Received: 30 June 2023, Accepted: 04 September 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 7, 1443-1452

Assigning the Probiotic Potential of Lactic Acid Bacteria Recovered from Popular Egyptian Fermented Artisanal Dairy Products

Mohamed E.A. Alnakip*, Engy El-Essely, Mohamed A. Bayoumi, Esmat I. Elsaid

Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Elsharkia, 4451, Egypt.

***Correspondence**
Corresponding author: Mohamed E.A. Alnakip
E-mail address: Alnakip.me@gmail.com

Abstract

Various artisanal dairy products such as kariesh cheese, Laban Rayeb (a type of fermented fluid milk), zabady (a type of artisanal yoghurt), Butter milk and soured cream (a type of cream separted from skim milk after overnight natural fermentation of milk in earthenware pots) are poular for human consumption in Egypt. However, they are manufactured from raw milk depending on natural fermentation by wild microflora without guaranteed heat treatment processing or addition of permissible additives. Thus, the current study included the microbiological investigations on 50 samples of each of afore-mentioned products (total of 250 samples) primarily to isolate and discriminate different lactic acid bacteria (LAB) flora and secondly to search for some LAB to be further considered as a probiotic culture. Accordingly, several characteristics were investigated including, their ability to resist and survive gastrointestinal tract conditions represented in gastric acidity (pH 3) and duodenal bile acids, and at the same time, ability to produce antimicrobial substances such as organic acids (lactic acid), hydrogen peroxide and diacetyl. At the same time, the isolates were tested for having safety or non-pathogenicity, which principally includes non-harboring of antibiotic resistance (AR) features or blood hemolysis activity. Three important technological properties including the salt tolerance, β-galactosidase production and milk acidification ability were tested for selected isolates as important features needed for optimum fermentation by LAB as starter or non-starter cultures that can be incorporated in dairy processing. Finally, the LAB strains were tested as inhibitors for bacteria of food-safety concern such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Our results showed that the examined fermented artisanal dairy products proved to harbor a wide variety of LAB microflora. After screening the probiotic, technological and safety related properties of 40 selected LAB isolates from examined products, 5 strains were proven to meet all required criteria, thus could be tested in future studies as promising strains to be incorporated in manufacture of various dairy products as starter and non-starter cultures.

KEYWORDS
Artisanal, Dairy, lactic acid bacteria, Probiotics, Safety, Inhibitory effects

INTRODUCTION

Among typical artisanal dairy products in Egyptian rural areas and villages, Karish cheese, artisanal yoghurt (known as Zabady), Laban Rayeb (a type of fermented liquid milk) and soured cream (a type of cream separted from skim milk after overnight natural fermentation of milk in earthenware pots) are extensively manufactured and are desired by a wide variety of consumers.

Karish cheese is a type of acid-coagulated cheeses made from defatted or skim milk in a special earthenware pots on which the partly skimmed milk sours and clots (Baraheem *et al*., 2007; Abou-Donia, 2008; Alnakip, 2009; Korish and Abd Elhamid, 2012). Later, the curd is poured onto a special mat which is tied and hung with its contents to allow the drainage of the whey over a period of 2-3 days until the desired texture of the cheese is obtained. Finally, the cheese is salted and cut into suitable pieces to be left for a few hours in the mat till no more whey drains out. Karish cheese can be ready to be consumed as a "fresh Karish" within 1-2 weeks or can be pickled in brine earthenware pots; the so called "Mish cheese" to maintain a valid shelf-life for up to a year (Abou-Donia, 1986; Baraheem *et al*., 2007; Abou-Donia, 2008). This cheese is one of the most popular types of soft cheese consumed in Egypt, especially in the countryside and among athletics owing to its high protein content (30%), low fat and lowered price (Ahmed *et al*., 2005; Abou-Donia, 2008; Alnakip, 2009; Korish and Abd Elhamid, 2012). Actually, the quantity of Karish cheese produced in Egypt, is unknown, however, it is believed that about 50% of the total milk produced is utilized for its manufacturing (Baraheem *et al*., 2007).

On the other hand, artisanal yoghurt is manufactured by various producers via inoculation of previously boiled milk with old yoghurt either from commercial factories or yoghurt produced locally from the previous days at the same processing unit (Alnakip, 2009). Thus, it differs from commercial yoghurt in the purity of starter as its manufacture not include the addition of starters in their pure form. Such method of production of artisanal yoghurt not guarantee the purity and the safety of starter as additive fermentation inoculum.

As inferred from production process, production facilities are quite primitive, the incubation conditions of those products are relatively uncontrolled accompanied with lack of strict hygienic measures and secure thermal treatment. Therefore, a wide variety of natural microflora particularly; lactic acid bacteria (LAB), are existing and contributing to fermentation process (Fahmy andYoussef., 1978; Dufour and Collin, 1995; El-Soda *et al*., 2003; El-Baradei *et al*., 2007; El-Baradei *et al*., 2008; Alnakip, 2009).

LAB are a group of Gram-positive non-sporing, non-respiring cocci or rods that are widely distributed in nature and mainly

ISSN: 2090-6277/2090-6269/

characterized by producing lactic acid as a major end product during the fermentation of carbohydrates (Axelsson, 2004). LAB represent 20-30% of total bacterial count in raw milk, but several factors as production conditions, breeding, season and the animal origin greatly affect their abundance and diversity (Gaya *et al*., 1999; Drakoularakou *et al*., 2003; Verdier-Metz *et al*., 2009). However, only LAB belonging to beneficial and nonpathogenic genera have traditionally been used in the food industry. From a practical, dairy-technology point of view, the following genera are considered the principally used LAB: *Enterococcus*, *Lactococcus*, *Pediococcus*, *Lactobacillus*, Leuconostoc and *Streptococcus*.

Currently, a great attention is arising towards healthy functional foods which combine both desirable aromatic characteristics and having microorganisms having health-promoting effects; the so called "Probiotics" (Zhang *et al*., 2003; Abou-Donia, 2008). In this sense, fermented dairy products are largely occupying advanced rank in consumers' interest globally. In Egypt particularly, the raw milk is frequently incorporated in manufacture of several artisanal dairy products such as kariesh cheese and artisanal yoghurt, mainly without heat-treatment or addition of permissible additives. The process is mainly dependent on the fermentation process adopted by existed wild lactic acid bacteria (LAB) flora, which include various types of beneficial probiotic bacteria (Abou-Donia, 2004; El-Baradei *et al*., 2008; Alnakip *et al*., 2016).

Probiotic as a term describes a group of microorganisms possessing beneficial potential for the host when taken in proper doses (FAO/WHO, 2007). The guidelines accredited by FAO/WHO for evaluating probiotics in foods impose that proper *in Vitro* investigations applied to guarantee their efficacy and public safety. There are many LAB strains that had been reported generally regarded as safe' (GRAS) status and used widely in commercial dairy products. Accordingly, to be considered as a probiotic strain, several properties must be possessed by bacterial strain including their ability to survive and resist gastrointestinal tract (GIT) conditions represented in gastric acidity and duodenal bile salts (Holzapfel *et al*., 1998), have pathogen antagonizing or competitor mechanisms via production of antimicrobial compounds such as organic acids (as lactic A., acetic A.), hydrogen peroxide, diacetyl, bacteriocins and bacteriocin-like compounds (Saarela *et al*., 2000), and possess a strong adhesive ability to intestinal epithelium (Monteagudo-Mera *et al*., 2012). Concurrently, safety of probiotic strain is a non-debatable concept, which principally includes non-harboring of antibiotic resistance or virulence factors (Monteagudo-Mera *et al*., 2011; Monteagudo-Mera *et al*., 2012).

Because the screening of microbial population in raw milk and artisanal dairy products is considerably neglected in Egypt and as a consequence of the potential role of lactobacilli in dairy industry, the aims of current study were isolation and typing of LAB from some artisanal fermented dairy products marketed in Egypt, testing for probiotic properties of selected LAB strains based on both resistance to gastrointestinal conditions and having antimicrobial activities, ensuring the human safety of selected LAB isolates through lack of antibiotic resistance and virulence factors and finally testing for the technological properties of isolates.

