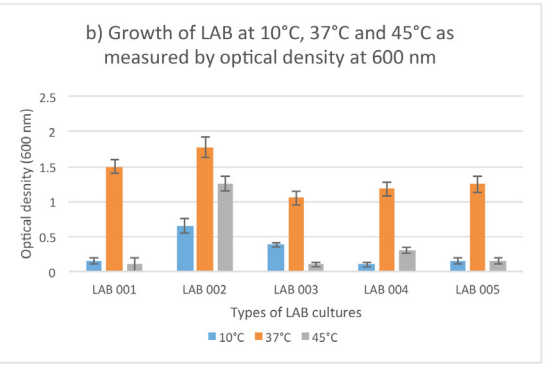
Table 3: Physiological characterisation of LAB

	Physiological Characterisation				
Physiological Tests	***Lactobacillus buchneri***	***Lactobacillus brevis 1***	***Leuconostoc mesenteroides***	***Lactobacillus acidophilus 3***	***Lactobacillus plantarum 1***
Fermentation Pattern	Heterofermentative	Heterofermentative	Heterofermentative	Homofermentative	Heterofermentative
Clear Zone	+	+	+	+	+
Acid Tolerance					
pH 4.4	++	++	+	+	++
pH 9.6	+	+	-	-	+
Temperature Tolerance˚C					
10˚C	+	+	+	-	+
37˚C	++	++	++	++	++
45˚C	-	++	-	+	+
Salt Tolerance					
1% NaCl	++	++	++	++	+
2% NaCl	++	++	+	++	++
3% NaCl	+	++	+	++	++
4% NaCl	-	++	+	++	+
5% NaCl	-	-	-	-	-
6% NaCl	-	-	-	-	-
Bile Salt Tolerance					
0.3% Bile Salt	++	++	++	++	++
0.5% Bile Salt	+	++	+	++	++
1% Bile Salt	+	++	+	+	++
Sugar Fermentation					
Glucose	-	++	++	++	++
Lactose	-	++	-	-	++
Mannitol	-	++	-	-	++
Sucrose	++	++	++	++	++

In Figure 2, the results revealed that all the LAB isolates were highly resistant to acidity conditions. At pH 4.4, all the LAB isolates grew significantly with high optical density value at 600 nm, ranging from 0.713 to 1.384 OD_{600}, indicating that all the LAB isolates were acid tolerant. It is interesting to report that five LAB isolates, *L. buchneri, L. brevis* 1, and *Leu. mesenteroides* sp. *mesenteroides* were able to survive at high pH or alkalinity conditions with an OD_{600} value of 0.452, 0.242 and 0.740, respectively. The regulation of the cytoplasmic pH helped LAB to adapt to the acidity or alkalinity conditions. Nyanga-Koumou *et al.* (2012) accepted that bacterial cytoplasmic pH is regulated by various cations transport systems to maintain normal and functionality of LAB's cellular activities. This allows acid resistant LAB to withstand the high pH of gastric juices also enables LAB to survive for longer period in acidic foods, such as kefir and sauerkraut.



*LAB 001: *L. buchneri*; LAB 002: *L. brevis* 1; LAB 003: *Leu. mesenteroides* ssp. *mesenteroides*; LAB 004: *L. acidophilus* 3 and LAB 005: *L. plantarum* 1



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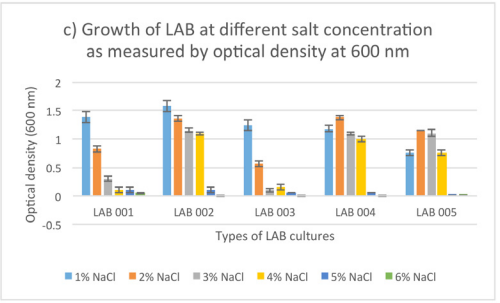

