Identification and Some Probiotic Potential of Lactic Acid Bacteria Isolated From Egyptian Camels Milk

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Abstract: A study was carried out to investigate the probiotic potential of isolated lactic acid bacteria from camel's row milk collected from Arabian camels (*Camelus dromedaries*) in Egypt. Eleven gram positive, catalase negative isolates were identified using API 20STREP identification system for the identification of cocci isolates and API 50CHL for bacilli. Isolates were identified as *Enterococcus faecium* (seven isolates), *Enterococcus durans* (one isolate), *Aerococcus viridians* (one isolate), *Lactococcus lactis* (one isolate) and *Lactobacillus plantarum* (one isolate). The probiotic potential of these isolates was investigated using *in vitro* antagonistic tests against *Salmonella typhi* ATCC 14028, *E.coli* ATCC 25922 and *Vibrio fluvialis* using agar spot test. All of the isolates were proved to be effective against those pathogens. Isolate ES08 was able to inhibit the growth of indicator pathogens with an average inhibition zone of 3.3, 3.7 and 2.0 cm in diameter against *Salmonella typhi*, *E.coli* and *Vibrio fluvialis*, respectively. All of the isolates showed resistance to stomach pH (pH 3.0), tolerance against 0.3% bile salts concentration and none of the isolates caused blood hemolysis. Isolate ES08 was farther identified by sequencing their 16S rRNA encoding gene.

[Eman Hamed and Aisha Elattar. **Identification and Some Probiotic Potential of Lactic Acid Bacteria Isolated From Egyptian Camels Milk**. *Life Sci J* 2013;10(1):1952-1961] (ISSN:1097-8135). http://www.lifesciencesite.com. 280

Key Words: Camels milk, Lactic Acid Bacteria, Probiotic potential.

1. Introduction

According to the FAO statistics (FAO, 2004), there are about 19 million camels in the World, of which 15 millions are found in Africa and 4 millions in Asia. About 79% of the world's population is found in Africa, and all are one-humped. Camel populations are more concentrated in North East Africa. In Egypt, their numbers were previously estimated as 230,000 camels (GOVS, 2005). Nowadays, camel milk is considered as one of the main source of animal protein in some Egyptian provinces. It was reported that patients suffering from chronic hepatitis had improved liver functions after drinking camel milk. Camel milk is also succefully used for stabilization of juvenile diabetes (Yagil, 1987). This is confirmed by the presence of insulin-like protein in camel milk (Beg *et al.*, 1986). In different countries of Africa (Egypt, Sudan and Somalia) there is a common belief that among herdsmen of camels, especially those grazing on herbs, that men who drink such camel milk become strong, swift and virile (El Agamy, 2006). In pastoral societies, milk is traditionally consumed predominantly in the form of fermented milk. Fermentation is the only means of preserving milk under warm condition (Mohamed *et al.*, 1990; Farah, 1993; Kamoun, 1990). In many arid areas, camels play a central role as milk suppliers where they are either home-consumed or sold (Yagil, 1982; Kamoun, 1995; Lhoste, 2004).

Micro-organisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the Lactic Acid Bacteria (LAB) which are used in making starter culture for dairy products. The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavour. The microbiological quality of milk and milk products is influenced by the initial flora of raw milk (Ritcher and Vadamuthu, 2001). When camel milk is left to stand, its acidity rapidly increases due to presence of LAB (Ohris and Joshi, 1961). It has also been recognized that LAB are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other microorganisms (Daeschel, 1989). Certain LAB strain characterized by their ability to transform lactose and improves the digestibility of fermented dairy products (Weinberg *et al.*, 2007) as well as their preservation (Abdelbasset and Djamila, 2008). They also employed for improvement of the taste, texture and viscosity in the manufacture of dairy products (Soukoulis *et al.*, 2007). The ability of LAB to produce probiotics (Temmerman et al., 2002) and stimulation of the immune system (Kalliomäki *et al.*, 2001) render this group of microorganisms' essential importance dairy industry. Bacteria used as probiotic adjuncts are commonly delivered in a food system and, therefore, upon oral administration, they begin

their journey from the stomach to the lower intestinal tract. Therefore, probiotic bacteria should have the ability to resist the digestion process in the stomach and the intestinal tract. A large number of lactic acid bacteria have been classified as probiotics. According to the definition adopted by the World Health Organization, probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host (Corsetti and Valmorri. 2011). Strains of the genera Lactobacillus, Bifidobacterium (Yateem *et al.*, 2008) and Enterococcus (Ogier and Serror (2008) are the most widely used and commonly studied probiotic bacteria. Today, there is a growing need for new strains of LAB that carry the probiotic traits mentioned above and with favorable health effects on human and animals. This can be obtained from other natural ecological niches which remain unexploited.

This study was undertaken to isolate and identify the lactic acid bacteria from row Camel's milk obtained from different locations in Egypt. The identification tests were applied using the phenotypic and genotypic methods. These isolates were investigated for bile salt and, acidic pH values resistant, hemolytic activities and bacteriocin production. Our goal is the selection of potential probiotic strains from camel's milk.

2. Materials and Methods

Sample collection

Bacterial strains were isolated from camel's milk samples collected from local lactating Arabian camels (*Camelus dromedaries*) in Egypt. A total of 21 camel milk samples were collected, five samples were collected from Mersa Matrouh, 14 samples from Wadi El Natrun area, and two samples from the farm of the Faculty of Veterinary Medicine - Menoufia University, Sadat city area. The samples were collected in sterile plastic containers and kept in ice box until delivery to the laboratory for the achievement of the isolation procedure.

Isolation of lactic acid bacteria

Each camel's milk sample was immediately cultured on MRS agar plates, and then serial dilutions were prepared from each sample. 1 ml of these dilutions was pour-plated in the MRS agar (de Man *et al.*, 1960). After incubation at 37°C for 48 hrs under anaerobic condition, individual different colonies were phenotypically selected.