MATERIALS AND METHODS

Collection of samples

A total of 250 samples of artisanal fermented dairy products including Kariesh cheese, Laban Rayeb, artisanal yoghurt (Zabady), Butter milk and soured cream (50 samples of each) were aseptically sampled from AlSharkia governorate markets, Egypt during the period from Decembre 2021 till Febraury 2023. All samples were aseptically collected in sterile containers and transported rapidly in a 4°C vehicle-mounted refrigerator to Laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University to be microbiologically examined within few hours acc. to accredited recommended guidelines.

Isolation of LAB and preliminary identification

The collected samples were adequately prepared and serially diluted according to recommended guidelines described by IDF (1992), Wehr and Frank (1992). Accordingly, 1 ml of each of Laban Rayed and Butter milk samples were mixed with 9 ml sterile distilled water and undergone serial dilution to be ready for culture. Meanwhile, for Kariesh cheese, zabady and soured cream, 11 grams of each sample were homogenized and thoroughly mashed in 99 ml of 0.1% sterile peptone water (40°C) inside a clean sterile mortar under sterile condition. After the mixture become homogenous, 1 ml from the solution was used for preparation of decimal dilution as previously described for laban rayeb and butter milk samples.

Following the serial dilution, 0.1 mL of each dilution was spread plated in duplicates on de Man, Rogosa, and Sharpe agar (MRS) (Difco Labs, Detroit, MI) adjusted to pH of 5.5 (De Man *et al*., 1960). MRS Plates were incubated anaerobically (BBL Gas pak plus Anaerobic Sys.) at 30°C for 48 h. Three colonies with distinct morphological differences were selected from each plate and further purified by re-streaking two successive times on fresh MRS plates. All isolates were maintained as frozen cultures in MRS broth and 50% glycerol at -20°C (Alnakip *et al*., 2016) for further investigations. The isolates were identified primarily based on Gram staining followed by biochemical identification based on Catalase, pH tolerance, Kliger's Iron Agar, Phenol tolerance, NaCl tolerance, sugar fermentation (Glucose, fructose, sucrose, xylose and lactose) and Casein digestion tests

Testing of probiotic properties for selected Lactobacillus sp. isolates (Prasad et al., 1998; Kamal et al., 2018)

The following tests were applied to 40 isolates corresponding to *Pediococcus pentosaceus* (n=5), *Lc. lactis* (n=5), *Lb. plantarum* (n=5), *Lb. rhamnosus* (n=5), *Lb. casei* (n=5), *Lb. brevis* (n=5), *Lb. acidophillus* (n=5) and *Lb. fermentum* (n=5) species; isolated from different products and selected randomly, to confirm their possession for probiotic properties.

Acidic pH tolerance

After cultivating LAB isolate overnight in MRS broth, bacterial pellets were harvested by centrifugation at 5000 rpm for 10 min followed by washing once in phosphate-saline buffer (pH 7.2). Simulated gastric juice acidity was carried out by adjusting the pH of different sterile MRS broth tubes to 2.0, 2.5 and 3.0 with concentrated HCl. Re-suspension of bacterial cells was done in different-pH MRS broth tubes followed by incubation at 37°C for 3 h. For screening tolerance, aliquots of 1 mL of different-pH MRS broth were taken periodically each hour for the determination of total viable count.

Bile salts tolerance

The bile tolerance for LAB isolates was determined by inoculation of 1 mL from over-night incubated MRS into MRS broth containing bile (0.3% w/v). The mixture was incubated at 37°C for

4 h. without agitation. For the tolerance assay, aliquots of 1 mL were removed each hour for determination of total viable count.

Determination of antimicrobial production (lactic acid, hydrogen peroxide and Diacetyl production capabilities) (AOAC, 1990)

For these measurements, the isolates were grown on MRS broth for 72 hours and samples taken at 12 hours interval.

Lactic acid

To 25 ml of broth culture of selected isolate, 3 drops of phenolphthalein were added as indicator. From the burette 0.1 N NaOH was slowly added to the sample until pink color appeared. Each ml of 0.1 NaOH is equivalent to 90.08 mg of lactic acid.

Hydrogen peroxide

Twenty-five milliliters of dilute sulphuric acid were added to 25 ml of the broth culture of the tested isolate. Titration was carried out with 0.1N potassium permanganate. Each ml of 0.1N potassium permanganate is equivalent to 1.070 mg of H_20_2. The decolourization of the sample was regarded as an end point.

Diacetyl

Twenty five milliliters of broth cultures were transferred into conical flasks and 7.5ml of hydroxylamine solution were used for the residual titration. The flasks were titrated with 0.1N HCl to a greenish-yellow end point using bromophenol blue as indicator. The equivalence factor of HCl to diacetyl is 21.5 mg.

Cell free supernatant (CFS) preparation and treatments

LAB isolates were anaerobically grown on MRS broth at 37 oC for 48 h. Incubated broths were then centrifuged (5000 rpm at 4°C for 10 minutes) and filtered using 0.45 µm pore membrane (Millipore). CFS kept refrigerated until further use. For exclusion of inhibitory effect of produced organic acids, CFS were initially neutralized using sterile 0.1N NaOH and named neutralized CFS (nCFS). To determine whether H_2O_2 and/ or bacteriocins have contributed to LAB's antimicrobial effect, CFS was divided into two portions; one was heated at 80°C for 10 min (hCFS) and the second was treated with proteolytic enzymes (pCFS) (trypsin, proteinase K and alpha chemotrypsin, 1 mg/mL) (Nair and Surendran 2000).

Antibacterial activity for LAB isolates (Kamal et al., 2018)

Pathogenic *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*Ps. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) (previously isolated and molecularly identified based on 16s rRNA GS (Alnakip, 2014; Sánchez-Rubio *et al*., 2016; Kamal *et al*., 2018) were used as indicator strains. The bacterial strains were inoculated into brain heart infusion broth (BD, Difco) and incubated at 37°Cfor 24 h prior to their incorporation.

The agar well diffusion method was used to detect the antibacterial property of the LAB isolates. One ml of each indicator organism was inoculated into Muller Hinton agar maintained at 50°C and then poured into a petri dish. After solidification, Wells (5 mm diameter) were cut and 35µl of cell-free supernatant (CFS) from each LAB isolate were added to each well. Cell-free supernatant CFS was prepared as follows: one ml. of LAB isolate was cultured overnight in 20 ml MRS broth then 1 ml. culture was sub-cultured overnight in 20 ml MRS broth. Cells were removed by centrifuging at 14,000 g for 5 min. The supernatant was filtered. Petri dishes were incubated at 37°C for 24 hrs. The antibacterial activity was determined by measuring the clear zone around the wells. Inhibitions zones indicated that isolates were able to produce antibacterial substances. In order to rule out possible inhibition effects by acidity, The CFS of these isolates were neutralized to pH 6 with 1 M NaOH. 35 µl of neutralized CFS were filtered and added to each well. Petri dishes were incubated at 37°C for 24 h. To determine whether H_2O_2 and/ or bacteriocins contributed to the inhibition of bacteria by LAB, Neutralized CFS were divided into two portions. One portion was heated at 80°C for 10 min. and the second was treated with proteolytic enzymes (trypsin, proteinase K and alpha chymotrypsin) (1 mg/ml).

Testing for the technological properties of selected LAB Isolates

Milk acidification ability

Acidification capability for an isolate was determined by the change in pH (ΔpH) during time. Fifty mL of UHT skim milk was inoculated with 1% of LAB culture (10^6 cfu/ ml) and incubated at 37°C. The pH was measured using a pH-meter (Micro pH). The acidification value was calculated as the difference between the value immediately after inoculation and values after incubation (ΔpH = pH zero time – pH at time). The LAB isolates were considered as fast, medium or slow acidifying when a ΔpH of 0.4 U was achieved after 3, 3-5 and > 5 h, respectively (Lombardi *et al*., 2002, Ayad *et al*., 2004).

