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Isolation and Identification of Lactic Acid Bacteria and Yeasts from Sameel Milk: A Saudi Traditional Fermented Milk

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ABSTRACT

Sameel milk is a traditional fermented milk product consumed mostly in nomadic areas of Saudi Arabia. Predominant microorganisms in the Sameel milk, responsible for milk fermentation, were isolated and identified. A total of 29 samples of Sameel milk were collected from three different villages in Eastern Region for analysis. Total aerobic mesophilic bacteria Lactobacilli, Lactococci, *Enterococcus* and Enterobacteriaceae were enumerated. A total of 112 Lactic Acid Bacteria (LAB) and 36 yeast were isolated from Sameel milk samples. The bacteria and yeast were identified by API 50 CHL and API 20C AUX identification systems, respectively. Mean counts of *Lactobacilli* and *Lactococci* were 7.4 and 7.7 $\log_{10}$ CFU mL^{-1}, respectively. While, the yeasts and *Enterococcus* counts were relatively low and accounted for 5.7 and 5.9 $\log_{10}$ CFU mL^{-1}, respectively. Low counts of *Enterobacteriaceae* were encountered (<2.0 $\log_{10}$ CFU mL^{-1}) in 20 samples, whereas the mean counts in other nine samples were detected at a relatively lower magnitude i.e., 3.7 $\log_{10}$ CFU mL^{-1}. The LAB species were identified as *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactococcus lactis* ssp *lactis*, *Lactobacillus brevis*, *Lactobacillus salivarius* and *Lactobacillus paracasei* ssp *paracasei*. The predominant yeast were *Candida lusitania*, *Cryptococcus laurentii*, *Saccharomyces cerevisiae* and *Candida kefyr*. The most frequently isolated species was *Lactobacillus plantarum* (50% of total isolates) followed by *Candida lusitania* (41%), *Lactobacillus pentosus* (26%), *Cryptococcus laurentii* (25%) and *Saccharomyces cerevisiae* (25%).

Key words: Traditional fermented milk, identification, lactic acid bacteria, yeasts, consumption

INTRODUCTION

Consumption of fermented milk has many advantages including enhanced nutritional value, digestibility, therapeutic benefits and safety against pathogens. Traditional Sameel milk is the most popular fermented milk especially in nomadic herders areas in Saudi Arabia. Sameel milk is prepared from unpasteurized whole sheep, goat, cow or camel milk. Fresh milk collected daily in a tanned goats or sheep leather bag container called Sameel containing small amount of fermented milk from previous batch as a starter culture. Some herbs called Depagh may added together with milk. It is left in the shadow place for 1-3 days depending on the ambient temperatures for spontaneous fermentation. The fermented milk may be consumed as such (straight fermented milk) when camel's milk has been used or as in the majority of cases (when used other types of milk), it is churned early in the morning to produce butter. After removing the butter, the sour buttermilk remaining named as "Sameel milk" is consumed fresh (Saleh, 2010). The main purposes of these processes is to produce butter. The microorganisms mainly Lactic Acid Bacteria (LAB) and yeasts inherent to this leather bag container (Sameel), milk and the air in the surrounding environment

are assumed to ferment the milk. The nomads, in general, prefer it due to its excellent natural acidic taste and aroma besides other functional benefits. Also, the people believe in its therapeutic value towards curing or protected from ailments such as diarrhoea and constipation as it contains LAB in different species. Moreover, LAB are also reported to colonize the human intestinal mucosa leading to beneficial effects (Fuller, 1992). Saleh (2010) detected some lactic acid bacteria in Sameel milk such as *Lactobacillus* and *Lactococcus* coupled with relatively low counts of yeast.

The nature of fermented products varies from one region to another. It depends on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Savadogo *et al*., 2004). Several investigators from other countries have isolated and identified many lactic acid bacterial species and yeasts from their traditional fermented dairy products. They found that the main LAB genera consisted of Lactobacilli, Lactococci and leuconostocs (Abdelgadir *et al*., 2001; Mathara *et al*., 2004, 2008; Lore *et al*., 2005; Sulieman *et al*., 2006; Luo *et al*., 2011). Whereas, they also found that the yeast species were *Saccharomyces*, *Candida*, *Zygosaccharomyces*, *Rhodotorula* and *Cryptococcus*.

From the documented literature, lack of scientific information was found about the traditional fermented milk in Saudi Arabia. In addition, no study has been initiated to identify the fermenting organisms of Sameel milk to the species level. Thus, the current study aimed at the isolation, identification and characterization of microorganisms that are responsible for fermentation of Sameel milk.