The purity of the isolates was checked by streaking again to fresh agar plates, followed by macroscopic and microscopic examinations. The strains displaying the general characteristics of lactic acid bacteria were chosen from each plate for further studies. The strains of lactic acid bacteria were stored without appreciable loss of properties in skimmed milk with 20% glycerol at -20°C.

Identification of isolates

All isolates were microscopically examined for Gram stain reaction, cell morphology and cellular arrangement. Catalase activity was examined by adding drop of 3 percent hydrogen peroxide on a clean microscopic slide. A visible amount of bacterial growth was added with the inoculating loop. Both were mixed and observed for gas bubble production. Only Gram-positive and Catalase negative isolates were identified at species level.

API 20STREP (Biomérieux, Marcy-l'Étoil, France) was used for cocci isolates identification according to the manufacturer's instructions. Results reading were done after 24 and 48 hrs at 37°C, on the other hand bacilli isolates were evaluated according to the carbohydrates fermentation profiles using API 50CHL (Biomérieux, Marcy-l'Étoil, France). The tests were also done according to the manufacturer's instructions and the results were interpreted after incubation at 37°C for 24 and 48 hrs. Identification of the isolates was done by the interpretation of the fermentation profiles using the computerized database program API WEB software V 1.2.1.

Determining the antagonistic activity of isolated LAB using in vitro tests

The antagonistic activity of the isolated LAB bacteria against *Salmonella typhi* ATCC 14028, *E. coli* ATCC 25922 (obtained from the High Institute of Public Health, Egypt) and *Vibrio fluvialis* (obtained from the National Institute of Oceanography and Fisheries, Egypt), was determined using agar spot test (Jacobsen *et al*., 1999).

Prior to conducting the test, the potential LAB isolates were propagated in MRS broth medium and incubated at 37°C for 24 h. For the agar spot test, 4 µL of each bacterial isolate were spotted on the surface of MRS agar medium and incubated at 37°C for 24 hrs to allow colonies to develop. Overnight culture of each test pathogen was inoculated (1% v/v) in 15 ml of soft Nutrient agar (containing 0.7% agar) and poured onto the inoculated MRS agar plates (Yavuzdurmaz, 2007). After incubation at 37°C for 24 hrs, the antimicrobial activity of tested strains was determined by measuring the diameter of the inhibition (clear) zones surrounding the colonies.

Resistance to acidic pH

The resistance of LAB isolates to acidic pH was performed according to (Nawaz *et al*., 2011). Each bacterial isolate was inoculated using 1% (v/v) of an overnight LAB culture, in sterile MRS broth adjusted to pH 2, 3 and 4 then incubated at 37ºC for 6 hrs. The absorbance at 620 nm using spectrophotometer (Optima, Japan) was monitored at hourly intervals. Control samples without acidification were also prepared and similarly handled.

Bile salts tolerance

Isolates were tested for their ability to grow in presence of different bile salts concentrations. For this purpose 0.1% and 0.3% (w/v) bile concentrations were selected. An aliquots of 15 ml sterile MRS broth containing 0, 0.1% and 0.3% of bile salts was inoculated with an overnight LAB culture and incubated at 37ºC for 4 hrs. The absorbance at 620 nm was monitored using spectrophotometer (Optima, Japan) at hourly intervals (Yavuzdurmaz, 2007).

Blood hemolysis

The hemolytic activity of isolates was determined according to Guttmann and Ellar (2000) on blood agar base (Biolife, Milano. Italy) plates containing 5% v/vs of sheep blood. After incubating the plates at 37 °C for 24 hrs, β-hemolytic, no haemolysis or γ and α-haemolysis reactions were recorded by the observation of a clear zone around the colonies, the non-hemolysed area under and around the colonies and the greenish zone, respectively.

Genotypic Identification using 16S rDNA

The genomic DNA of the presumptive LAB strain was isolated using the DNA extraction and purification kit according to the manufacturer instructions (Fermentas, UK). DNA preparations were then analyzed by electrophoresis in 1% agarose gel.

The PCR reaction mixture contained 5 µL of template DNA, 2 µL of reverse primer (10 mM), 2 µL of forward primer (10 mM), 2 µL of dNTP (25 mM), 4 µL of $MgCL_2$ (25 mM), 5 µL of PCR buffer (10X) and 1µL Taq polymerase, Distilled water was added to obtain 50 µL final volume in the PCR tube. The primers used for PCR amplification of 16S rRNA gene were S-C-Act-235-a-S-20 F: 5´-CGCGGCCTATCACTTGTTG-3´ and S-C-Act-878-a-A-19 R: 5´-CCGTACTCCCCAGGCGGGG-3´ (Jaatinen *et al*., 2008), with expected product size of 400 bp. The cycling program was 95ºC for 5 min, 35 cycles at 95ºC for 30 sec, 50ºC for 30 sec and 72ºC for 2 min. At the end, the reaction was incubated at 72ºC for 10 min.

Gel electrophoresis was carried out by using 1% agarose gel prepared in TAE buffer (2.0 M Tris base, 1.0 M glacial acetic acid and 0.05 M EDTA at pH 8).

The DNA samples were loaded in the gel after mixing with the loading dye (a solution of 0.1% bromophenol blue, 50% glycerol, 0.1 M EDTA pH 8 and 1% SDS), and the voltage was then applied (90 v/cm) after soaking in TAE buffer. The DNA was visualized using a UV transilluminater (Bio-Rad, USA) after staining the gel with ethidium bromide (10 mg/ml) for 20 min.

Sequencing of PCR product was made by the sequencing facility offered by the U.S.B. American Company through SIGMA-Egypt.

