Sjournals
Scientific Journals
Scientific Journal of Microbiology (2013) 2(2) 31-42
ISSN 2322-2948

Contents lists available at Sjournals
Scientific Journal of Microbiology
Journal homepage: www.Sjournals.com

Original article

Isolation and identification of microorganisms and antibacterial activity of *Laban Zeer*, an Egyptian traditional fermented milk product

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ARTICLE INFO

Article history:
Received 28 January 2013
Accepted 21 February 2013
Available online 28 February 2013

Keywords:
Laban Zeer
Traditional Egyptian fermented milk
LAB
Yeasts
Antibacterial
Pathogens

ABSTRACT

Laban Zeer is a traditional Egyptian fermented milk product. The microorganisms of Laban Zeer were isolated and identified to species level, as well as the antibacterial activity of *Laban Zeer* was also studied against pathogenic bacteria. Total viable microorganisms, including, lactic acid bacteria (LAB), aerobic mesophilic bacterial, *Enterococus and Enterobacteriaceae* were enumerated. A total forty eight LAB and twenty eight yeast isolates were isolated from four *Laban Zeer* samples and identified by API 50 CHL and API 20C AUX identification system, respectively. The avenger of LAB counts were 7.4 cfu/g, while yeast and *Enterococus* counts were 4.67 and 4.39 cfu/g, respectively. It is noted that the count of bacteria belonging to the family of *Enterobacteriaceae* was not detected in all tested samples. The LAB species were identified as *Leuconostoc mesenteroides* subsp. *cremoris, Lb. rhamnosus, Lb. plantarum, Lb. paracasei* subsp *paracasei, Lb. delbercii* subsp *bulgaricus, Lb. curvatus* subsp *curvatus* and *Lb acidophilus.* The isolated yeasts were identified as *Sccharomyces cervisiae*, *Candida kefyr*, *Candida utilis* and *Rhodotorula mucilaginosa.* The most frequently isolated species was found to be *Leuconostoc mesenteroides* subsp. *cremoris* (37.5%), *Lb. rhamnosus* (20.8%), *Sccharomyces cervisiae* (41.9%) and *Candida kefyr* (29.0%). The antimicrobial activities of *Laban Zeer* were evaluated *in vitro* using an agar well diffusion method and *in situ* method. The major supernatants of *Laban Zeer* samples inhibited the growth of pathogenic bacteria, belonging to *Escherichia*, *Pseudomonas*, *Salmonella*, *Listeria* and *Staphylococcus* genera in

various degrees. The *in situ* method was performed by the inoculation of *Staph. aureus* and *E. coli* in *Laban Zeer* samples separately at an initial level around of 6 log cfu/ml. The count of *Staph. aureus* and *E. coli* were not detected after 12 and 3 days of refrigerated storage period, respectively in samples number 2 and 3. *Laban Zeer* is not suitable environment for growth and activity of such pathogen and would eliminate the pathogen early enough before the products were made ready for consumption. The microorganisms isolated from *Laban Zeer* can be used widely in the food fermentation industry as starter culture and bio-preservatives due to their broad inhibition spectrum.

1. Introduction

Lactic fermentation has been known from a long time as an inexpensive food preservative technology. Some strains of lactic acid bacteria (LAB) may increase the safety and quality of fermented products due to production of different antimicrobial compounds, which can prevent the growth of pathogenic and spoilage bacteria (Ahmadova et al., 2013).

In Egypt, fermented dairy products have been traced back at least to the pharaoh era of 4000 BC (Ross et al., 2002). *Laban Zeer* is Egyptian popular traditional fermented milk. It is also known as earthenware stored *Laban Khad* or concentrated buttermilk, it made mainly from buffalo milk, and sometimes from caw, goat, ewe milk or mixer between them. In Upper Egypt, farmers collect the milk, daily in a tanned goat's leather bag container called *Kerbah* which containing residue amount of fermented milk from previous batch as starter culture. The milk left on ambient temperature to spontaneously ferment for periods determined by experience (depend on ambient temperature). *Kerbah* is blown by air before closing tightly and shaking until the fat globules coalesce. After removing the butter granules, the remainder is called *Laban Khad* or sour buttermilk. It is then stored in an amphora-shaped earthenware vessel called *Zeer*. During the storage some whey permeates through the porous container walls thus concentrating the product. After each addition of a new lot of fresh sour buttermilk, some salt is added to the contents of each *Zeer*. The *Laban Zeer* is used for the manufacture of *Kishk* (made of *Laban Zeer* and boiled, dried and crushed wheat). Sometimes used in a salad or to make beverages after dilution with water; semisolid consistency, acid and salty pleasant taste (El-Gindy, 1983; Kurmann et al., 1992 and Abou-Donia, 2008).