Salt resistance

MRS broth tubes containing 4% sodium chloride were inoculated with a loopful of 18 h. active culture of a selected isolate. The tubes were incubated at 37°C and the growth was observed after 48 h.

Study of β-Galactosidase production

20 mg/ml stock solution of X-Gal in dimethyl sulfoxide (DMSO) was Prepared. X-Gal stock solution was added to the molten agar at 45°C (5 ml./1liter). Active cultures (16-18 h) were spotted on modified MRS agar medium (lactose was used instead of glucose as a carbon source) and the plates were incubated at 37°C for 48 h anaerobically. Appearance of blue colonies indicated positive results.

Testing of safety-related properties for LAB isolates

The examination of safety-related properties for selected LAB isolates was assessed as previously described (Prasad *et al*., 1998; Bernardeau *et al*., 2008; Verdenelli *et al*., 2009; Gheytanchi *et al*., 2010; Sireswar *et al*., 2017; Kamal *et al*., 2018; Alnakip *et al*., 2019) based on the following criteria:

Hemolytic activity

The assessment of possessing blood hemolysis activity was evaluated on Columbia agar plates (Oxoid) supplemented with 5% sheep blood which were incubated at 37°C for 24 h (Lombardi *et al*., 2004, De Vuyst and Leroy, 2007).

Antibiotic resistance of LAB isolates

The isolates were inoculated into MRS broth individually and

incubated at 37°C for 24 h. MRS agar were inoculated by the cultures of LAB isolates (10^6 CFU/ml), mixed well, poured into sterile Petri plates and allowed to solidify. Different antimicrobials discs were placed and pressed on the top of the MHA plates. The plates were incubated at 37°C overnight (Alnakip *et al*., 2023). The absence of a growth inhibition zone around discs indicated resistance.

Statistical analysis

All experiments were done in triplicates. Significant differences were considered at p value of < 0.05. Results were descriptively analyzed using SAS software. Results were reported as Mean value ± Standard error of mean.

RESULTS AND DISCUSSION

Since ancient times, the Egyptian dairy markets in rural areas and villages have been characterized by the permanent presence of artisanal fermented dairy products. The manufacture process of such dairy products is primitive without addition of any starter, meanwhile the fermentation process is mainly dependent on native wild microflora (Fahmy and Youssef., 1978; Dufour and Collin, 1995; El-Soda *et al*., 2003; El-Baradei *et al*., 2007; El-Baradei *et al*., 2008; Alnakip, 2009, Alnakip *et al*., 2023). The nature of artisanal dairy products, often varied between different areas depending on the local indigenous microflora, particularly LAB. The composition of LAB in the previously studied dairy products in certain region differed from similar styles of same products produced elsewhere, pointing to the importance of natural contamination by native inhabitant flora from specific geographical localities (Alnakip *et al*., 2016). Interestingly, strict European food safety law had resulted in lowering flexibility in food production and would finally lead to the disappearance of a number of geographical and artisanal dairy products and their related indigenous M.Os (Hawaz, 2014; Alnakip *et al*., 2016).

Isolation and screening of microorganisms from naturally occurring environments is the most powerful means for obtaining useful isolates for commercial and research purposes. Some characteristics of LAB such as texture and flavor formation are very important to the food industry due to their applicability for a variety of products. The dairy industry used well-defined single strain or multiple strain starter cultures to obtain dairy products of constant and high quality. So, a continuous need exists for the isolation of new strains with superior natural qualities (Holzapfel *et al*., 1998; El-Soda *et al*., 2003; Bernardeau *et al*., 2008).

Microbiological isolation and identification of LAB from examined samples

In the current study, 50 samples of each of Laban rayeb, artisanal Yoghurt (Zabady), butter milk, soured cream and Kariesh

Table 1. LAB count/ml in examined samples

Samples	No. of samples	LAB				
		Positive		LAB count/ml		
		No.	%	Min.	Max.	Mean ±S.E.M.
Laban Rayeb	50	50	100	8.8×10^5	9.3×10^7	$6.9\times10^6\pm0.39\times10^4$
Kariesh cheese	50	50	100	8.5×10^6	6.4×10^7	$2.6\times10^7\pm0.33\times10^3$
Zabady	50	50	100	9.2×10^5	9.8×10^6	$7.3\times10^5\pm0.81\times10^3$-
Butter milk	50	50	100	6.9×10^5	7.8×10^7	$8.2\times10^6\pm2.5\times10^3$
Soured Cream	50	50	100	5.5×10^6	9.5×10^7	$7.9\times10^7\pm0.39\times10^4$

Table 2. Incidence of LAB in artisanal Egyptian dairy products

Species	Laban Rayeb		Kariesh cheese		Zabady		Butter milk		Soured Cream	
	No	%	No	%	No	%	No	%	No	%
E. faecalis	17	11.33	23	15.33	22	14.67	14	9.33	9	6
E. faecium	8	5.33	12	8	14	9.33	12	8	12	8
E. hirae	6	4	5	3.33	8	5.33	8	5.33	4	2.67
E. durans	4	2.67	7	4.67	2	1.33	8	5.33	11	7.33
E. casselifavus	4	2.67	6	4	0	0	7	4.67	9	6
E. saccharolyticus	3	2	2	1.33	0	0	5	3.33	1	0.67
Pediococcus pentosaceus	4	2.67	2	1.33	0	0	5	3.33	0	0
Lc. garviae	0	0	1	0.67	8	5.33	2	1.33	3	2
Lc. lactis	22	14.67	15	10	24	16	14	9.33	18	12
Lb. plantarum	14	9.33	12	8	14	9.33	8	5.33	12	8
Lb. rhamnosus	12	8	12	8	3	2	11	7.33	11	7.33
Lb. casei	11	7.33	7	4.67	6	4	8	5.33	14	9.33
Lb. brevis	6	4	9	6	4	2.67	3	2	6	4
Lb. acidophillus	8	5.33	12	8	31	20.67	6	4	12	8
Lb. fermentum	16	10.67	11	7.33	9	6	19	12.67	11	7.33
Leuc. mesentroides	1	0.67	3	2	1	0.67	2	1.33	3	2
Non-Identified	14	9.33	11	7.33	4	2.67	18	12	14	9.33
Total	150	100	150	100	150	100	150	100	150	100

cheese were randomly collected from different vendors, rural areas and artisanal markets in Alsharkia governorate, Egypt. Primarily, the microbiological assays included the isolation and identification of LAB flora in examined samples. The results of LAB counts/ml in examined samples are illustrated in Table 1. The kariesh cheese samples showed to harbor the highest LAB count ($2.6\times10^{7}\pm0.33\times10^{3}$), meanwhile the lowest LAB count was reported in Zabady samples ($7.3\times10^{5}\pm0.81\times10^{3}$).

Table 2 shows the discrimination of LAB isolates from different dairy products based on the biochemical assays, among LAB isolates, *L. lactis* predominated in Laban rayeb samples, *Lb. casei* predominated in both karish cheese and soured cream samples, *Lb. acidophilus* predominated in Zabady samples and finally *Lb. plantarum* predominated in butter milk samples. Our results were similar to previous studies (Wouters *et al*., 2002; El-Soda *et al*., 2003; Alnakip, 2009; Alnakip, 2014; Alnakip *et al*., 2016).