3. Results and Discussion

Isolation of lactic acid bacteria

From the twenty one collected samples, a total of 60 isolates randomly picked, after the original characterization. They are all gram positive bacteria, moreover, a lot of catalase positive bacteria and yeast were observed. The presence of yeast in the tested samples is possibly a result of contamination from udder skin, as previously mentioned by Yavuzdurmaz, (2007). Only eleven isolates were stable after purification and sub-culture (Table1), consequently, they were applied in this study. They were Gram positive cocci or rods, catalase negative and non spore forming. Ten of them (91%) were found to be cocci with spherical morphology and appeared mostly as forming chains or groups therefore they tentatively referred to *lactococcus.* Only one isolate (9%) was bacilli mostly appeared as short rod, pairs or single cells and this could cautiously determined as derivatives of the genus *Lactobacillus*. Brasca *et al*. (2008) purified 92 isolates of lactic acid bacteria using frozen camel's milk. These isolates were classified as 55.43% and 44.56% of cocci and rods isolates, respectively. While Ashmaig *et al*. (2009) isolated 24 LAB from 12 samples of gariss (fermented camel's milk) in the Sudan. The isolates were classified into 66.6% rods and 33.3% cocci. Also, Khay *et al*. (2011) isolated a total of 450 cultures from 25 samples of dromadedary milk collected from Laâyoune region of Morocco. Out of these, 30 were determined to be lactic acid bacteria.

Physiological and biochemical identification of the isolated lactic acid bacteria

Based on phenotypic, biochemical characteristics and interpretation of the API database, 11 strains were satisfactorily identified, of which 10 (cocci) were identified using the enzymatic and carbohydrate fermentation profile API 20 STREP and one isolate (rod shaped) was identified using API 50 CHL. The biochemical profiles of the tested strains and the suggested identification are shown in Tables (2 and 3).

All isolates fermented lactose, trehalose and ribose. Only isolate (EW01) failed to ferment arabinose and mannitol. All the isolates did not ferment raffinose and did not utilize inulin or sorbitol. Only two isolates (EW02 and LS07) could not utilize esculine. All of the isolates were not able to hydrolysis hippuric acid.

Table 1. Morphological characterization of the bacterial isolates.

Isolate Number	Shape	Gram staining	Catalase reaction
EW01	Cocci	G +ve	-ve
EW02	Cocci	G +ve	-ve
EW03	Cocci	G +ve	-ve
AW04	Cocci	G +ve	-ve
ES05	Cocci	G +ve	-ve
ES06	Cocci	G +ve	-ve
LS07	Cocci	G +ve	-ve
ES08	Cocci	G +ve	-ve
ES09	Cocci	G +ve	-ve
ES10	Cocci	G +ve	-ve
LS11	Short rods	G +ve	-ve

Table 2. Analysis results of isolated LAB using API 20 STREP* and API 50 CHL**.

Strain	Identification	Confidence (%)
EW01*	*Enterococcus durans*	83.20
EW02*	*Enterococcus faecium*	85.40
EW03*	*Enterococcus faecium*	85.80
AW04*	*Aerococcus viridans*	95
ES05*	*Enterococcus faecium*	85.80
ES06*	*Enterococcus faecium*	85.80
LS07*	*Lactococcus lactis* subsp. *cremoris*	42.80
ES08*	*Enterococcus faecium*	85.80
ES09*	*Enterococcus faecium*	85.80
ES10*	*Enterococcus faecium*	85.80
LS11**	*Lactobacillus plantarum*	99.9

On the other hand, they could produce acetoin as they gave positive results in the Voges Proskauer test. All isolates did not show blood hemolysis. Based on API 20 STREP identification, 7 isolates which represent 63.6% of the experimental isolates were identified as *Enterococcus faecium*, one isolate (9%) as *Enterococcus durans*, one isolate (9%) as *Aerococcus viridans*, one isolate (9%) as *Lactococcus lactis* and one isolate (9%) as *Lactobacillus plantarum*. The presence of *E. faecium* and *E. durans* in raw camel's milk and cheese is common (Rodríguez *et al*., 1995; Freitas *et al*., 1999) and this observation was in agreement with our results. Enterococcal strains, mainly those of *E. faecium* are frequently present in various food systems and their technological and probiotic benefits are widely recognized (Giraffa, 1995). The isolates, which were identified as *E. faecium* produced acid from mannitol and arabinose (Durlu-Ozkaya *et al*., 2001). The confidence results were highly (83.2% – 99.9%) for all the isolates except for the isolate LS07 (42.8%) which was not clearly identified but it could be referred to *Lactococcus lactis* subsp. *cremoris* (Table.2).

Enterococci represented a large part of the bacterial microflora of this work as a total of 7 isolated strains were identified as *E. faecium*. This group of bacteria plays a major role in the ripening and aroma development in butter and many types of traditional cheeses (Centeno *et al*., 1996 and Malek *et al.*, 2012). In addition, Egyptian Ras and Domiatti cheese were made in the presence and absence of selected *E. faecium* strains. The cheese containing *E. faecium* exhibited higher levels of free fatty acids and amino acids. The organoleptic evaluation of the different cheeses revealed a preference to the *E. faecium* containing cheeses, which suggest a desirable role of this microorganism during the ripening of Egyptian cheeses (El Soda. 2002) In addition to these technological aspects, clinical research on enterococci emphasizes that the safety of dairy products containing enterococci should be carefully addressed before use (Giraffa, 2003). Contrary to other lactic acid bacteria, some strains of enterococci are not considered as "Generally Recognized As Safe" (GRAS) microorganisms (Cariolato *et al.*, 2008). And their detection in water is regarded as an indicator of fecal contamination. So, enterococci are generally considered as having an ambiguous status concerning their safety assessment procedure (Oigier and Serror. 2008).

On the other hand, *Lactobacillus* phenotypes were represented by only one out of the eleven isolates, this may be due to a possibility that camel's milk is not an adequate medium for their growth, their sensitivity to natural inhibitors which represent in the milk or to the lack of essential growth factors (Benkerroum *et al*., 2003). The experimental isolate (LS07) that fermented lactose, ribose, mannitol, trehalose and arabinose but not raffinose, inulin and sorbitol was identified as *Lactococcus lactis.* The isolation of *Lactococcus lactis* from camel's milk was also reported by Hardie (1986) and Khay *et al*. (2011). *Lactococcus lactis* subsp. *Lactis* and *Lactococcus lactis* subsp. *cremoris* are important in food technology (Garvie, 1984; Sandine, 1985; Salama *et al*., 1991) as the bacteriocin producing lactococcal strains have been used successfully in starter cultures for cheese to improve the safety and quality of the product (Maisnier-Patin *et al*., 1992; Delves-Broughton *et al*., 1996 and Ryan *et al*., 1996). The experimental isolate (AW04) that gave positive reactions for hydrolysis of esculin and acidification of lactose and trehalose was identified as *Aerococcus viridans* (MacFaddin, 1980).