Throughout the fermentation of *Laban Zeer*, lactic acid bacteria and yeasts give the characteristic taste and flavor of *Laban Zeer* by producing of lactic acid, ethanol, carbon dioxide and some other organic components. The finished product has a low pH value of 3.5-3.8 is a poor medium for pathogens and spoilage organisms (Morcos et al., 1973; Beuchat, 1983; Odunfa, 1985; Kurmann et al., 1992 and Tamime and O'Connor 1995).

However, in recent years several studies have been carried out on the acid tolerance and survival characteristics of some pathogenic bacteria in different acidic and/or acidic fermented foods (Skovgaard 2007; Alvarez-Ordonez et al., 2009 and Montet et al., 2009). From this perspective, it is important to determine the surviving condition of some pathogenic bacteria in acidic fermented food such as *Laban Zeer*.

The microbiology of *Laban Zeer* or *Kishk* in Egypt were studied in earlier work by Morcos et al., (1973); Beuchat, (1983) and Tamime and O'Connor (1995) including tentative identification of the predominant microorganisms. The dominant bacteria were *Lactococcus lactis, Lactobacillus brevis, Lactobacillus casei* and *Lactobacillus plantarum*.

From the documented literatures, it seems that very little work has been attempted to identify the fermenting organisms of *Laban Zeer* to the species level. In addition, no study has been initiated on the antibacterial activity of *Laban Zeer*. In this background, the present work aims at the isolation and identification of lactic acid bacteria that ferment *Laban Zeer* and its antibacterial activity against some pathogenic bacteria

2. Materials and methods

2.1. Sample collection

Four *Laban Zeer* samples were collected in sterile plastic bags from Tutun village, Atsa city of Faiyum government, Egypt during August, 2012. The collected sample considered few, because this oldest industry is almost extinct. The samples were refrigerated stored until analysis.

2.2. Indicator pathogenic bacterial strains

Three Gram negative and four Gram positive pathogenic bacterial strains were used for detection of the antibacterial activity of *Laban Zeer*. The bacterial strains which used in these studies and its sources are presented in Table 1.

Table 1
Pathogenic Bacterial species used in these studies.

No.	Name of bacterial species	Species number	Gram Stain
1	*Escherichia coli*	ATCC* 25922	G-
2	*Pseudomonas aeruginosa*	ATCC 27853	G-
3	*Salmonella enterica*	ATCC 13076	G-
4	*Listeria monocytogenes*	ATCC 7644	G+
5	*Staphylococcus saprophyticus*	ATCC 15305	G+
6	*Staphylococcus aureus*	ATCC 25923	G+
7	*Staphylococcus aureus*	ATCC 29213	G+