Testing of Probiotic properties of Selected Lactobacilli

LAB composes the largest group of probiotic bacteria. A successful potential probiotic strain must possess a number of desirable properties. Among important properties is the tolerance to acid and bile as well as possessing antimicrobial potential mediated by secretion of antimicrobial substances such as organic

Table 3. Resistance of LAB to acidity and bile salts

No.	Strain	Bile salts				Acid (pH 3)			
		Count (Log)				Count (Log)			
		0 h	1 h	2 h	3h	0 h	1 h	2 h	3h
1	*Pediococcus pentosaceus*	9.55±0.04	8.31±0.06	8.01±0.08	6.07±0.09	8.81±0.01	8.72±0.80	7.89±0.11	7.52±0.19
2	*Pediococcus pentosaceus*	9.86±0.09	9.88±0.04	7.54±0.23	6.41±0.16	9.25±0.05	8.69±0.01	8.97±0.17	7.49±0.55
3	*Pediococcus pentosaceus*	9.46±0.18	9.02±0.19	7.99±0.24	6.96±0.22	9.67±0.01	8.83±0.38	7.57±0.08	7.14±0.38
4	*Pediococcus pentosaceus*	9.47±0.81	9.31±0.48	8.26±0.08	7.28±0.41	8.95±0.08	8.88±.0.67	7.46±0.02	7.25±0.44
5	*Pediococcus pentosaceus*	9.24±0.02	8.92±0.06	7.69±0.18	6.84±0.06	9.07±0.82	8.74±0.04	7.50±0.41	7.23±0.18
6	*Lc. lactis*	9.60±0.34	9.24±0.01	7.81±0.32	6.94±0.032	9.41±0.17	9.1±0.17	7.99±0.13	7.84±0.15
7	*Lc. lactis*	10.25±0.22	8.95±0.61	7.89±0.14	6.95±0.017	9.17±0.12	8.81±0.01	7.88±0.13	7.69±0.09
8	*Lc. lactis*	9.21±0.03	9.04±0.11	7.97±0.22	7.04±0.19	9.11±0.21	8.65±0.02	7.89±0.17	6.90±0.03
9	*Lc. lactis*	10.36±0.07	9.02±0.07	8.90±0.21	6.92±0.29	9.30±0.08	8.94±0.27	8.07±0.39	7.98±0.17
10	*Lc. lactis*	10.23±0.44	8.93±0.04	7.89±0.03	6.81±0.06	9.04±0.16	8.54±0.32	7.96±0.66	6.46±0.08
11	*Lb. plantarum*	9.55±0.04	9.31±0.06	8.01±0.08	6.07±0.09	8.81±0.01	8.72±0.80	7.99±0.11	7.82±0.19
12	*Lb. plantarum*	9.22±0.09	8.12±0.04	7.54±0.23	6.41±0.16	10.25±0.04	8.69±0.01	8.07±0.16	7.49±0.53
13	*Lb. plantarum*	9.46±0.18	9.02±0.19	7.99±0.24	6.96±0.22	9.83±0.01	8.83±0.38	7.97±0.08	7.04±0.45
14	*Lb. plantarum*	9.47±0.81	9.31±0.48	8.26±0.08	7.08±0.41	8.85±0.08	8.18±.0.67	7.86±0.02	7.56±0.44
15	*Lb. plantarum*	9.24±0.02	8.92±0.05	7.69±0.18	6.84±0.06	9.07±0.82	8.74±0.04	7.90±0.41	7.67±0.17
16	*Lb. rhamnosus*	9.30±0.54	9.24±0.01	7.81±0.32	6.94±0.032	9.71±0.17	9.12±0.17	7.67±0.14	7.33±0.12
17	*Lb. rhamnosus*	9.25±0.22	8.95±0.61	7.89±0.14	6.95±0.017	9.87±0.12	8.81±0.01	7.88±0.13	7.19±0.05
18	*Lb. rhamnosus*	9.21±0.03	9.04±0.04	7.97±0.22	7.34±0.19	9.41±0.21	8.65±0.02	7.98±0.17	6.87±0.02
19	*Lb. rhamnosus*	9.36±0.07	9.02±0.07	8.90±0.21	6.92±0.29	9.39±0.08	8.94±0.27	8.07±0.39	7.94±0.17
20	*Lb. rhamnosus*	9.23±0.44	8.93±0.04	7.89±0.03	6.81±0.06	9.94±0.16	8.54±0.32	7.96±0.66	7.96±0.03
21	*Lb. casei*	10.55±0.04	9.31±0.06	8.01±0.08	6.07±0.09	8.81±0.01	8.12±0.80	7.99±0.11	7.12±0.19
22	*Lb. casei*	9.29±0.05	8.33±0.04	7.54±0.23	6.41±0.16	10.34±0.09	8.69±0.01	8.07±0.18	7.49±0.53
23	*Lb. casei*	9.46±0.18	9.02±0.19	7.99±0.24	6.96±0.22	9.98±0.01	8.83±0.38	7.97±0.08	7.24±0.38
24	*Lb. casei*	10.47±0.81	9.31±0.48	8.26±0.08	7.08±0.41	8.95±0.08	8.88±.0.67	7.86±0.02	7.25±0.24
25	*Lb. casei*	9.24±0.02	8.92±0.06	7.69±0.18	6.84±0.06	9.07±0.82	8.74±0.04	7.90±0.41	7.13±0.18
26	*Lb. brevis*	9.37±0.57	9.24±0.01	7.81±0.32	6.94±0.032	9.41±0.17	9.1±0.17	7.99±0.14	7.14±0.15
27	*Lb. brevis*	9.25±0.22	8.95±0.61	7.89±0.14	6.95±0.017	9.17±0.12	8.81±0.01	7.88±0.13	7.69±0.08
28	*Lb. brevis*	9.21±0.03	7.04±0.08	6.97±0.22	6.04±0.19	9.11±0.21	8.65±0.02	7.13±0.17	6.34±0.03
29	*Lb. brevis*	9.36±0.07	9.02±0.07	8.90±0.21	6.92±0.29	9.30±0.08	8.94±0.27	8.07±0.39	6.94±0.17
30	*Lb. brevis*	9.23±0.44	8.93±0.04	7.89±0.03	6.81±0.06	9.04±0.16	8.54±0.32	7.96±0.66	7.46±0.04
31	*Lb. acidophillus*	9.55±0.04	9.31±0.06	8.01±0.08	6.07±0.09	8.81±0.01	8.72±0.80	7.99±0.11	7.82±0.19
32	*Lb. acidophillus*	9.17±0.12	8.84±0.04	7.54±0.23	6.41±0.16	8.95±0.07	8.69±0.01	8.07±0.17	6.49±0.45
33	*Lb. acidophillus*	9.46±0.18	9.02±0.19	7.99±0.24	6.96±0.22	10.23±0.01	8.98±0.38	7.97±0.08	6.14±0.18
34	*Lb. acidophillus*	9.47±0.81	9.31±0.48	7.26±0.08	7.23±0.41	9.95±0.08	8.09±.0.67	7.86±0.02	7.25±0.44
35	*Lb. acidophillus*	9.24±0.02	8.92±0.06	7.69±0.18	6.84±0.06	9.07±0.82	8.74±0.04	7.70±0.41	7.23±0.18
36	*Lb. fermentum*	9.33±0.12	9.24±0.01	7.81±0.32	6.94±0.032	9.41±0.17	9.1±0.17	7.99±0.14	7.44±0.15
37	*Lb. fermentum*	9.25±0.22	8.95±0.61	7.89±0.14	6.95±0.017	9.17±0.12	8.81±0.01	7.88±0.13	7.69±0.09
38	*Lb. fermentum*	9.21±0.03	10.04±0.04	7.97±0.22	7.04±0.19	9.11±0.21	8.65±0.02	7.89±0.17	6.80±0.03
39	*Lb. fermentum*	9.36±0.07	9.02±0.07	8.90±0.21	6.92±0.29	9.30±0.08	8.94±0.27	8.07±0.39	7.23±0.19
40	*Lb. fermentum*	9.23±0.44	8.93±0.03	7.89±0.03	6.81±0.06	9.74±0.16	8.54±0.32	7.96±0.66	7.44±0.04

acids, hydrogen peroxide and Diacetyl. Besides, other safety related properties such as lack of antibiotic resistance and blood hemolysis are considered as essential characters for selection of potential probiotic strain (Prasad *et al.*, 1998; Bernardeau *et al.*, 2008; Verdenelli *et al.*, 2009; Gheytanchi *et al.*, 2010; Sireswar *et al.*, 2017; Kamal *et al.*, 2018; Alnakip *et al.*, 2019). Finally, the potential strains proposed to be incorporated in dairy processing should have salt tolerance, acidification ability and preferably, if possess β-Galactosidase production ability (Kamal *et al.*, 2018).