Probiotic properties

Antagonestic activity

LAB have been shown to inhibit the growth of many enteric pathogens *in vitro* and have been used for treatment of a broad range of gastrointestinal disorders in both humans and animals (Rolfe, 2000). Therefore, experimental isolates were tested against the indicator microorganisms such as, *Salmonella typhi* ATCC 14028, *E. coli* ATCC 25922 and *Vibrio fluvialis* (Fig. 1.). The diameter of inhibition zones (Table 4) showed that all of the isolates have antibacterial effect on the indicator microorganisms. Isolate ES10 gave the largest inhibition zone against *E. coli* with a diameter of 3.8 cm followed by isolate ES08 which gave an inhibition zone with a diameter of 3.7 cm, while isolates ES05 and ES09 were less effective against *E. coli* and gave inhibition zones with almost the same diameter (3.1 cm). On the other hand, isolate ES08 was the most effective isolate against *Salmonella typhi* as it gave an inhibition zone with a diameter of 3.3 cm followed by LS07 (3 cm). Isolates LS11 and EW02 were the lowest effective with inhibition zones of 2.4 and 2.3 cm respectively. While, against *Vibrio fluvialis*, ES08 and ES10 were the most effective strains as they gave clear zones with a diameter of 2 cm and strains ES05 and ES09 were the lowest effective as they gave clear zones with 1 cm in diameter. The inhibitory action of LAB bacteria is mainly due to the accumulation of main primary metabolites such as lactic and acetic acids, ethanol and carbon dioxide. Additionally, LAB are also capable of producing antimicrobial compounds such as formic and benzoic acids, **hydrogen peroxide**, diacetyl, acetoin and bacteriocins. The production levels and the proportions among those compounds depend on the strain, medium compounds and physical parameters (Tannock, 2004).

Table 3. Biochemical identification of the ten cocci isolates using API 20 STREP identification system.

Strain	TIME	VP	HIP	ESC	PYRA	αGAL	βGUR 4	βGAL	PAL	LAP	ADH	RIP	ARA	MAN	SOR	LAC	TRE	INU	RAF	AMD	GLYG	β-hem
EW03	4h	+	-	+	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
AW04	4h	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	-	+	+	+	+	-	+	+	-	-	-	-	-
EW01	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	-	-	-	+	+	-	-	-	-	-
EW02	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	24_h	+	-	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
ES05	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
ES06	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
LS07	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	-	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
ES08	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
ES09	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-
ES10	4h	+	-	-	+	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-
	24_h	+	-	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	-	-	-	-

+ = positive reaction, - = negative reaction, test was done under anaerobic conditions at 37ºC /4 and 24 hrs.

Table 4. Antagonistic activity of isolated LAB against *Salmonella typhi* ATCC 14028, *E. coli* ATCC 25922 and *Vibrio fluvialis* as determined by the agar spot test.

Isolate number	Inhibition zones diameter in cm		
	Salmonella typhi	***E. coli***	***Vibrio fluvialis***
EW01	2.5	3.5	1.2
EW02	2.3	3.4	1.4
EW03	2.5	3.5	1.6
AW04	2.7	3.4	1.7
ES05	2.5	3.1	1.0
ES06	2.9	3.6	1.4
LS07	3.0	3.3	1.8
ES08	3.3	3.7	2.0
ES09	2.5	3.1	1.0
ES10	2.8	3.8	2.0
LS11	2.4	3.4	1.8

LAB has shown to possess inhibitory activities mostly towards Gram positive pathogens and closely related bacteria due to the bactericidal effect of protease sensitive bacteriocins (Jack *et al*., 1995). LAB strains are mostly inactive against Gram-negative bacteria due to the resistance conferred by the outer membrane. However, inhibitory effects of nisin (Cutter and Siragusa, 1995), bacteriocin produced by *Lactobacillus paracasei* subsp. *paracasei* (Caridi, 2002), bacteriocin ST151BR produced by *Lactobacillus pentosus* ST151BR (Todorov and Dicks, 2004), thermophylin produced by *Streptococcus thermophillus* (Ivanova *et al*., 1998) and some enterocins produced by enterococcus sp. (Jennes *et al*., 1999) on Gram-negative bacteria through their synergetic effects with other antimicrobials has gained increased interest (Helander *et al*., 1997). LAB were also able to control the growth of Gram negative pathogens including food borne pathogens by the production of **organic acid**s and **hydrogen peroxide** (Lu and Walker, 2001 and Ito *et al*., 2003).

Resistance to low pH

One of the major selection criteria for probiotic strains is to be resistant to low pH (Chou and Weimer, 1999; Quwehand, *et al*., 1999). Since, they have to pass through the stressful conditions of stomach to reach the small intestine. Although in the stomach, pH can be as low as 1.0, however, *in vitro* assays pH 3.0 has been preferred, due to the fact that a significant decrease in the viability of strains is often observed at pH 2.0 and below (Prasad, *et al*. 1998). For selection of strains resistant to low pH, MRS broth with pH-adjusted to 4.0, 3.0 and 2.0 were used. Berrada *et al.* (1991) mentioned that the time from entrance to release from the stomach was reported to be 90 min and the bactericidal effect of the acid is evident at pH values below 2.5 (Maffei and Nobrega, 1975). After the examination, all the isolates survived in pH 3.0 were taken to the next step. Experiments were run twice. The growth was monitored by measuring the O.D at 620 nm.