MATERIALS AND METHODS

Collection of sameel milk samples: Twenty nine Sameel milk samples were collected from three different villages of Al-Ahsa, Eastern Region of Saudi Arabia (Ten from Eastern villages, E1-10, ten from Riyadh road villages, R1-10 and nine from Qatar road villages, Q1-9). The Sameel milk was collected aseptically from the mouth of leather bag container to sterile stomacher polyethylene bags. The samples were kept in ice boxes and transported within 2 h to the laboratory and stored in refrigerator (5±1°C) for one day until analysis.

Determination of pH: The pH was determined using a Grison pH meter (GPL21) (Herisau, Switzerland) after calibration using standard buffers (Metrohm Ion Analysis, Herisau, Switzerland) at pH 4 and 7.

Microbial enumeration and isolation: Ten milliliter from each fermented milk sample was transferred aseptically into 90 mL peptone water solution and mixed thoroughly. Serial dilutions (10^{-1}-10^{-8}) were performed and 1 mL aliquot of the appropriate dilution was incubated in triplicate on universal and selective media. Plate count agar (Oxoid CM0325) incubated at 30°C for 72 h for enumeration of total aerobic mesophilic bacteria. MRS agar (Oxoid CM0361) incubated anaerobically at 30°C for 48 h for enumeration of *Lactobacilli*. M17 agar (Oxoid CM0785) incubated anaerobically at 30°C for 48 h for enumeration of *Lactococci*. Kanamycin aesculin azide agar (KAA) (Oxoid CM0591) incubated at 37°C for 48 h for enumeration of *Enterococcus*. Violet red bile agar (VRBA) (Oxiod CM 0107) incubated anaerobically at 37°C for 24 h for enumeration of *Enterobacteriaceae*. Acidified potato dextrose agar (PDA) (Oxoid CM0139) was incubated at 30°C for 48 h for enumeration of yeasts and moulds, (0.1 mL of the appropriate dilution spread plated on this medium). The anaerobic condition was performed in anaerobic jars (Biolab) with gas generating kits (Oxiod BR0038B). Representative bacterial colonies were isolated randomly from

plates of MRS, M17 and KAA agar. Isolates were cultivated in its selected broth medium and incubated at 30°C for 24 h. The isolates were purified by streak plating using the same medium. Gram positive catalase negative of LAB were purified by re-streaking on MRS and M17 agar. The bacterial isolates were re-suspended and stored in its selected medium containing 15% glycerol at -18°C. Representative yeast colonies on PDA were examined by phase contrast microscopy and purified by successive streaking on PDA. The pure yeast isolates were stored on slants at 4°C.

Identification of lactic acid bacteria: Gram positive catalase negative bacteria were examined microscopically (cell morphology and arrangements). The rods and cocci bacteria were presumptively identified as Lactic Acid Bacteria (LAB) according to Gerhartdt *et al.* (1981). The growth of bacteria at 10, 15 and 45°C in MRS and M17 broth was evaluated visually after 24, 48 and 72 h of incubation. Hetro and homo-fermentative activity (using MRS and M17 broth with inverted Durham tubes) and production of ammonia from arginine were carried out as described by Harrigan and McCance (1986). Salt tolerance was performed by using MRS and M17 containing 6.5% (w/v) NaCl with incubation at 37°C for 72 h. The ability to ferment carbohydrate substrates was studied using the API 50 CH galleries and API 50 CHL medium (BioMerieux, Marcy l'Etoile, France) system, which enabled identification of the LAB isolates to species level. All the tests were performed according to the manufacture's instructions as follows. A swab of each LAB isolates grown on MRS agar plates (incubated anaerobically at 37°C for 48 h) was suspended in API 50 CHL medium. Using sterile pipette, homogenized suspension of the cells in the medium was distributed into each of the 50 wells on the 50 CH strips. All wells were overlaid with sterile paraffin oil (Merck) to affect anaerobiosis. The strips were moistened and covered as recommended by the manufacturer and incubated at 37°C. The changes in color from violet were monitored daily for 2 days. The strips were read after the incubation time and each test was interpreted: positive (+) samples were denoted by a change in color to yellow; no change in color indicated negative (-) samples and the samples that changed to another color were doubtful (±). The first strip was used as a negative control well. An esculin hydrolysis (revealed by a change to darker color or black) was represented by a positive sign while a negative sign represented no change. The APILAB PLUS database (BioMerieux) was used to interpret the result.