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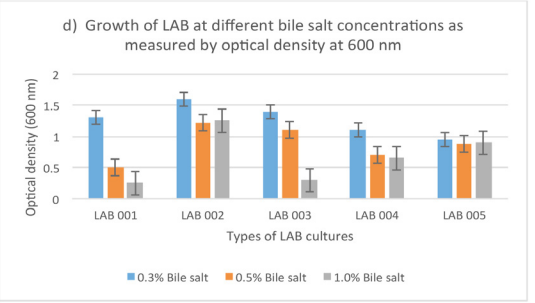

*LAB 001: *L. buchneri*; LAB 002: *L. brevis* 1; LAB 003: *Leu. mesenteroides* ssp. *mesenteroides*; LAB 004: *L. acidophilus* 3 and LAB 005: *L. plantarum* 1

Figure 2: Growth of LAB isolates at different conditions measured by optical density at 600 nm (a: different pH, b: different temperatures, c: different salt concentrations and d: different bile salt concentrations)

Sensory Evaluation

Table 4 represents the sensory analysis for the kefir milk samples on six sensory attributes, which includes appearance, colour, odour, taste, sourness, and overall acceptance. Cow milk kefir has the highest mean score of 6.80 for appearance, 7.23 for colour, 6.80 for odour, 7.27 for taste, 6.07 for sourness and 6.70 for overall acceptance as compared with coconut and soy milk kefirs. This indicates that most of the panelists preferred cow milk than coconut milk kefir and soy milk kefir in the sensory evaluation on each attribute.

Table 4: Sensory analysis of kefir milks

Sensory Analysis (Mean Score ± Standard Deviation)						
Sample of Kefir Milk	**Appearance**	**Colour**	**Odour**	**Taste**	**Sourness**	**Overall Acceptance**
Coconut Milk Kefir	5.571.91[b]	5.771.72[b]	5.53 1.46[b]	5.170.83[b]	6.032.19[a]	5.201.06[b]
Cow Milk Kefir	6.801.52[a]	7.231.30[a]	6.800.96[a]	7.270.69[a]	6.071.28[a]	6.700.65[a]
Soy Milk Kefir	5.370.85[b]	5.131.00[b]	5.030.96[b]	5.001.20[b]	5.071.51[a]	5.030.49[b]

*Means with different superscript indicate statistically significant difference (n = 30)

Overall, panelists like the colour and taste of cow milk kefir moderately with high overall acceptance than coconut and soy milk kefirs, but like the appearance, odour, and sourness of cow milk kefir slightly. Based on the results, cow milk kefir differed significantly (p<0.05) with coconut and soy milk kefirs in the evaluation of appearance, colour, odour, texture and overall acceptance, but shows no significant difference (p>0.05) with coconut and soy milk kefirs in the evaluation of sourness. Besides, there is no significant difference (p>0.05) between coconut and soy milk kefirs in the evaluation in all attributes. According to Irigoyen, 2004, kefir products from dairy animals such as cow, goat, sheep, camel, buffalo contain pH around 4.0, alcohol from 0.5% to 2% and fat content from 3.3 to 7%. The cow milk Kefir taste is acidic, prickly, and slightly yeasty. The sharp acid and yeasty flavour, together with the prickly sensation contributed by the carbon dioxide produced by the yeast flora can be considered as the typical kefir flavour. Furthermore, during fermentation, vitamins B1, B12, calcium, amino acids, folic acid and vitamin K, increase in the cow milk kefir (Otles & Cadingi, 2003).

Conclusion

From this study, five different strains of LAB have been isolated from different milk substrates (cow milk, coconut milk and soy milk) of kefir milks which are *L. buchneri, L. brevis, Leu. mesenteroides, L. acidophilus* and *L. plantarum*. This study showed that the different milk substrates have significant effect on the physiochemical and sensory properties of three different kefir milks. Further investigation should be focused on the probiotic characteristics of kefir milks and their effects on gut microbiota. Our findings are relevant and would benefit manufacturers in the probiotic industry to produce**,** as alternative probiotic products for dairy and non-dairy consumers (lactose intolerant and vegetarian).