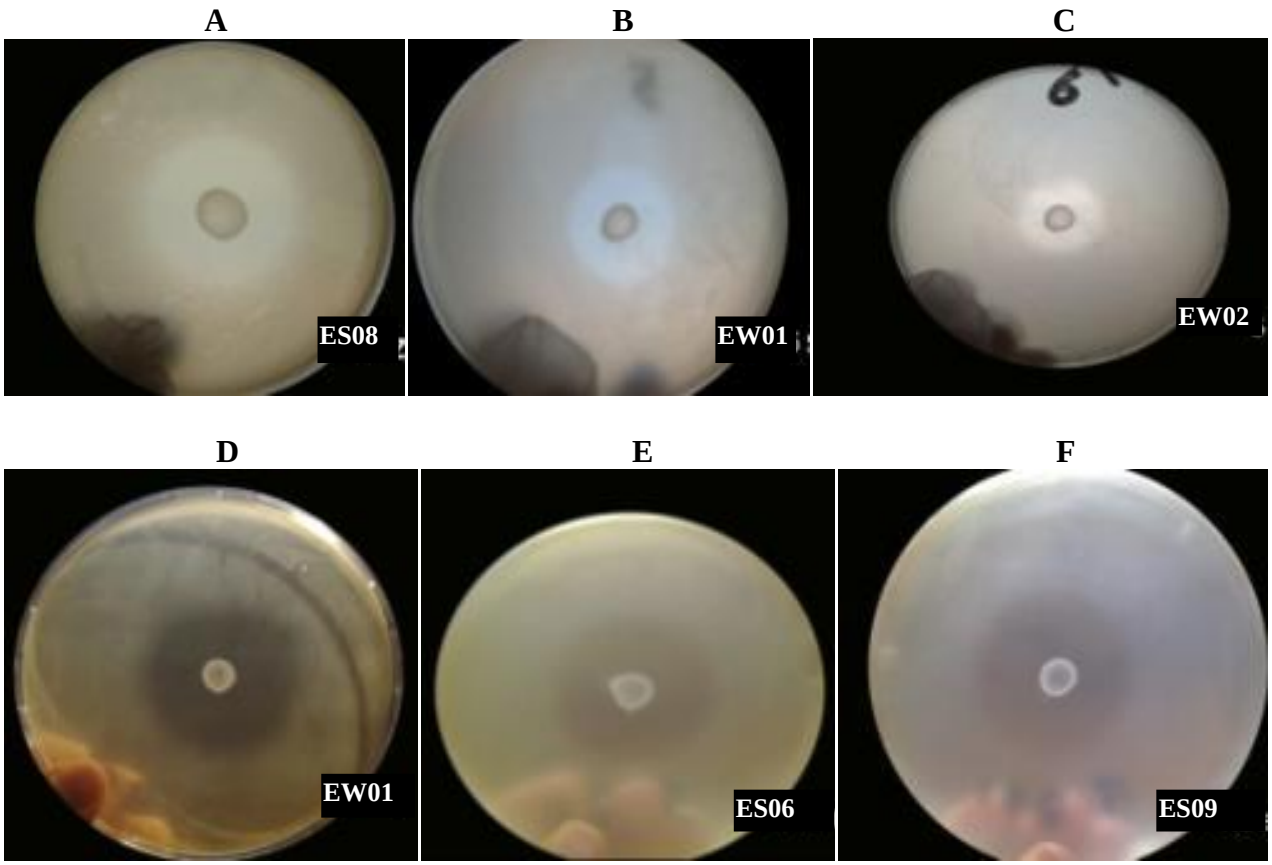

Fig. 1. Antagonistic effects of isolated LAB against *Salmonella typhi* (A, B), *Vibrio fluvialis* (C) and *E. coli* (D, E, F) using agar spot test.

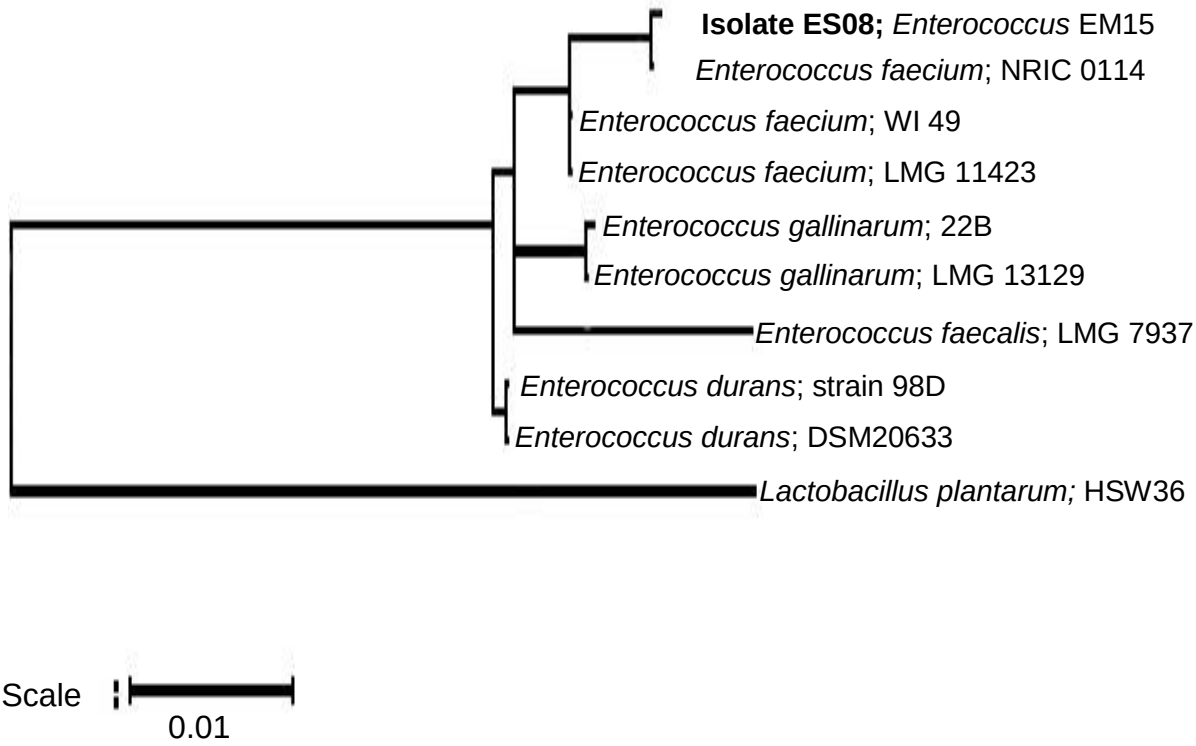

Fig. 2. Phylogenetic analysis of ES08 isolate based on partial sequence of 16s rRNA gene. The scale indicated substitution per site.