Potential probiotic bacteria should resist stressful acidic conditions of the stomach to reach to the small intestine (Sireswar *et al.*, 2017; Kamal *et al.*, 2018). The resistance to acidic condition of stomach is usually determined *in Vitro* by detection of resistance to pH 3 for a 3 hours' time; nearly equal to that consumed by stomach for digestion (Prasad *et al.*, 1998). The results presented in Table 3 showed that all randomly selected LAB strains survived the test period of 3 h at pH 3.0 without a critical decrease in survival percentage. *L. lactis* (strain no. 9) and *Lb. rhamnosus* (strain no. 20) showed the highest acid tolerance. While other strains showed variable survival percentages. Nearly similar results were reported by Satish Kumar *et al.* (2011) and Kamal *et al.* (2018) who observed ability of *Lb. plantarum* and *Lb. rhamnosus* strains to survive gastric acidity. Also, a study by Maragkoudakis *et al.* (2006) showed that all of the tested *Lactobacillus* strains of dairy

Table 4. Testing of safety-related concerns in selected lactobacilli

No.	LAB Strains	Resistance of examined LAB isolates against selected antibiotics.												Blood haemolysis
		AMP	C	CEF	Amk	E	GN	CP	S	TE	CX	VA	CT	
1	*Ped. pentosaceus*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
2	*Ped. pentosaceus*	S	R	R	R	R	R	R	R	R	S	R	R	γ-hemolysis
3	*Ped. pentosaceus*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
4	*Ped. pentosaceus*	S	R	S	R	R	R	S	R	R	S	R	R	γ-hemolysis
5	*Ped. pentosaceus*	R	S	S	S	R	S	S	S	S	S	R	S	γ-hemolysis
6	*Lc. lactis*	S	R	S	S	S	R	R	R	R	S	R	S	γ-hemolysis
7	*Lc. lactis*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
8	*Lc. lactis*	R	R	R	R	R	R	R	R	R	S	R	R	γ-hemolysis
9	*Lc. lactis*	S	R	S	R	R	R	S	R	R	S	R	S	γ-hemolysis
10	*Lc. lactis*	S	R	S	S	R	R	R	R	R	S	R	S	γ-hemolysis
11	*Lb. plantarum*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
12	*Lb. plantarum*	S	R	S	R	R	S	S	R	S	S	S	R	γ-hemolysis
13	*Lb. plantarum*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
14	*Lb. plantarum*	S	R	S	R	R	R	S	R	R	S	R	R	γ-hemolysis
15	*Lb. plantarum*	S	R	S	S	R	R	S	S	R	S	R	S	γ-hemolysis
16	*Lb. rhamnosus*	R	R	R	S	S	S	S	R	S	S	R	R	γ-hemolysis
17	*Lb. rhamnosus*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
18	*Lb. rhamnosus*	R	R	R	R	R	R	R	R	R	S	R	R	γ-hemolysis
19	*Lb. rhamnosus*	S	R	S	R	R	R	S	R	R	S	R	S	γ-hemolysis
20	*Lb. rhamnosus*	S	R	S	S	R	R	S	R	R	S	R	S	γ-hemolysis
21	*Lb. casei*	S	R	S	R	R	S	S	S	R	R	S	S	γ-hemolysis
22	*Lb. casei*	S	R	R	S	S	R	R	S	R	S	S	R	γ-hemolysis
23	*Lb. casei*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
24	*Lb. casei*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
25	*Lb. casei*	S	S	S	S	S	S	S	S	R	S	R	S	γ-hemolysis
26	*Lb. brevis*	R	R	R	S	S	R	R	R	R	S	R	S	γ-hemolysis
27	*Lb. brevis*	R	S	R	S	S	R	S	S	S	S	S	S	γ-hemolysis
28	*Lb. brevis*	R	R	R	R	R	R	R	R	R	S	R	R	γ-hemolysis
29	*Lb. brevis*	S	R	R	S	R	R	S	S	R	S	R	R	γ-hemolysis
30	*Lb. brevis*	S	R	S	R	S	R	S	S	S	S	R	R	γ-hemolysis
31	*Lb. acidophillus*	S	R	R	S	S	S	R	S	S	S	S	S	γ-hemolysis
32	*Lb. acidophillus*	S	R	R	R	R	R	R	R	R	S	R	R	γ-hemolysis
33	*Lb. acidophillus*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
34	*Lb. acidophillus*	S	R	R	R	R	S	S	R	R	S	S	S	γ-hemolysis
35	*Lb. acidophillus*	R	S	S	R	S	R	S	S	R	S	S	S	γ-hemolysis
36	*Lb. fermentum*	R	R	R	S	S	S	S	S	R	S	R	S	γ-hemolysis
37	*Lb. fermentum*	S	S	S	S	S	S	S	S	S	S	S	S	γ-hemolysis
38	*Lb. fermentum*	R	R	R	S	R	R	S	R	R	S	R	S	γ-hemolysis
39	*Lb. fermentum*	S	R	S	R	S	R	S	R	S	S	R	S	γ-hemolysis
40	*Lb. fermentum*	S	R	S	S	R	R	S	R	R	S	R	S	γ-hemolysis

R: resistant, S: susceptible, AMP: Ampicillin, C: Chloramphenicol, CEF; Ceftriaxone sodium, E: Erythromycin,
CN: Gentamycin, CP; Cefoperazone, S: Streptomycin, TE: Tetracyclin, CX; Cefoxetine, VA: Vancomycin, CT; Cefotaxim

origin were resistant to pH 3 during the 3 hours period.

The mean bile concentration in small intestine was suggested to be 0.3% (w/v) and the staying time of food in it was believed to be 4 h (Prasad *et al*., 1998). In turn, these conditions were adopted in this study to explore the ability of strains to resist intestinal condition. Like resistance to pH 3, the results presented in Table 3 showed that all randomly selected LAB strains could survive test period of 3 h at pH 3.0 without a critical decrease in survival percentage. *Pediococcus pentosaceus* (strain no. 4) and *Lb. rhamnosus* (strain no. 18) showed the highest tolerance. While other strains showed variable survival percentages. Similar to our results, all of the tested *Lactobacillus* strains of dairy origin were resistant to 0.3% bile salts concentration in 4 h in a study by Maragkoudakis *et al*. (2006).

It was stupendous that that all randomly selected LAB strains of artisanal dairy origins could survived the simulation to gastrointestinal stressful conditions without a critical decrease in survival percentage. This may be attributed mainly to the artisanal manufacturing in the same places for long periods, with microbes gaining the ability to coexist in this environment. Also, some of the artisanal products that were examined, such as artisanal yoghurt and soured cream; made in pottery pots, which are used successively for manufacture and cannot be fully cleaned, enabling microorganisms to hide between their pores, which gives them greater resistance capabilities. Likewise, the butter milk which is produced in wooden barrels allow for the same results as gained from earthen ware pots.

Testing of safety-related concerns in selected lactobacilli

Antibiotic susceptibility of LAB

The absence of antibiotic resistance is a crucial issue when selecting a probiotic strain because strain possessing antibiotic resistance may transfer such feature to gut microbiome (Maragkoudakis *et al*., 2006; Sireswar *et al*., 2017; Alnakip *et al*., 2019). Among tested 40 randomly selected isolates, 10 isolates showed sensitivity towards all selected antibiotics. As shown in Table 4, these isolates included *Pediococcus pentosaceus* (Isolates no. 1,3), *Lc. lactis* (isolate no. 7), *Lb. plantarum* (isolates no. 11, 13), *Lb. rhamnosus* (isolates no. 17), *Lb. casei* (isolates no. 23, 24), *Lb. acidophilus* (isolate no. 33) and *Lb. fermentum* (Isolate no. 37). Therefore, they could be chosen as a potential probiotic for further examination to be used in the production of different types of artisanal cheeses and fermented dairy products. Various literatures that examined LAB isolated from different artisanal dairy samples for antibiotic resistance concluded that the results of antibiotic resistance vary from study to study, product to another and from geographical region to another.