Identification of yeasts: Primary classification of colonies from the PDA agar plates was based on colony characteristics (pigmentation and shape), formation of ascospores, presence of budding cells and hyphae or pseudohyphae. The methods described by Harrigan and McCance (1986) were followed. Identification of the yeast isolates to species level was done using the API 20C AUX (BioMerieux, Marcy l'Etoile, France) system of carbohydrate assimilation profiles. The tests were performed according to the manufacture's instructions as follows. A swab of each yeast isolates grown on PDA agar plates (incubated at 37°C for 48 h) was suspended in API C medium. Using sterile pipette, homogenized suspension of the cells in the medium was distributed into each of the 20 wells on the API 20C AUX strips. Strips were moistened and covered as recommended by the manufacturer and incubated at 29±2°C. After 48 h of incubation or 72 h (if the tests, in particular glucose, are not clear-cut after 48 h), the grows were compared in each strip to the first strip, which was used as a negative control well. The well having more turbidity than the control, indicates a positive reaction (+), while well having a clear medium indicates a negative reaction (-). The APILAB PLUS database (BioMerieux) was used to interpret the result.

RESULTS AND DISCUSSION

Enumeration of microorganisms: Table 1 shows the microbial counts of Sameel milk samples obtained from different villages. *Lactobacilli* and *Lactococci* were the dominant LAB in all tested samples with average viable counts of 7.4 and 7.7 $\log_{10}$ CFU mL^{-1} with a range of 6.3-8.7 and 6.5-8.9 $\log_{10}$ CFU mL^{-1}, respectively, with the corresponding average aerobic mesophilic bacterial counts of 6.6 $\log_{10}$ CFU mL^{-1}. Actually, the average counts of LAB (*Lactobacilli* and *Lactococci*) of Sameel milk was similar to that of yoghurt, which normally was 6-8 log CFU mL^{-1} (Gou, 2003). Many potential health or nutritional benefits from some species of LAB were reported as: improved nutritional value of food, control of intestinal infections, improved digestion of lactose, control of some type of cancer and control of serum cholesterol levels (Gilliland, 1990). The mean number of yeast (5.7 $\log_{10}$ CFU mL^{-1}) was lower than either the counts of *Lactobacilli* or *Lactococci* by almost two log-cycle. The samples having low pH values (≤4.2) contained higher number of yeast than other samples. The mean of *Enterococcus* count was 5.9 $\log_{10}$ CFU mL^{-1} with a range of 3.3-6.9 $\log_{10}$ CFU mL^{-1} giving an indication that this group of LAB may contribute to

Table 1: Microbial viable counts ($\log_{10}$ CFU mL^{-1}) and pH of Sameel milk samples from Eastern villages (E1-E10), Riyadh road villages, (R1-R10) and Qatar road villages, (Q1-Q9)

Samples	Aerobic mesophilic bacteria	*Lactobacilli*	*Lactococci*	Yeasts	*Enterococcus*	*Enterobacteriaceae*	pH
E1	6.5	7.8	8.5	7.7	6.3	<2.0	4.1
E2	6.3	8.4	8.1	5.6	6.2	<2.0	4.5
E3	7.9	7.0	8.5	4.5	4.1	2.1	4.6
E4	7.8	6.8	7.3	3.1	3.8	5.8	5.0
E5	6.9	8.2	7.6	7.0	6.9	<2.0	4.2
E6	7.3	7.6	8.0	7.3	6.7	<2.0	4.1
E7	5.9	8.5	7.8	6.9	6.5	<2.0	4.1
E8	5.3	7.6	8.7	7.5	6.8	<2.0	4.1
E9	7.6	8.7	8.3	5.1	6.4	<2.0	4.5
E10	7.7	6.9	8.0	6.1	4.3	3.2	4.4
R1	6.3	7.4	6.5	4.0	6.3	<2.0	4.5
R2	6.8	6.6	6.7	4.7	6.5	<2.0	4.5
R3	5.0	7.0	8.8	5.6	7.0	<2.0	4.4
R4	6.4	6.4	6.8	3.9	3.5	5.7	4.8
R5	6.18	8.1	6.5	5.3	6.3	<2.0	4.5
R6	6.3	7.5	7.5	5.3	6.5	<2.0	4.4
R7	6.2	6.3	7.3	6.5	6.4	<2.0	4.4
R8	7.3	6.4	8.9	5.8	6.8	<2.0	4.5
R9	5.4	6.5	8.0	4.7	3.3	4.5	4.7
R10	6.4	7.5	6.8	5.0	6.6	<2.0	4.5
Q1	5.5	7.3	6.8	5.1	6.6	<2.0	4.6
Q2	7.2	6.5	7.5	5.7	6.9	<2.0	4.6
Q3	6.9	7.0	7.6	5.9	6.8	<2.0	4.5
Q4	7.8	7.7	8.3	6.4	6.5	<2.0	4.3
Q5	7.1	8.5	7.6	5.3	6.3	2.1	4.6
Q6	7.0	8.1	7.8	6.6	5.3	3.2	4.3
Q7	5.8	8.3	8.1	7.1	5.1	3.1	4.2
Q8	6.4	7.9	6.9	5.8	5.0	2.6	4.6
Q9	5.3	6.8	8.1	6.2	6.8	<2.0	4.4
Mean	6.6	7.4	7.7	5.7	5.9	3.7	4.5
Standard deviation	0.8	0.7	0.7	1.1	1.1	1.5	0.2