*American Type Culture Collection

2.3. Enumeration and isolation of microorganisms

Ten gram of each sample was transfered aseptically into 90 ml of pepton water (Oxoid, CM009, UK) and serially diluted (10^{-1} – 10^{-7}) using normal salin solution. 1 ml of each seven dilutions were then inoculated on plates in duplicate. Total aerobic mesophilic counts were obtained by spread plating on plate count agar (Oxoid CM0325, UK) and incubating at 30°C for 48 h. Lactic acid bacterial counts were enumerated on doule layers of de Man Rogosa and Sharpe, MRS agar (Oxoid CM0361, UK) and anerobically incubating at 37°C for 48 h. The anaerobic condition was performed in anaerobic jars (Biolab) with gas generating kits (Oxiod BR0038B). Kanamycin aesculin azide agar, KAA (Oxoid CM0591, UK) incubated at 37°C for 48h for enumeration of *Enterococus*. Violet red bile agar, VRBA (Oxiod CM 0107, UK) incubated anaerobically at 37°C for 24h for enumeration of *Enterobacteriaceae*. Yeast and mould were determined by spread plating on acidified ptato dextrose agar, PDA (Oxoid CM0139) and incubating for 48 h at 30°C. Representative bacterial colonies were isolated randomly from plats of MRS and KAA agar. Isolates were cultivated in its selected broth medium and incubated at 30°C for 24h. The isolates were purified by streak plating using the same medium. Gram positive catalase negative of bacteria were purified by re-streaking on MRS agar. The bacterial isolates were re-suspended and storage in its selected medium containing 15% glycerol at -18°C. Representative yeast colonies on PDA were examined by phase contrast microscopy and purified by successive streaking on PDA. The pure yeast isolates were stored on slants at 4°C.

2.4. Identification of LAB and yeasts

Gram positive and catalase negative of bacteria were microscopic examined (cell morphology and arrangements). Rods and cocci bacteria were presumptively identified as lactic acid bacteria (LAB) (Gerhardt et al., 1981). Growth at 10, 15 and 45°C in MRS broth was evaluated visually after 24, 48 and 72h of incubation. Hetro- and homo-fermentative activity (using MRS broth with inverted Durham tubes) and production of ammonia from arginine were carried out as described by Harrigan and McCance (1986). Salt tolerance was performed by using MRS containing 6.5% (w/v) NaCl with incubation at 37°C for 72h. Ability to ferment carbohydrate substrates was studied using the API 50 CH galleries and API 50 CHL medium (BioMérieux, Marcy l'Etoile, France) system, which enabled identification of the LAB isolates to species level.

For identification of yeast, primary classification of colonies from the PDA agar plates was based on colony characteristics (pigmentation and shape), formation of ascospores, present budding cells and hyphae or pseudohyphae. The methods described by Harrigan and McCance (1986) were followed. Identification of the yeast

isolates to species level was done using the API 20C AUX (BioMerieux, Marcy l'Etoile, France) system of carbohydrate assimilation profiles.

2.5. Evaluation of antibacterial activity of *Laban Zeer*

2.5.1. *In vitro* evaluation by agar well diffusion assay

The well diffusion method was used for determination of *in vitro* antibacterial activity of cell free supernatants of *Laban Zeer* according to (Tagg and McGiven, 1971). An actively growing indicator bacteria in a soft nutrient agar (Oxoid, Hampshire, UK) of 24 h culture at 35°C were spread on the surface of a nutrient agar plate medium (thickness of 5 mm) with a sterile glass spreaders (hockey sticks) which was rotated several times. The plates were left for about 30 min to ensure an even distribution of inoculum. The *Laban Zeer* samples were centrifuged at 3500×g for 10 min. The clear supernatants were removed and filter-sterilized by 0.2 µm syringe filter. Three holes were punched out of the agar, by using a sterile cork borer of 6 mm diameter. The well was filled by 100 µL of the sterilized cell free supernatants of *Laban Zeer*. The plates were incubated until zones of inhibition have clearly developed. The diameter of the complete inhibition zones was measured, including the well diameter. Zones are measured to the nearest whole number in millimeter, using transparent ruler. The antibacterial activity of *Laban Zeer* was compared to the activity of both antibiotics, chloramphenicol (Riyadh Pharma, Saudi Arabia) and sulfadimidin (interchemie, Holland). The results are presented as the means of duplicate.