Testing of LAB strains for possessing blood hemolysis capability

Hemolysis on blood agar is one of the safety tests that ensure safe administration and non-hemolytic activity is considered as a safety prerequisite for the selection of a probiotic strain (De Vuyst and Leroy, 2007). Hemolysis was evaluated using Columbia blood agar plates containing 5% (v/v) sheep blood, and incubated at 37°C for 48 h. Characteristics of haemolysis on blood agar were shown as β-, α-, and γ-hemolysis. Our results presented in table 4 showed that all examined strains exhibit γ-hemolysis (no hemolysis). Thus, these isolates could not exhibit any pathogenicity and regarded as safe organisms due to their non-hemolytic activity. Considering the possessing of antibiotic resistance patterns, the aforementioned 10 isolates that lack AR as well as lack of β-haemolysis feature could be promising probiotic strains.

Antibacterial activity of LAB isolates against S. aureus and/or E. coli.

The antibacterial effect of LAB isolates was tested against 4-previuosly isolates of food-safety concerns; *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae* which were kindly provided by Sánchez-Rubio *et al*. (2016); Kamal *et al*. (2018) and Alnakip *et al*. (2019).

The antibacterial effect of LAB is associated with production of several substances having antimicrobial properties such as organic acids (lactic and acetic acid), hydrogen peroxide, acetaldehyde, diacetyl, reuterin, carbon dioxide, bacteriocins and bacteriocin-like substances (Prasad *et al*., 1998; Saarela *et al*., 2000; Sireswar *et al*., 2017). To determine whether acidity, bacteriocins and/ or H_2O_2 contributed to the inhibition effect by LAB, CFS were prepared from each of the previous 150 LAB isolates showing antibacterial activities against afore-mentioned food-borne pathogens and then neutralized to pH 6.5 to exclude the antibacterial effect of acidity. Neutralized supernatants were examined for antibacterial effect as the previous method. Table 6 shows the diameters of the inhibition zones around wells previously filled with CFS from the examined LAB isolates. Among all selected LAB isolates, it could be seen that only 2.93%, 3.86%, 2.53% and 2.26% of LAB strains could inhibit the growth of either of *S. aureus*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*, respectively (Table 5). The inhibitory effect of other LAB varied with different food-borne pathogens and appeared to be strain-dependent.

Testing of technological properties for selected LAB strains acidifying ability

A rapid decrease in pH during the initial step of cheese preparation is of definitive importance in the manufacture of cheese because it is very essential for coagulation and prevention or

Table 5. Antibacterial activity of LAB isolates.

Type of product	No. of LAB isolates	LAB isolates having antibacterial effect							
		S. aureus		*E. coli*		*K. pneumoniae*		*Ps. aeruginosa*	
		No.	%	No.	%	No.	%	No.	%
Laban Rayeb	150	5	3.33	6	4	6	4	5	3.33
Kariesh cheese	150	7	4.67	8	5.33	3	2	5	3.33
Zabady	150	3	2	4	2.67	3	2	3	2
Butter milk	150	4	2.67	4	2.67	6	4	3	2
Soured Cream	150	3	2	7	4.67	1	0.67	1	0.67
Total	750	22	2.93	29	3.86	19	2.53	17	2.26

reduction of the growth of adventitious microflora (Gomes and Malcata, 1998; Sireswar *et al*., 2017). The fast-acidifying strains are good candidates in the dairy fermentation process as primary starter organisms. Whereas, the poor acidifier strains can be used as adjunct cultures based on their other important properties, e.g., proteolytic and autolytic activity (Saarela *et al*., 2000). With respect to the acidifying activities of the selected 40 LAB strains in our study, 19 (47.5%) LAB strains showed a fast acidification activity meanwhile, the remaining 21 (52.5%) strains exhibited medium acidification activity. Similar results were obtained by (Ayad *et al*., 2004; Kamal *et al*., 2018).

Salt tolerance

Because addition of salt in cheese manufacture essential, thus probiotic strains should possess the ability to grow in the presence of 4-6.5% NaCl to ensure their application in dairy industry as primary starter cultures, or as adjunct cultures based on other technological properties. Salt tolerance of strains was measured as positive growth after 48 h, incubation periods in liquid MRS medium containing Nacl 4%. In our study, All LAB strains could grow well at Nacl 4% as shown in table 6. These results are similar to those reported by (Ayad *et al*., 2004).

Table 6. Testing criteria of Probiotic properties of Selected Lactobacilli.

No.	Isolate	Source	Testing of antimicrobial production capability			Antibacterial activity of neutralized supernatant. (Inhibition zone in mm)				Effect of heating, addition of trypsin& proteinase K on the antibacterial activity of neutralized CFS			Testing of technological properties for selected LAB Isolates		
			Lactic A.	H_2O_2	Diacetyl	*S. aureus*	*E. coli*	*K. pneumoniae*	*Ps. aeruginosa*	Heating (80°c/10min.)	Heating (100°c/30min.)	Treatment with trypsin & proteinase K	Acidifying ability	NaCL 4% tolerance	β- Galactosidase production
1	*Ped. pentosaceus*	Laban Rayeb	11.37	15.12	17.67	8	9	8	9	+	-	-	Medium	+	++
2	*Ped. pentosaceus*	Laban Rayeb	8.3	6.12	9.98	8	7	--	--	-	-	-	Medium	-	-
3	*Ped. pentosaceus*	Zabady	11.8	14.23	12.35	--	11	--	8	+	-	+	Fast	+	++
4	*Ped. pentosaceus*	Kariesh cheese	10.3	16.7	11.34	10	11	8	7	+	-	-	Fast	+	-
5	*Ped. pentosaceus*	Kariesh cheese	11.47	15.65	19.33	6	6	8	7	-	-	+	Medium	+	++
6	*Lc. lactis*	Laban Rayeb	5.4	9.3	7.67	9	9	--	--	+	-	-	Medium	-	+
7	*Lc. lactis*	Kariesh cheese	11.89	16.03	14.77	9	7	6	7	-	-	+	Fast	+	++
8	*Lc. lactis*	Zabady	12.34	14.57	16.89	8	--	--	--	-	-	+	Fast	+	+
9	*Lc. lactis*	Butter milk	10.98	12.34	12.21	--	8	9	8	+	-	-	Medium	+	++
10	*Lc. lactis*	Soured Cream	13.43	16.76	19.06	11	8	--	--	+	-	-	Fast	+	-
11	*Lb. plantarum*	Laban Rayeb	10.44	8	14.56	8	6	6	8	+	+	-	Medium	-	++
12	*Lb. plantarum*	Kariesh cheese	7.34	4.54	3.42	9	13	11	11	-	-	-	Medium	-	+
13	*Lb. plantarum*	Kariesh cheese	6.43	5.03	2.43	--	9	8	9	+	-	+	Fast	-	++
14	*Lb. plantarum*	Butter milk	10.88	13.23	14.35	--	11	9	8	+	-	-	Fast	+	+
15	*Lb. plantarum*	Soured Cream	11.3	10.7	10.22	11	9	9	9	-	-	+	Medium	+	++
16	*Lb. rhamnosus*	Laban Rayeb	9.98	16.54	15.76	--	--	--	7	+	-	-	Medium	+	+
17	*Lb. rhamnosus*	Kariesh cheese	4.98	6.54	3.76	--	--	--	12	-	-	-	Medium	-	+
18	*Lb. rhamnosus*	Zabady	7.18	6.33	5.98	11	12	9	7	-	-	-	Medium	+	+
19	*Lb. rhamnosus*	Butter milk	6.34	5.34	5.28	8	9	9	10	+	-	-	Medium	+	+
20	*Lb. rhamnosus*	Kariesh cheese	6.65	6.89	5.06	--	11	--	9	-	-	-	Fast	+	++
21	*Lb. casei*	Laban Rayeb	5.76	5.61	8.76	--	13	8	--	-	-	-	Medium	+	++
22	*Lb. casei*	Kariesh cheese	11.77	13.22	12.56	11	11	9	10	-	-	-	Medium	-	+
23	*Lb. casei*	Zabady	7.3	6.14	10.65	11	13	9	8	+	-	+	Fast	+	++
24	*Lb. casei*	Butter milk	12.33	16.76	10.9	9	8	9	7	+	-	+	Fast	+	+
25	*Lb. casei*	Soured Cream	11.89	14.65	11.34	11	12	8	11	-	-	+	Medium	+	++
26	*Lb. brevis*	Soured Cream	11.47	14.35	9.35	9	9	8	8	+	-	-	Medium	+	++
27	*Lb. brevis*	Kariesh cheese	11.37	9.12	10.67	11	9	12	9	-	-	+	Fast	+	++
28	*Lb. brevis*	Butter milk	10.3	8.32	9.22	12	7	8	9	-	-	+	Fast	+	+
29	*Lb. brevis*	Butter milk	10.81	12.43	11.45	11	13	8	11	+	-	-	Medium	+	+
30	*Lb. brevis*	Soured Cream	10.3	13.7	14.56	11	--	--	8	+	-	-	Fast	+	++
31	*Lb. acidophillus*	Laban Rayeb	6.76	4.89	4.67	11	13	11	10	-	-	-	Medium	-	+
32	*Lb. acidophillus*	Kariesh cheese	6.76	9.83	3.65	12	13	9	11	-	-	-	Medium	-	+
33	*Lb. acidophillus*	Zabady	10.76	11.11	9.89	7	9	7	--	+	-	+	Fast	+	++
34	*Lb. acidophillus*	Butter milk	11.22	12.45	10.34	12	13	9	8	+	-	+	Fast	+	+
35	*Lb. acidophillus*	Soured Cream	10.3	14.87	8.87	12	11	7	9	-	-	+	Medium	+	++
36	*Lb. fermentum*	Soured Cream	6.78	3.67	6.89	9	--	--	7	-	-	-	Medium	-	-
37	*Lb. fermentum*	Kariesh cheese	12.34	7.89	11.23	8	7	9	9	+	-	+	Fast	+	++
38	*Lb. fermentum*	Zabady	11.05	11.43	10.93	9	8	9	8	-	-	+	Fast	+	+
39	*Lb. fermentum*	Butter milk	7.76	6.98	5.43	9	12	11	12	-	-	+	Medium	-	-
40	*Lb. fermentum*	Soured Cream	10.39	16.87	13.34	14	8	9	12	+	-	-	Fast	+	+