fermentation of these fermented milk. The counts of *Enterobacteriaceae* were under the detection level in twenty samples, whereas the mean counts in other nine samples were detected at a relatively lower numbers 3.7 $\log_{10}$ CFU mL^{-1} with a range of 2.1-5.7 $\log_{10}$ CFU mL^{-1}. The pH values of these twenty samples were ≤4.5 with the exception of two samples (Q1 and Q2). The *Enterobacteriaceae* counts of one sample (E4) were 5.8 $\log_{10}$ CFU mL^{-1} with a yeast count of 3.1 $\log_{10}$ CFU mL^{-1} at pH 5.0. A definite correlation was also observed in all tested samples that the *Enterobacteriaceae* counts decreased and yeast counts increased with the decrease of pH value. This observation suggests a possible interaction between bacteria and yeasts involved in Sameel milk fermentation. Presence of low counts of *Enterobacteriaceae* has also been reported (Mathara *et al*., 2004; El-Baradei *et al*., 2008; Zhang *et al*., 2008). The lactic acid produced by this LAB together with other metabolites and the strong competitive effects of the LAB population may be responsible mainly for the extended shelf life of fermented products with sufficiently low pH. There were wide variations in the counts of microorganisms compared with findings of similar studies on naturally fermented milks in various part of the world. These variations may be due to the difference in the fermentation conditions and types of milk in China, According to Sun *et al*. (2005), Luo *et al*. (2005) and Zhang *et al*. (2008), the average counts of LAB were 7.4, 8.74 and 9.18 $\log_{10}$ CFU mL^{-1} while the mean counts of yeasts were 6.44, 7.48 and 8.33 $\log_{10}$ CFU mL^{-1}, respectively. In East region of Africa, Mathara *et al*. (2004) and Sulieman *et al*. (2006) found the mean counts of *Lactobacillus* as 7.4 and 7.54 $\log_{10}$ CFU mL^{-1}, *Lactococcus* as 5.92 and 7.9 $\log_{10}$ CFU mL^{-1} and the yeasts counts were 5.9 and 6.0 $\log_{10}$ CFU mL^{-1}, respectively.

Identification of LAB to genus level: A total of one hundred and twelve LAB strains were isolated from the traditional Sameel fermented milk and identified to genus level based on cell morphology, gas production from glucose, growth behavior at 10, 15 and 45°C and in presence of 6.5% NaCl and at pH 9.6 according to Wood and Holzapfel (1995) and Stiles and Holzapfel (1997). All the LAB isolates were gram positive and catalase negative. Out of these, 82% belonged to the genus *Lactobacillus*, 10% to *Lactococcus* and 8% to *Enterococcus*. The spontaneous fermentation of unheated milk takes advantage of the action of naturally occurring mixed microflora inherent in the milk (Sanders, 1991). The sources of these LAB genera in the spontaneously fermented milk are from exterior of the udder, dairy utensils, dust, grass, cattle dung and feedstuffs (Sharpe, 1981; Teuber and Geize, 1981).