2.5.2. *In situ* evaluation by Inoculation pathogen in *Laban Zeer*

Pure colonies of *Escherichia coli* ATCC 25922 as Gram negative and *Staphylococcus aureus* ATCC 25923 as Gram positive bacteria were taken from VRBA and Staph 110 agar medium (Oxoid, CM0145, UK) respectively. The colonies were suspended in 0.85% NaCl. The concentration of bacterial cells was adjusted by measuring the optical density (Lahtinen et al., 2007). The optical density at 600 nm (OD 600) of 0.5±0.01 means 3-4×10^8 CFU/ml. So, inoculation of 100 g sample with 0.5 mL of this individual suspension would theoretically give an initial contamination rate of around 1.5×10^6 cell per gram. The samples were then stored in refrigerator at 5±1°C for 15 days and the count of *E. coli* or *Staph. aureus* were monitored after 1, 3, 6, 9, 12 and 15 days of storage. Ten 10 g from the inoculated sample was diluted with 90 mL peptone water and serially diluted. A 1 mL of the appropriate dilution was pour plated onto VRBA or Staph 110 agar medium for counting of *E. coli* or *Staph. aureus*, respectively.

2.6. Determination of pH

pH values were determined using a Grison pH meter (GPL21) (Herisau, Switzerland) after calibration using standard buffers (Metrohm Ion Analysis, Herisau, Switzerland) at pH 4 and 7.

2.7. Statistical analysis

Three independent experiments were performed. All analysis and enumeration were done in duplicate. All data were analysed by ANOVA using the general models procedure of SAS (1989). Differences among means wear tested for significance (P>0.05) by Duncan's multiple range test.

3. Results and discussion

3.1. Viable count of microorganisms in *Laban Zeer*

The pH values and the microbial counts of *Laban Zeer* are shown in table 2. LAB were the dominating microorganisms in all tested samples with average values of 7.4±0.49 $\log_{10}$ cfu/g. Aerobic mesophilic bacterial numbers were usually tow logarithmic units lower than those for LAB. Also, yeasts were present in considerably high numbers in the samples with average values of 4.67 $\log_{10}$ cfu/g. This high numbers of LAB and yeasts, coupled with the low values of pH (3.9) and medium salty may be responsible for the sour taste, flavor and unique aroma of *Laban Zeer.* However, *Enterobacteriaceae* counts were not detected in all *Laban Zeer* samples. These findings may be due to the low pH values (ranged from 3.7-4.1), in addition, the presence of salt in samples. Akabanda et al., (2010), found the *Enterobacteriaceae* numbers decreased with the decreasing of pH values in Ghanaian traditional fermented milk.

The counts of LAB in *Laban Zeer* were similar to other fermented milk products. Other researchers such as Obodai and Dodd (2005) found LAB counts between 8 and 10 log cfu/ ml in a fermented milk product (*nyarmie*) in Accra, Ghana. Akabanda et al., (2010) found the LAB count of 8.3 log cfu/ml in *Nunu*, a Ghanaian traditional fermented milk, Beukes et al. (2001) also found LAB counts of 8.88 log cfu/ml on MRS at 42°C and 8.85 log cfu/ml on M17 from some fermented milks of South Africa. Savadogo et al (2004) also found LAB numbers between 4.3 and 8.6 log cfu/ml in Fulani fermented milk. Similar results were found in Tanzanian fermented milk (Isono et al., 1994), and Zimbabwean fermented milk products (Feresu and Muzondo, 1990).

Table 2
pH values and viable counts of microorganisms (log_{10} cfu/g) of *Laban Zeer*

Samples	pH	Aerobic mesophilic bacteria	Lactic acid bacteria	*Enterococus*	*Enterobacteriaceae*	Yeasts
1	3.8	5.72	7.94	4.69	ND	5.56
2	4.0	5.84	7.63	4.72	ND	4.95
3	4.1	5.61	7.22	4.92	ND	5.16
4	3.7	5.69	6.81	5.40	ND	3.0
Mean	3.9	5.72	7.40	4.93	-	4.67
SD	0.18	0.10	0.49	0.33	-	1.14