In this study, all the isolates classified as *E. faecium* 63.6% survived in pH 3.0 for 3 hours. This result is in accordance with the study of Strompfová and Lauková (2007) which found that *E. faecium* can survive at pH 3.0 for 3 h. Accordingly, it could be suggested that the *E. faecium* phenotypes isolated in this study could be used as probiotic.

Isolate LS11, followed by isolate EW01, seemed to be the most stable isolates as they could maintain themselves and increase the cell density at pH 3. Isolates 6, EW03, AW04, ES05, ES06, ES08 and ES10 showed a good stability at pH 3 for 3 hours. Isolates ES09 and LS07 were more sensitive to low pH than the other isolates.

Tolerance against bile salts

The isolated bacterial phenotypes were tested for their ability to grow in the presence of bile salts. Although the bile concentration of the human gastrointestinal tract varies, the mean intestinal bile concentration is believed to be 0.3% w/v and the staying time is suggested to be 4 hrs (Prasad, *et al*., 1998). The isolates showed a good stability at 0.1% concentration indicated by the increase in the optical density. At 0.3% concentration, none of the isolates showed a marked increase in the optical density; instead they could survive at this concentration for 4 hours. Similar results were observed by Ouled-Haddar *et al*. (2012) who reported an increase in cell viability by alginate microencapsulation. In addition, Strompfova *et al*. (2004) reported that *E. faecium* isolated from dogs can tolerate up to 1% bile for 24 hours.

Bile tolerance is an important characteristic of bacteria to survive in small intestine. Bile resistance of some strains is related to specific enzyme activity, bile salt hydrolase (BSH) which helps to hydrolyse conjugated bile, thus reducing its toxic effect (Du Toit *et al*., 1998). Hydrolyzation of bile salt by enzyme hydrolases (BSHs) had been explained by Tanaka *et al*. (2000), which can be found in *Lactobacillus* sp. (De *et al*., 1995) and *Enterococcus* sp. (Agus, 2003).

Molecular phylogeny of the selected isolate

Isolate ES08 was selected according to its probiotic properties. This isolate was farther identified by partial sequencing of the gene coding for the 16S rRNA. The phylogenetic relationship of the experimental sequence and its close relatives was analyzed through the facilities of the Ribosomal Database Project (http://rdp.cme.msu.edu/) and summarized in a dendrogram (Fig.2). Therefore, the

confirmation of the identified isolate was as *Enterococcus faecium.*

Conclusion

As a conclusion, the results obtained from this study demonstrated the potential probiotic ability of the isolated LAB species from camel's milk. In addition it is recommended that these species can be further studied according to selection criteria like stimulation of immunological system, antibiotic resistance and adhesion to epithelium tissue.

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References

1. Abdelbasset, M. and Djamila, K. (2008). Antimicrobial activity of autochthonous lactic acid bacteria isolated from Algerian traditional fermented milk "Raïb". *Afr. J. Biotechnol*. 7, 2908-2914.
2. Agus, W. (2003). Investigation into the influence of a bacteriocin-producing *Enterococcus* strain on the intestinal microflora, PhD. Thesis, Graduate School, Karlsruhe University, Germany, p. 124.
3. Ashmaig, A., Hasan, A. and El Gaali, E. (2009). Identification of lactic acid bacteria isolated from traditional Sudanese fermented camel's milk (Gariss). *Afr. J. Microbiol. Res.* 3, 451-457.
4. Benkerroum, N., Boughdadi, A., Bennani, N. and Hidane, K. (2003). Microbiological quality assessment of Moroccan camel's milk and identification of predominating lactic acid bacteria. *World J. Microbiol. Biotechnol.* 19, 645-648.
5. Beg, O. U., Von Bahr-Linstrom, H., Zaidi, Z. H. and Jornvall, H. (1987). Characterization of a heterogenous camel milk whey non-casein pro-protein. *Fed. Eur. Biochem. Soc. Lett.* 216, 270-274.
6. Berrada, N., Lemeland, J. F. and Laroche, G. (1991). *Bifidobacterium* from fermented milks: Survival during gastric transit. *J. Dairy Sci*. 74, 409-413.
7. Brasca, M., Lodi, R. and Morandi, S. (2008). Metabolic Characteristics of Lactic Acid Bacteria from Camel Milk "5th IDF symposium on cheese ripening", 9 -13 March, Bern, Switzerland, p. 139.
8. Caridi A. (2002). Selection of *Escherichia coli* inhibiting strains of *Lactobacillus paracasei* subsp. *paracasei*. *J. Ind. Microbiol. Biotechnol*. 29, 303-308.
9. Cariolato, D., Andrighetto, C., Lombardi, A., 2008. Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. Food Control 19, 886–892.
10. Centeno, J. A., Menéndez, S. and Rodriguez-Otero, J. L. (1996). Main microbial flora present as natural starters in Cebreiro raw cow's-milk cheese (Northwest Spain). Int. Food Microbiol. 33,307-313.
11. Chou, L. S. and Weimer, B. (1999). Isolation and characterization of acid and bile tolerant isolates from strains of *Lactobacillus acidophilus*. J. Dairy Sci. 82, 23-31.
12. Corsetti. A. and Valmorri S. 2011. *Lactobacillus* spp.: *Lactobacillus plantarum. In* Encyclopedia of Dairy Sciences, Second Edition, 1, pp. 111-118
13. Cutter, C. N. and Siragusa, G. P. (1995). Population reductions of Gram-negative pathogens following treatments with nisin and chelators under various conditions. J. Food. Protect. 58, 977-983.
14. Daeschel, M.A., 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol., 43: 164-166.
15. De, S., Van, H. L., Vande, W. M., Christiaens, H. and Verstracte, W. (1995). Significance of bile salt hydrolytic activities of Lactobacilli. J. Appl. Bacteriol. 78, 292-301.
16. De Man, J. D., Rogosa, M. and Sharpe, M. E. (1960). A Medium for the cultivation of Lactobacilli. J. Appl. Bacteriol. 23, 130–135.
17. Delves-Broughton, J., Blackburn, P., Evans, R. J. and Hugenholtz, J. (1996). Application of the bacteriocin, nisin. Anton. Leeuw. Int. J. Gen. Mol. Microbiol. 69, 193-202
18. Du Toit, M., Franz, C., Schillinger, U., Warles, B. and Holzappfel, W. (1998). Characterization and selection of probiotic lactobacilli for a preliminary minipig-feeding trail and their effect on serum cholesterol level, faeces moisture contents. Int. Food Microbiol. 40, 93-104.
19. Durlu-Ozkaya, F., Xanthopoulos, V., Tunail, N. and Litopoulou-Tzanetaki, E. (2001). Technologically important properties of lactic acid bacteria isolated from Beyas cheese made from raw ewes' milk. J. Appl. Microbiol. 91, 861-870.
20. El-Sayed I. El-Agamy. 2006. Camel Milk. In Handbook of Milk of Non-Bovine Mammals. Edited by Young W. Park and George F.W. Haenlein. Blackwell Publishing. pag: 297-344
21. El Soda. M. 2002. Selection of *Enterococcus faecium* strains for Egyptian cheesemaking. Abstract in congree Enterococci in Foods Functional and Safety Aspects. 30-31 May 2002, Berlin. Germany.
22. FAO, (2004). FAO-STAT DATA, Food and Agricultural Organization, Statistical Databases at http://earthtrends.wri.org/text/agriculture-food/variable-334.html
23. Farah, Z. (1993). Composition and characteristics of camel milk. J. Dairy Res. 60, 603-626.