Identification of LAB to species level: The identification of LAB to species level which were isolated from Sameel milk are shown in Table 2. A total of 112 LAB were isolated. The *Lactobacilli* were the predominate LAB in most of the tested samples. Fifty six *Lactobacillus* strains were identified as *Lactobacillus plantarum* with frequency occurrence of 50%. They all could grow in MRS at pH 3.9 and in the presence of 6.5% NaCl. The presence of a high numbers of *Lactobacillus plantarum* strains could be due to the addition of some herbs (Depagh) with milk during fermentation processing. The *Lactobacillus plantarum* strains are known to be commonly associated with plant based food fermentations (Stiles and Holzapfel, 1997; Wood and Holzapfel, 1995; Adebayo-tayom and Onilude, 2008). Presence of a high number of *Lactobacillus plantarum* are in close agreement with findings of Mathara *et al*. (2004) who identified 130 out of 339 isolates as *Lactobacillus plantarum*. Adebayo-tayom and Onilude (2008) recorded 30.8% *Lactobacillus plantarum* of LAB isolated from plant origin fermented food. Medina *et al*. (2001) identified 92%

Table 2: Identification of LAB isolated from Sameel milk

	No. of strains					
Carbohydrate	56	29	13	6	5	3
Control	-	-	-	-	-	-
Glycerol	+	+	-	+	±	+
Erythritol	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-
L-Arabinose	+	+	-	+	+	+
D-Ribose	+	+	+	+	+	+
D-Xylose	-	+	-	+	+	-
L-Xylose	-	-	-	-	-	-
D-Adonitol	-	-	-	-	-	-
Methyl-βD-Xylopyranoside	-	-	-	-	-	-
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-Acetylglucosamine	+	+	+	+	+	+
Amygdalin	+	-	-	+	-	+
Arbutin	+	+	+	+	-	+
Esculin ferric citrate	+	+	+	+	+	+
Salicin	+	+	+	+	-	+
D-Cellobiose	+	+	+	+	-	+
D-Maltose	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	-
D-Sucrose	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+
Inulin	-	-	-	-	-	-
D-Melezitose	+	-	-	+	-	+
D-Raffinose	-	+	+	-	+	-
Amidon	+	-	-	-	-	±
Glycogen	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-
Gentiobiose	+	+	+	+	-	+
D-Turanose	-	-	-	-	-	-
D-Lyxose	-	-	-	-	-	-
D-Tagatose	+	+	+	+	-	+
D-Fucose	-	-	-	-	-	-
L-Fucose	-	+	+	±	+	-
D-Arabitol	-	-	-	-	-	-

Table 2: Continue

	No. of strains					
Carbohydrate	56	29	13	6	5	3
L-Arabitol	-	-	-	-	-	-
Potassium gluconate	±	+	-	+	+	+
Potassium 2-ketogluconate	-	-	-	-	-	-
Potassium 5-ketogluconate	-	-	-	-	-	-
Identity	*Lactobacillus plantarum*	*Lactobacillus pentosus*	*Lactococcus lactis* ssp. *lactis*	*Lactobacillus brevis*	*Lactobacillus salivarius*	*Lactobacillus paracasei* ssp. *paracasei*

-: Negative result, +: Positive result, ±: Doubtful result as described in material and methods

of all *Lactobacilli* in ewe's milk and cheese as *Lactobacillus plantarum*. From a total of 100 isolates from fermented milk, Isono *et al.* (1994) identified only four strains as *Lactobacillus plantarum*. Cueto *et al.* (2007) identified 15 out of 36 LAB isolates as *Lactobacillus plantarum*.

In this study, twenty nine strains were identified as *Lactobacillus pentosus* with a frequency of 26%. All of these strains are able to grow in MRS at pH 3.9 and in the presence of 6.5% NaCl. However, *Lactobacillus pentosus* has not been previously reported in traditional fermented milk, but it has been isolated from plant materials and fermented food (Osawa *et al.*, 2000; Sawitzki *et al.*, 2007). Whereas, Cueto *et al.* (2007) identified only one strain of *Lactobacillus pentosus* from 36 LAB strain isolated from traditional fermented milk. Thirteen strains were identified as *Lactococcus lactis* ssp *lactis*, six as *Lactobacillus brevis*, five as *Lactobacillus salivarius* and three as *Lactobacillus paracasei* ssp *paracasei*. All of these LAB were frequently found in the various traditional fermented milk in many countries of the world (El-Soda *et al.*, 2003; Lore *et al.*, 2005; Sulieman *et al.*, 2006; Cueto *et al.*, 2007; El-Baradei *et al.*, 2008; Kayagil and Candan, 2009). A very little is known about its source and role in the Sameel milk products which needs further studies.