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References

Bourrie, B. C. T., Willing, B. P., & Cotter, P. D. (2016). The microbiota and health promoting characteristics of the fermented beverage kefir. *Frontiers in Microbiology*, *7*, 647. https://doi.org/10.3389/fmicb.2016.00647.

Chapot-Chartier, M.P., & Kulakauskas, S. (2014). Cell wall structure and function in lactic acid bacteria. *Microbial Cell Factories*, 13(Suppl 1), S9. https://doi.org/10.1186/1475-2859-13-S1-S9.

Dadkhah, S., Pourahmad, R., Assadi, M. M., & Moghimi, A. (2011). Kefir production from soymilk. *Annals of Biological Research*, *2*(6): 293-299.

Elzeini, H.M, Ali, A.A., Nasr, N.F., Awad, A.A., & Hassan, A.A. (2017). Morphological and rheological identification of cocci lactic acid bacteria. *Journal of Microbial and Biochemical Technology*, *9*(1): 519-526.

Font de Valdez, G., Rollán, G., Gerez, C. L., & Torino, M., I. (2011). Microbial applications in the biopreservation of cereal products.

Protective Cultures, Antimicrobial Metabolites and Bacteriophages for Food and Beverage Biopreservation, Woodhead Publishing Series in Food Science, Technology and Nutrition. pp. 348-363.

Garcia Fontan, M.C., Martinez, S., Franco, I., & Carballo, J. (2006). Microbiological and chemical changes during the manufacture of Kefir made from cows' milk, using a commercial starter culture**.** *International Dairy Journal*, *16*, 762-767.

Gaware, V., Kotade, K., & Dolas, R. (2011). The magic of Kefir: A review history of Kefir. *Pharmacology online*, *1*, 376-386.

Goyal, R. (2012). Characterisation of the *Lactobacillus* isolated from different curd samples. *African Journal of Biotechnology*. *11*(79): 14448-14452.

Granato, D., Branco, G.F., Nazzaro, F., Cruz, A.G., & Faria, J.A. (2010). Functional foods and non-dairy probiotic food development: Trends, concepts, and products. *Comprehensive Review in Food Science and Food Safety*, *9*, 292-302.

Gulel, S. (2014). Molecular identification and probiotic properties of *Lactobacillus acidophilus* group isolates from Turkish Kefir. M. Sc. Thesis. Middle East Technical University, Turkey.

Hasali, N. H. M., Zamri, A. I., Lani, M. N., Mubarak, A., & Suhaili, Z. (2015). Identification of lactic acid bacteria from Meliponine honey and their antimicrobial activity against pathogenic bacteria. *American-Eurasian Journal of Sustainable Agriculture*. *9*(6): 1-7.

Hass, R., Schnepps, A., Pichler, A., & Meixner, O. (2019) Cow milk versus plant-based milk substitutes: A comparison of product image and motivational structure of consumption. *Sustainability, 11*, 5046; 1-25. doi:10.3390/su11185046.

Irigoyen, A., Arana, I., Castiella, M., Torre, P., & Ibanez, F.C. (2005). Microbiological, physicochemical, and sensory characteristics of kefir during storage. *Food Chemistry*, *90*(4): 613-620.

Lani, M., Nor, N., Ramli, N., Radhuan, Z., Rizan, M., Lokman, N., & Hassan, Z. (2015). Effects of incorporation of lactic acid bacteria on microbiological quality and shelf life of raw 'Satar'. In Beneficial Microorganisms in Food and Nutraceuticals (pp. 81-109). Springer, Cham.

Lee, A.L.C.N., Lani, M.N., Alias, R. & Hassan, Z. (2019). Lactic acid bacteria isolated from locally produced vinegars and their antibacterial activity against foodborne bacteria. UMT *Universiti Malaysia Terengganu Journal of Undergraduate Research, 2*, 1-7.