3.2. Identification of LAB isolated from *Laban Zeer*

The identified LAB isolated from *Laban Zeer* samples are listed in Table 3. Forty eight LAB isolates were identified phenotypically. The dominant lactic acid bacteria identified are the genera of *Lactobacillus* (30 strains) followed by *Leuconostoc* (18 strains). The dominance of *Lactobacillus* among the isolated strains is consistent with the findings of El-Shafei et al., (2002). However, large populations of a single strain of *Leuconostoc mesenteroides* subsp. *cremoris* with frequency occurrence (37.5%) formed the dominant cultivable population in *Laban Zeer*. Bacteria of the genus *Leuconostoc* are incorporated into dairy starter cultures due to their ability to produce important metabolites such as diacetyl and CO_2 from citric acid (Björkroth and Holzapfel, 2006 and Cogan and Jordan, 1994). Diacetyl is the primary source of aroma and flavor compounds in a variety of fermented milk products including buttermilk, butter and various cheese types (Björkroth and Holzapfel 2006). Ten strains were identified as *Lactobacillus rhamnosus,* six as *Lactobacillus plantarum,* four strains as *Lactobacillus paracasei* subsp *paracasei, Lactobacillus delbercii* subsp *bulgaricus* and *Lactobacillus curvatus* subsp *curvatus* for each. The presence of *Lactobacillus rhamnosus, Lactobacillus paracasei* subsp *paracasei* and *L. acidophilus* in *Laban Zeer* samples are beneficial and could be used in combination with *L. delbrueckii* subsp*. bulgaricus* in the preparation of yoghurt as probiotic culture with improved organoleptic characteristics and enhanced therapeutic benefits (Akabanda et al., 2010). Furthermore, *Lactobacillus acidophilus* is a natural inhabitant of mammalian gastrointestinal systems. These species are of considerable industrial and medical interest, because *Lactobacillus rhamnosus, Lactobacillus paracasei* subsp *paracasei* and *L. acidophilus* are believed to play an important role in human health and nutrition by its influence on the intestinal flora (Roy et al., 2001).

In this study, six strains were identified as *Lactobacillus plantarum* with frequency occurrence 12.5%. The presence of *Lactobacillus plantarum* in *Laban Zeer* may be due to contamination of milk with plant food materials during lactation of milk. *Lactobacillus plantarum* strains are known to be commonly associated with plant based food fermentations (Adebayo-tayo and Onilude 2008). Presence of *Lactobacillus plantarum* is in close agreement to that of Mathara et al., (2004) they identified 130 out of 339 isolates as *Lactobacillus plantarum*. Morcos et al., (1973); Beuchat, (1983) and Tamime and O'Connor (1995) found different LAB predominant species in *Laban Zeer* or *Kishk*, such as, *Lactococcus lactis, Lactobacillus brevis* and *Lactobacillus casei*. These differences may accord with change of sites, milk types, season of production and climate. It is reported that traditional fermented milk in regions with a cold climate favor the growth of mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp.* whereas, in warm regions, thermophilic bacteria like *Lactobacillus* and *Streptococcus* prevailed (Savadogo *et al*., 2004*)*. Mohammed et al., (2009) reported that the *Lactobacillus delbrueckii* subsp*. lactis, Lactobacillus fermentum, Enterococcus faecium Lactobacillus delbrueckii* subsp*. bulgaricus, Lactobacillus paracasei* subsp*. paracasei, Lactobacillus plantarum* and *Lactococcus lactis* subsp*. lactis* were the predominant species in Egyptian dairy

products. In this study, the all of the identified LAB were frequently found in various traditional fermented milk in Egypt or in other countries of the world (Feresu and Muzondo 1990; Beukes et al., 2001; El Soda et al., 2003; Mathara et al., 2004 and Duskova and Karoiskova, 2013).

Table 3
Identification of LAB isolated from *Laban Zeer* using API 20C AUX

Name of species	No. of isolates
Leuconostoc mesenteroides subsp. *cremoris*	18
Lactobacillus rhamnosus	10
Lactobacillus plantarum	6
Lactobacillus paracasei subsp *paracasei*	4
Lactobacillus delbercii subsp *bulgaricus*	4
Lactobacillus curvatus subsp *curvatus*	4
Lactobacillus acidophilus	2

Table 4
Identification of yeasts isolated from *Laban Zeer*

Name of species	No. of isolates
Sccharomyces cervisiae	13
Candida kefyr	9
Candida utilis	6
Rhodotorula mucilaginosa	3