Identification of yeasts: The identification of yeast strains isolated from Sameel milk are illustrated in Table 3. Thirty six yeast species were identified. The predominant species were *Candida lusitania* (15 strains), *Cryptococcus laurentii* (9) and *Saccharomyces cerevisiae* (9). While *Candida kefyr* (3) were identified less often. When compared to other studies, it seems that different yeast species were predominant in different fermented milk products. For example, *Saccharomyces cerevisiae*, *Candida lusitania*, *Candida colliculosa* and *Saccharomyces dairenensis* were the predominant strains in Zimbabwean traditional fermented milk (Gadaga *et al.*, 2000). *Debaryomyces hansenii* was the predominant strain in Sardinian ewe's dairy products (Cosentino *et al.*, 2001). Abdelgadir *et al.* (2001), in Sudan and Shuangquan *et al.* (2006) in Mongolia, found that the predominant yeast strains in the fermented milk were *Saccharomyces cerevisiae* and *Candida kefyr*. *Candida lusitania* has been mentioned as an important spoilage yeast in yoghurt (Green and Ibe, 1987; Jakobsen and Narvhus, 1996) and the species has been considered as one of the characteristic yeasts in dairies. This aspect, however, raises concerns about its suitability as a possible starter culture in milk fermentations (Gadaga *et al.*, 2000). Additionally, none of the strains could utilize lactose as a carbon source which are an important technological properties in milk fermentation. Nine isolates were identified as *Cryptococcus laurentii*. Except D-sorbitol, these species were able to utilize all tested sugars including lactose. Therefore, such

Table 3: Identification of yeasts isolated from Sameel milk

	Number of strains			
Characteristics	15	9	9	3
Colony colour	Cream	White	White	White
Colony shape	Ovoid	Spherical	Ovoid	Ovoid
Ascospores	Absent	Absent	Present	Absent
Budding cells	Present	Present	Present	Present
Hyphae/pseudohyphae	Absent	Absent	Absent	Absent
Substrate fermentation				
Control	-	-	-	-
D-Glucose	+	+	+	+
Glycerol	+	+	-	+
Calcium 2-Keto-Gluconate	+	+	-	-
L-Arabinose	-	+	-	+
D-Xylose	-	+	-	-
Adonitol	+	+	-	-
Xylitol	-	+	-	-
D-Galactose	-	+	+	+
Inositol	-	+	-	-
D-Sorbitol	+	-	-	-
Methyl-αD-Glucopyranoside	-	+	-	-
N-Acetyl-Glucosamine	+	+	-	-
D-Cellobiose	+	+	-	-
D-Lactose	-	+	-	+
D-Maltose	+	+	+	-
D-Sucrose	+	+	+	+
D-Trehalose	+	+	-	-
D-Melezitose	+	+	-	-
D-Raffinose	-	+	+	+
Identity	*Candida lusitania*	*Cryptococcus laurentii*	*Saccharomyces cerevisiae*	*Candida kefyr*

-: Negative result, +: Positive result, ±: Doubtful result as described in material and methods

yeast could be applicable in fermented dairy product. Some researchers isolated and identified *C. lusitania* and *Cryptococcus laurentii* from traditional fermented milk. Gadaga *et al.* (2000), identified 11 strains as *C. lusitania* and one strain as *Cryptococcus laurentii* isolated from Zimbabwean traditional fermented milk. Kebede *et al.* (2007), isolated and identified *Cryptococcus laurentii* strains from south African spontaneously fermented milk in plastic bowl container. *Saccharomyces cerevisiae* fermented sucrose and raffinose in addition to glucose, galactose and maltose but not lactose. The growth response obtained could be explained by the utilization of the trace amounts of glucose and galactose in milk (Rosenthal, 1991). In other investigation, *S. cerevisiae* has been isolated from traditional fermented milk (Gadaga *et al.*, 2000; Abdelgadir *et al.*, 2001; Cosentino *et al.*, 2001; Shuangquan *et al.*, 2006).

From the isolates, *Candida kefyr* were identified. Although *C. kefyr* has a low incidence in the samples but they are known to be important in dairy products (Fleet, 1990; Seiler and Busse, 1990) and their presence in the milk sample could be important.

After identifying the dominant flora in Sameel milk fermentation, there is need for investigation of their technological properties to select the most appropriate strains as starter culture for a controlled and optimized process. In reality, some strains of *Lactobacillus paracasei* ssp. *paracasei*

are probiotic culture (Tharmaraj and Shah, 2004; Vlieger *et al*., 2009). However, the presence of *Lactobacillus paracasei* ssp. *paracasei* in Sameel milk increases its therapeutic value. Isolation of some strains of this species will provide an opportunity for further investigation.

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