β-galactosidase Production

β-galactosidase had been widely used for industrial as well as medical applications. In dairy industries, β-galactosidase had been used to prevent crystallization of lactose, improve sweetness and increase the solubility, flavor and digestibility of the milk products (Gheytanchi *et al*., 2010). Enzymatic hydrolysis of lactose by β-galactosidase is one of the most popular technologies to produce lactose reduced milk and related dairy products for consumption by lactose intolerant people (Gheytanchi *et al*., 2010; Sumathy *et al*., 2012). In our study, the selected 40 LAB strains were screened for their β-galactosidase activity with X- gal and colonies with blue color were regarded as bacteria containing β-galactosidase enzyme as shown in Table 6.

CONCLUSION

kariesh cheese, Laban Rayeb, zabady, Butter milk and soured cream are among popular artisanal fermented dairy products that are manufactured in Egyptian rural areas and villages. These products have been proven to harbor a wide variety of LAB microflora. After screening the probiotic, technological and safety related properties of 40 selected LAB isolates from the afore-mentioned fermented artisanal dairy products, 5 strains were proven to meet all required criteria, these isolates were corresponded to *Ped. pentosaceus*, *Lb. plantarum* (isolate no.13), *Lb. casei* (isolates no.23, 24), *Lb. acidophilus* (isolate no.33, 34) and *Lb. fermentum* (isolate no.37). Consequently, these isolates could be tested in future studies as promising strains to be incorporated in manufacture of various dairy products as starter and non-starter cultures.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abou-Donia, S.A., 1986. Egyptian Domiati Soft White Pickled Cheese. New Zealand J. Dairy Sci. Technol, 21, 3,167-190.

Abou-Donia, S.A., 2008. Origin, History and Manufacturing Process of Egyptian Dairy Products: An Overview. Alexandria J. Food Sci. Technol. 5, 51-62.

Abou-Donia, S.A., 2004. Recent Developments in Zabady and Egyptian Labneh Research: A Review. Egypt. J. Dairy Sci. 32, 1-16.

Ahmed, N., El Soda, M., Hassan, A., Frank, J., 2005. Improving the textural properties of an acid-coagulated, karish cheese using exopolysaccharide producing cultures. LWT-Food Sci. Technol. 38, 843-847.

Alnakip, M.E., 2014. Development of molecular methods of bacterial identification in food products by means of genomic and proteomic tools, PhD. Retrieved from www.eulc.edu.eg.

Alnakip, M., 2009. Prevalence of gram positive bacteria in milk and some dairy products. M.V.Sc. thesis, Zagazig University. Retrieved from www.eulc.edu.eg.

Alnakip, M.E., Youssef, M.Y., Abd-Elaal, S.F., Bayoum, M.A., 2023. Screening of Food-borne *Staphylococcus aureus* and *E. coli* Pathogens in Artisanal White Soft Cheese in Delta Region, Egypt. J. Adv. Vet. Res. 13, 1203-1209.

Alnakip, M.E., Quintela-Baluja, M., Böhme, K., Caamaño-Antelo, S., Bayoumi, M.A., Kamal, R.M., Merwad, A.M., Calo-Mata, P., Barros Velázquez, J., 2019. Molecular characterization and typing the methicillin resistance of *Staphylococcus* spp. isolated from raw milk and cheeses in northwest Spain: A mini survey. Int. Dairy J. 89, 68-76.

Alnakip, M.E., Rhouma, N.R., Abd-Elfatah, E.N., Quintela-Baluja, M., Böhme, K., Fernández-No, I., Bayoumi, M.A., Abdelhafez, M.M., Taboada Rodriguez, A., Calo-Mata, P., Barros-Velázquez, J., 2020. Discrimination of major and minor streptococci incriminated in bovine mastitis by MALDI-TOF MS fingerprinting and 16S rRNA gene sequencing. Res. Vet. Sci. 132, 426-438.

Alnakip, M.E, Mohamed, A.S., Kamal, R.M., Elbadry,S. 2016. Diversity of Lactic acid Bacteria Isolated from Raw Milk in Elsharkia Province, Egypt. Japanese J. Vet. Res. 64 S23-S30.

AOAC (Association of Official Analytical Chemist), 1990. Official Methods of Analysis, Association of Official Analytical Chemists. 15th Ed. Gaithersburg, USA: AOAC Press; 1990

Axelsson, L., 2004. Lactic Acid Bacteria. In: Classification and Physiology. In . Vol. 139, 1-66: MARCEL DEKKER AG.

Ayad, E.S., Nashat, N., El-Sadek, H., Metwaly, M. El-Soda.,M., 2004. Selection of Wild Lactic Acid Bacteria Isolated from Traditional Egyptian Dairy Products According to Production and Technological Criteria. Food Microbiol. 21, 715-725.

Baraheem, Omar H., Hoda A. El-Shamy, Wafaa M. Bakr, Gomaa, N.F., 2007. Bacteriological Quality of some Dairy Products (Kariesh Cheese and Ice Cream) in Alexandria. J. Egypt Public Health Assoc. 82, 491-510.

Bernardeau, M., Jean Paul, V., Segolene, H., Micheline, G., 2008. Safety Assessment of Dairy Microorganisms: The *Lactobacillus* Genus. Inter.J. Food Microbiol.126, , 278-285.

De Man, J.C., deM Rogosa, Elisabeth Sharpe, M., 1960. A Medium for the Cultivation of Lactobacilli. J. Appl. Bacteriol., 23, 130-135.

De Vuyst, L. Leroy, F., 2007. Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. J. Molec. Microbiol. Biotechnol. 13, 194-199.