Maekawa, T., & Hajishengallis, G. (2014). Topical treatment with probiotic *Lactobacillus brevis* CD2 inhibits experimental periodontal inflammation and bone loss. *Journal of Periodontal Research*, *49*(6), 785–791.

Magalhães, K. T., Pereira, G. V. de M., Campos, C. R., Dragone, G., & Schwan, R. F. (2011). Brazilian kefir: structure, microbial communities and chemical composition. *Brazilian Journal of Microbiology*, *42*(2), 693–702.

McKevith, B., & Shortt, C. (2003). Fermented milks: Other relevant products. *Encyclopedia of Food Sciences and Nutrition*, 2383–2389. https://doi.org/10.1016/B0-12-227055-X/00458-2.

Mattila-Sandholm, T., Myllärinen, P., Crittenden, R., Mogensen, G., Fondén, R., & Saarela, M. (2002). Technological challenges for future probiotic foods. *International Dairy Journal*, *12*(2–3), 173–182.

Nyanga-Koumou, A. P., Ouoba, L. I. I., Kobawila, S. C. & Louembe, D. (2012). Response mechanisms of lactic acid bacteria to alkaline environments: A review. *Critical Reviews in Microbiology*, *38*(3), 185–190. https://doi.org/10.3109/1040841X.2011.640978.

Oktari, A., Supriatin, Y., Kamal, M., & Syafrullah, H. (2017). The Bacterial endospore stain on Schaeffer Fulton using variation of methylene blue solution. *Journal of Physics: Conference Series, 812* (2017), 012066. https://doi.org/10.1088/1742-6596/812/1/012066.

Otles, S., and Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition*, *2*(2): 54-59.

Oner, Z., Karahan, A.G., & Cakmakci, M. L. (2010). Effects of different milk types and starter cultures on kefir. *Gida*, *35*(3), 177-182.

Ouoba, L. I. I., Nyanga-Koumou, C. A. G., Parkouda, C., Sawadogo, H., Kobawila, S. C., Keleke, S., & Sutherland, J. P. (2009). Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. *Journal of Applied Microbiology*, *108*(6), 2019–2029.

Pine, L., Hoffman, P.S., Malcolm, G.B., Benson, R.F., & Keen, M.G. (1984). Determination of catalase, peroxidase, and superoxide dismutase within the genus *Legionella*. *Journal of Clinical Microbiology*, *20*(3), 421-429.

Pyar, H., & Kok-Khiang, P. (2014) Characterization and identification of *Lactobacillus acidophilus* using biolog rapid identification system. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(1), 189-193.

Raja, B. R., & Arunachalam, K. D. (2011). Market potential for probiotic nutritional supplements in India. *African Journal of Business Management*, *5*(14), 5418–5423.

Rattanachaikunsopon, P., & Phumkhachorn, P. (2010). Lactic acid bacteria: their antimicrobial compounds and their uses in food production. *Scholars Research Library Annals of Biological Research*, *1*(4), 218–228.

Rhaiem, N., Chahboun, N., Inekach, S., & Ouhssine, M. (2016) Identification and characterisation of lactic acid bacteria isolated from cow milk and olives brine. *Journal of Materials and Environmental Science*, *7*(5), 1504-1509.

Ruiz-Rodríguez, L., Bleckwedel, J., Eugenia Ortiz, M., Pescuma, M., & Mozzi, F. (2017). Lactic Acid Bacteria. In: Industrial Biotechnology: Microorganisms, Volume 1. (pp. 395–451). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA.

Sefidgar, S. A. A., Gharekhani, S., & Ghasempour, M. (2014). Identification of yeasts and bacteria isolated from Iranian kefir drink. *Jundishapur Journal of Microbiology*, *7*(2), e10707.

Wojtowski, J., Dankow, R., Skrzyper, R., & Fahr, R. D. (2003). The fatty acid profile in kefirs from sheep, goat and cow milk. *Milchwissenschaf*, 58(11/12), 633–636.