24. Freitas, A. C., Pintado, A. E., Pintado, E. M. and Malcasa, F. X. (1999). Role of dominant microflora of Picante cheese on proteolysis and lipolysis. Int. Dairy J. 9, 593-603.
25. Garvie, E. I. (1984). Separation of species of the genus *Leuconostoc* and differentiation of the Leuconostocs from other lactic acid bacteria. In: Methods In Microbiology. Bergan, T. (ed.). Academic press, London, pp. 147-178.
26. Giraffa, G. (1995). Enterococcal bacteriocins: their potential as anti-*Listeria* factors in dairy technology. Food Microbiol. 12, 291–299.
27. Giraffa, G., 2003. Functionality of enterococci in dairy products. International Journal of Food Microbiology 88, 215–222.
28. GOVS (2005). General organization for veterinary services: Animal report of general organization for veterinary services, ministry of agriculture Egypt.
29. Guttmann, D. M., and Ellar, D. J. (2000). Phenotypic and genotypic comparisons of 23 strains from the *Bacillus cereus* complex for a selection of known and putative *B. thuringiensis* virulence factors. FEMS Microbiol Lett. 188, 7–13.
30. Hardie, J. M. (1986). Genus *Streptococcus*. In: Bergey's Manual of Systematic Bacteriology, Vol. 2. Sneath, P. H. A. (ed.), Williams and Wilkins, London, pp. 1043-1047.
31. Helander, I. M., Von Wright, A. and Mattila-Sandholm, T. M. (1997). Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. Trends. Food. Sci. Technol. 8, 146-150.
32. Ito, A., Sato, Y., Kudo, S., Sato, S., Nakajima, H. and Toba, T. (2003). The screening of hydrogen peroxide-producing lactic acid bacteria and their application to inactivating psychrotrophic food-born pathogens. Curr. Microbiol. 47, 231-236.
33. Ivanova, I., Miteva, V., Stefanova, T. S., Pantev, A., Budakov, I., Danova, S., Moncheva, P., Nikolova, I., Dousset, X. and Bouvaval, P. (1998). Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. Int. J. Food. Microbiol. 42, 147-158.
34. Jack, R. W., Tagg, J. R. and Ray, B. (1995). Bacteriocins of gram-positive bacteria. Microbiol. Rev. 59, 171-200.
35. Jacobsen, C. N., Nielsen, V. R., Hayford, A. E., Moller, P. L. and Michaelsen, K. F. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. 65, 4949-4956.
36. Jennes, W., Dicks, L. M. T. and Verwoerd, D. J. (1999). Enterocin 012, a bacteriocin produced by *Enterococcus gallinarum* isolated from the intestinal tract of ostrich. J. Appl. Microbiol. 88, 349-357
37. Kalliomäki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P. and Isolauri, E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. The Lancet. 357, 1076-1079.
38. Kamoun, M. 1990. Cheese production from camel milk. Options Mediterrannees, Ser. Nr. 12, p. 119–124.
39. Kamoun M (1995). Le lait de dromadaire: production, aspects qualitatifs et aptitude à la transformation. Options méditerranéennes, Séries séminaires, 13: 81-103.
40. Khay, E., Idaomar, M., Castro, L. P., Bernárdez, P. F., Senhaji, N. S. and Abrini, J. (2011). Antimicrobial activities of the bacteriocin-like substances produced by lactic acid bacteria isolated from Moroccan dromedary milk. Afr. J. Biotechnol. 10, 10447-10455
41. Lhoste F (2004). Lait de chamelle pour l'Afrique. Atelier sur la filière cameline en Afrique, Niamey, 5-8 novembre 2003, Animal production and health, Publication FAO, Rome.
42. Lu, L. and Walker, W. A. (2001). Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. Am. J. Clin. Nutr. 73, 1124S-1130S.
43. MacFaddin, J. F. (1980). Biochemical Tests for Identification of Medical Bacteria, 2nd ed. Williams and Wilkins, Baltimore
44. Maffei, H. V. and Nobrega, F. J. (1975). Gastric pH and microflora of normal and diarrhoeic infants. *Gut* 16, 719-726.
45. Maisnier-Patin, S., Deschamps, N., Tatini, S. R., Richard J (1992). Inhibition of *Listeria monocytogenes* in Camembert cheese made with nisinproducing starter. Le Lait. 72, 249-263.
46. Malek, R. El-Attar, A., Mohamed, M. Anwar, S. El-Soda, M. and Béal, C. 2012. Technological and safety properties display biodiversity among enterococci isolated from two Egyptian cheeses, "Ras" and "Domiati". International Journal of Food Microbiology 153. pp: 314–322.
47. Mohamed, M. A., Larsson-raznikiewicz, M. and Mohamud, M. U. (1990). Hard cheese making from camel milk. Milchwissenschaft. 45, 716–718.
48. Nawaz, M., Wang, J., Zhou, A., Ma, C., Wu, X. and Xu, J. (2011). Screening and characterization of new potentially probiotic lactobacilli from breast-fed healthy babies in Pakistan. Afr. J. Microbiol. Res. 5, pp. 1428-1436.
49. Ogier JC and Serror P (2008) Safety assessment of dairy microorganisms: The Enterococcus genus. International Journal of Food Microbiology 126: 291–301.
50. Ohris, S.P. and B.K. Joshi, 1961. Composition of camel milk. Ind.Veterinary j., 38: 514-516.
51. Ouled-Haddar, H., Idoui, T., Sifour, M., Guezira, M. and Bouthabet, M. (2012). Isolation, characterization and microencapsulation of