Drakoularakou, A.P., Kehagias, C., Karakanas, P.N., Koulouris, S., Timbis, D., 2003. A Study of the Growth of *Lactobacillus acidophilus* in Bovine, Ovine and Caprine Milk. Inter. J. Dairy Technol. 56, 59-61.

Dufour, J., Collin, S., 1995. Bacteriological Studies on Domiati Cheese. Egyptian J. Dairy Sci., 23, 239-247.

El-Baradei, G., Delacroix-Buchet, A., Ogier, JC., 2008. Bacterial Biodiversity of Traditional Zabady Fermented Milk. Inter. J. Food Microbiol. 121, 295-301.

El-Baradei, G., Delacroix-Buchet, A., Ogier, J.C., 2007. Biodiversity of Bacterial Ecosystems in Traditional Egyptian Domiati Cheese. Appl. Environ. Microbiol. 73, 1248-1255.

El-Soda, M., El-Ziney, M., Awad, S., Osman, G., Omran, N., Gamal, G., Ezzat, N., El-Shafei, H., 2003. A Culture Collection of Lactic Acid Bacteria Isolated from Raw Milk and Traditional Egyptian Dairy Products. Egypt. J. Dairy Sci. 31, 23-42.

Fahmy, T. K., Youssef, L.M., 1978. Incidence of Streptococci and Lactobacilli in Domiati Cheese. Agric. Res. Rev. 56, 149-152.

FAO/WHO, 2007. Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food. Guidelines for the Evaluation of Probiotics in Food: Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food, London, Ontario, Canada, April 30 and may 1, 2002. Ftp.Fao.Org/Es/Esn/Food/Wgreport2.Pdf.Accessed 16.

Gaya, P., Babín, M., Medina, M., Nuñez, m., 1999. Diversity among Lactococci Isolated from Ewes' Raw Milk and Cheese. J. Appl, Microbiol. 87, 849-855.

Gheytanchi, E., Fariba, H., Bahareh, K., Jamileh, N., Farahnaz, M., 2010. Study on B-Galactosidase Enzyme Produced by Isolated Lactobacilli from Milk and Cheese. Afri. J. Microbiol. Res. 4, 454-458.

Gomes, A., Xavier Malcata, F., 1998. Development of Probiotic Cheese Manufactured from Goat Milk: Response Surface Analysis Via Technological Manipulation. J. Dairy Sci. 81, 1492-1507.

Hawaz, E., 2014. Isolation and Identification of Probiotic Lactic Acid Bacteria from Curd and *in Vitro* Evaluation of its Growth Inhibition Activities Against Pathogenic Bacteria. African J. Microbiol. Res. 8, 1419-1425.

Holzapfel, W.H., Petra, H., Johannes, S., Ulrich, S., in't-Veld, J., 1998. Overview of Gut Flora and Probiotics. Inter. J. Food Microbiol., 41, 85-101.

IDF. 1992. Milk and Milk Products. Preparation of Samples and Dilutions Microbiological Examination. Brussels. Belgium.

Kamal, R. M., Alnakip, M.E., Abd El Aal, S.F., Bayoumi, M.A., 2018. Bio-Controlling Capability of Probiotic Strain *Lactobacillus rhamnosus* against some Common Foodborne Pathogens in Yoghurt. Inter. Dairy J. 85, 1-7.

Korish, M., Abd Elhamid, A.M., 2012. Improving the Textural Properties of Egyptian Kariesh Cheese by Addition of Hydrocolloids. Inter. J. Dairy Technol. 65, 237-242.

Lombardi, A., Dal Maistro, L., De Dea, P., Gatti, M., Giraffa, G., Neviani, E., 2002. A polyphasic approach to highlight genotypic and phenotypic diversities of *Lactobacillus helveticus* strains isolated from dairy starter cultures and cheeses. J. Dairy Res. 69, 139-149.

Lombardi, ,A., Monica, G., Lucia, R., Sandra, T., Christian, A., Giorgio, G., 2004. Characterization of *Streptococcus* macedonicus strains isolated from artisanal Italian raw milk cheeses. Inter. Dairy J., 14, 967-976

Maragkoudakis, P., Georgia, A., Christos, M., George, K., Bruno, P., Effie, T., 2006. Probiotic Potential of *Lactobacillus* Strains Isolated from

Dairy Products. Inter. Dairy J. 16, 189-199.
Monteagudo-Mera, A., Caro, I., Rodríguez-Aparicio, L.B., Rúa, J., Ferrero, M.A., García-Armesto, M.R., 2011. Characterization of Certain Bacterial Strains for Potential use as Starter Or Probiotic Cultures in Dairy Products. J. Food Protection 74, 1379-1386.
Monteagudo-Mera, A., Leandro, R., Javier, R., Honorina, M., Nicolás, N., María, R., Miguel, Á., 2012. *In Vitro* Evaluation of Physiological Probiotic Properties of Different Lactic Acid Bacteria Strains of Dairy and Human Origin. J. Functional Foods 4, 531-541.
Nair, S., P., Surendran., P. K., 2000. Studies on Lactic Acid Bacteria from Tropical Fish and Shellfish. Diss. Microbiology, Fermentation and Biotechnology Division Central Institute of Fisheries Technology, Faculty of Marine Sciences.
Prasad, J., Harsharanjit, G., John, S., Pramod, K.G., 1998. Selection and Characterisation of *Lactobacillus* and *Bifidobacterium* Strains for use as Probiotics. Inter. Dairy J. 8, 993-1002.
Saarela, M., Gunnar, M., Rangne, F., Jaana, M., Tiina, M., 2000. Probiotic Bacteria: Safety, Functional and Technological Properties. J. Biotechnol. 84, 197-215.
Sánchez-Rubio, Marta, Mohamed EA Alnakip, Mohamed Abouelnaga, Amaury Taboada-Rodríguez, Marín-Iniesta, F., 2016. Use of Thermosonication for Inactivation of *E. coli* O157: H7 in Fruit Juices and Fruit Juice/Reconstituted Skim Milk Beverages. ISHS Acta Horticulturae 1194: VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits - Ethical and Technological Issues, 1164, 267-274. https://doi.org/10.17660/ActaHortic.2018.1194.39.
Satish Kumar, R., Kanmani, P., Yuvaraj, N., Paari, K.A., Pattukumar, V., Arul., V., 2011. *Lactobacillus plantarum* AS1 Binds to Cultured Human Intestinal Cell Line HT-29 and Inhibits Cell Attachment by Enterovirulent Bacterium *Vibrio parahaemolyticus*. Lett. Appl. Microbiol. 53, 481-487.
Sireswar, S., Gargi D., Sreesoundarya, T.K., Dipon, S., 2017. Design of Probiotic-Fortified Food Matrices Influence their Antipathogenic Potential. Food Biosci. 20, 28-35.
Sumathy, R., Vijayalakshmi, M., Deecaraman, M., 2012. Study on B-Galactosidase of *Lactobacillus* sp. from Milk Products and its Applications. Inter. J. Appl. Biol. Pharmaceutical. Technol. 3, 138-148.
Verdenelli, M.C., Francesca G., Stefania S., Carla O., Cinzia C., Alberto C., 2009. Probiotic Properties of *Lactobacillus rhamnosus* and *Lactobacillus* Paracasei Isolated from Human Faeces. Eur/ J. Nutrition 48, 355-363.
Verdier-Metz, I., Valerie, M., Celine D., Marie-Christine M., 2009. Do Milking Practices Influence the Bacterial Diversity of Raw Milk?. Food Microbiol. 26, 305-310.
Wehr, H., Frank, J. F., 2004. Standard Methods for the Examination of Dairy Products. American Public Health Association. https://ajph.aphapublications.org/doi/10.2105/9780875530024ch01
Wouters, T.M., Eman, H.E.A., Hugenholtz, J., Smit, G., 2002. Microbes from Raw Milk for Fermented Dairy Products. Inter. Dairy J. 12, 91-109.
Zhang, X., Richard, Kilmer, L., Muhammad, A., 2003. A Descriptive Analysis of Egypt and Saudi Arabia Who Import United States Dairy Products. Inter.Agriculture Trade and Policy Center, Univ.Florida.