3.3. Identification of yeasts

Yeasts appear to be commonly associated with traditional fermented dairy products and have been reported in several studies (Beukes et al., 2001; Gadaga et al., 2001; Mathara et al., 2004 and Akabanda et al., 2010). The identified yeast strains isolated from *Laban Zeer* are presented in Table 4. Four yeast species were identified from thirty one isolates, 13, identified as *Sccharomyces cervisiae*, 9, as *Candida kefyr*, 6, as *Candida utilis* and 3, as *Rhodotorula mucilaginosa.* The predominance of *Sccharomyces cervisiae* and *Candida kefyr* observed in our study is in agreement with results reported for other traditional fermented milk products (Gadaga et al., 2001; Abdelgader, et al., 2001 and Shuangquan et al., 2006). Other studies reported different yeast species predominant in its traditional fermented milk products. Soliman and Aly, (2011), found *Trichosporon cutaneum* and *Candida catenulate* to be the most dominant species associated with in Egyptian *Karish* cheese, but *Sccharomyces cervisiae* was also frequently isolated, Nahvi and Moeini (2004) reported *kluyveromyces lactis* and *kluyveromyces marxiamus* to be a major part of the yeast flora in dairy products. *kluyveromyces lactis* and *K. marxiamus* were never recovered in our study. This fact suggests that the composition of the yeast flora is likely to differ according to sites and countries of production. *Sccharomyces cervisiae* and *Candida kefyr* may play an essential role in flavor development during fermentation of *Lanan Zeer*. Fleet, (1990) reported that *Candida famata* and *C. kefyr* convincingly emerge as the most prevalent yeasts in dairy products. Co-culturing of typical lactic acid bacteria species with representative yeast strains has been shown to slightly enhance bacterial growth, to produce specific organic acids and a majority of aroma compounds (Alvarez-Martin et al., 2008). Indeed, a symbiosis between yeasts and lactic acid bacteria has been suggested: whereby the bacteria provide the acidic condition favorable for the growth of yeasts. Additionally, Alvarez-Martin et al. (2008) reported that yeast growth can be essential to the development of the typical texture and aroma profiles of certain fermented milk products, the outcome of their strong proteolytic and lipolytic activity.

Table 5
Diameter of *Laban Zeer* inhibition zone against pathogenic bacteria.

***Laban Zeer* samples**	**Diameter of inhibition zone (mm)**						
	***Escherichia coli* ATCC25922**	***Pseudomonas aeruginosa* ATCC 27853**	***Salmonella enterica* ATCC *13076***	***Listeria monocytogenes* ATCC *7644***	***Staphylococcus saprophyticus* ATCC 15305**	***Staphylococcus aureus* ATCC 25923**	***Staphylococcus aureus* ATCC *29213***
1	10 ± 1^{d}	ND	10 ± 0^{c}	14 ± 1^{d}	12 ± 2^{c}	ND	9 ± 0^{cd}
2	13 ± 2^{c}	13 ± 1^{b}	12 ± 0^{b}	19 ± 1^{c}	16 ± 1^{b}	10 ± 1^{c}	10 ± 0^{c}
3	13 ± 1^{c}	12 ± 2^{b}	12 ± 1^{b}	17 ± 2^{c}	15 ± 1^{b}	9 ± 0^{c}	9 ± 0^{cd}
4	11 ± 2^{cd}	12 ± 2^{b}	10 ± 1^{c}	12 ± 0^{d}	12 ± 0^{c}	ND	8 ± 0^{d}
Chl	44 ± 1^{a}	33 ± 1^{a}	48 ± 0^{a}	60 ± 0^{a}	ND	46 ± 0^{a}	46 ± 1^{a}
Sul	30 ± 1^{b}	ND	26 ± 0^{b}	30 ± 0^{b}	38 ± 0^{a}	32 ± 0^{b}	33 ± 1^{b}

$^{a-d}$ Mean values (±SD; n = 3) within the same column bearing different superscripts are significantly different (P>0.05)
Chl: chloramphenicol, 25 mg/ml
Sul: sulfadimidin, 100 mg/ml
ND: Not Detcted