probiotic *Lactobacillus curvatus* G7 from chicken crop. J. Sci. Technol. 2, 47- 53.
52. Prasad, J., Gill, H., Smart, J., and Gopal, P. K. (1998). Selection and Characterization of *Lactobacillus* and *Bifidobacterium* strains for use as probiotic. Int. Dairy J. 8, 993-1002.
53. Ritcher. R. L and R. Vadamuthu., 2001. Microbiology Examination of Food. 4th Ed. American Public Health Association, pp: 483-485.
54. Rodríguez, M. M. L., Tornadijo, M. E., Carballo, J. and Sarmiento, R. M. (1995). Microbiological study of León raw cow-milk cheese, a Spanish craft variety. J. Food. Protect. 58, 998-1006.
55. Rolfe, D. R. (2000). The role of probiotic cultures in the control of gastrointestinal health. J. Nutr. 130, 396S–402S
56. Ryan, M. P., Rea, M.C., Hill, C. and Ross, R. P. (1996). An application in cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147. Appl. Environ. Microbiol. 62, 612-619.
57. Quwehand, A. C., Kirjavainen, P. V., Shortt, C. and Salminen, S. (1999). Probiotics: Mechanisms and established effects. Int. Dairy J. 9, 43-52.
58. Salama, M., Sandine, W. and Giovannoni, S. (1991). Development and application of oligonucleotide probes for identification of *Lactococcus lactis* subsp. *cremoris*. Appl. Environ. Microbiol. 57, 1313–1318.
59. Sandine, W. E. (1985). The streptococci: milk products. In: Bacterial Starter Cultures for Food. Gilliland S. E. (ed.), CRC Press, Boca Raton, Florida. pp. 5-23.
60. Soukoulis, C., Panagiotidis, P., Koureli, R. and Tzia, C. (2007). Industrial yogurt manufacture: monitoring of fermentation process and improvement of final product quality. J. Dairy Sci. 90, 2641-2654.
61. Strompfova, V. and Laukova, A. (2007). *In vitro* study on bacteriocin production of Enterococci associated with chickens. Anaerobe. 13, 228-237.
62. Strompfova, V., Laukova, A. and Ouwehand, A. C. (2004). Selection of enterococci for potential canine probiotic additives. Vet. Microbiol. 100, 107-114.
63. Tannock, G. W. (2004). A special fondness for lactobacilli. Appl. Environ. Microbiol. 70, 3189-3194.
64. Tanaka, H., Hashiba, H., Kok, J. and Mierau, I. (2000). Bile salt hydrolase of *Bifidobacterium longum*: biochemical and genetic characterization. Appl. Environ. Microbiol. 66, 2502-2512.
65. Temmerman, R., Pot, B., Huys, G. and Swings, J. (2002). Identification and antibiotic susceptibility of bacterial isolates from probiotic products. Int. J. Food Microbiol. 81, 1-10.
66. Todorov, S. D. and Dicks, L. M. T. (2004). Effect of medium components on bacteriocin production by *Lactobacillus pentosus* ST151BR, a strain isolated from beer produced by the fermentation of maize, barley and soy flour. World. J. Microbiol. Biotechnol. 20, 643-650.
67. Weinberg, Z., Shatz, O., Chen, Y., Yosef, E., Nikbahat, M., Ben-Ghedalia, D. and Miron, J. (2007). Effect of lactic acid bacteria inoculants on *in vitro* digestibility of wheat and corn silages. J. Dairy Sci. 90, 4754-4762.
68. Yagil, R. (1982). Camels and camel milk Animal Production and Health Paper No. 26. FAO, Rome. 26, 14-19.
69. Yagil, R. (1987). Camel milk. A review. *Int. J. Anim. Sci.* 2, 81
70. Yateem, M.T. Balba, T. Al-Surrayai, B. Al-Mutairi and Al-Daher,R. 2008. Isolation of lactic acid bacteria with probiotic potential from camel milk. International Journal of Dairy Science. 3 (4): 194-199.
71. Yavuzdurmaz, H. (2007). Isolation, characterization, determination of probiotic properties of lactic acid bacteria from human milk. M.Sc. Thesis, The graduate School of Engineering and Sciences of Izmir Institute of Technology.

2/2/2013