3.4. Inhibition of pathogenic bacteria by *Laban Zeer* using agar well diffusion method

The supernatants of *Laban Zeer* were screened for their ability to inhibit a variety of pathogens compared to antibiotics (chloramphenicol and sulfadimidin) by agar diffusion method (Table 5). The major pathogenic either Gram negative or positive bacteria were inhibited to different extents by all the tested samples, with *Listeria monocytogenes* being the most susceptible and *Staphylococcus aureus* ATCC 25923 was more resistance. *Laban Zeer* number 2 and 4 recorded significantly higher inhibition zone than other samples against *Salmonella enterica, Listeria monocytogenes, Staphylococcus saprophyticus* and *Staphylococcus aureus* ATCC 25923. No inhibitory activity was found against *Staphylococcus aureus* ATCC 25923 by samples number one and four as well as against *Pseudomonas aeruginosa* by the sample number one and sulfadimidin. The chloramphenicol did not inhibit *Staphylococcus saprophyticus*, while all tested sample inhibited it.

This inhibitory activity of *Laban Zeer* may was due to the production of organic acids particular, acetic acid and lactic acid. In general, those organic acids have a strong inhibitory activity against Gram negative bacteria (Makras and Vuys 2006). Nevertheless, it was found that several LAB strains produced antibacterial substances, deferent from organic acids. These compounds, present in concentrated culture supernatant, were mostly active against a narrow range of Gram positive and Gram negative bacteria. The production of specific antibacterial compounds by our LAB isolates (Table 3) has been reported previously. For instance, the bacteriocin isolated from *Leuconostoc mesenteroides* subsp. *cremoris* showed strong killing activity against several pathogenic bacteria, including Gram-positive bacterium *Listeria innoqua*, *Listeria monocytogenes*, *Bacillus cereus strains* and Gram-negative bacterium *Pseudomonas fluorescens* (DÜndar, 2006). Futher, Sarika et al., (2010) found that the cell-free supernatant of *L. rhamnosus* inhibited the growth of *Bacillus brevis*, *B. pumilus*, *B. subtilis, Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Vibrio harveyi*, *Acinetobacter* sp. and *Arthrobacter* sp. Also, Fazeli et al., (2009) stated that the *Lactobacillus plantarum* strains could inhibit growth of *S. typhimurium.* Bendali et al., (2011) observed antibacterial activity of culture supernatant of *Lactobacillus paracasei* subsp *paracasei* against pathogens, both Gram-negative and Gram-positive: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Enterococcus faecalis* and *Staph. aureus*.

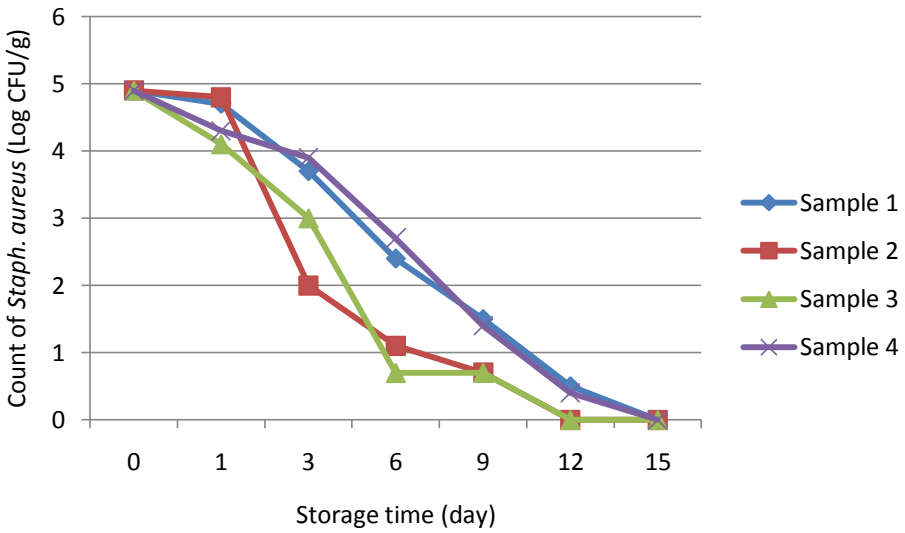

Fig. 1. Survival of *Staph*. *aureus* ATTCC 25923 in *Laban Zeer* during storge time.

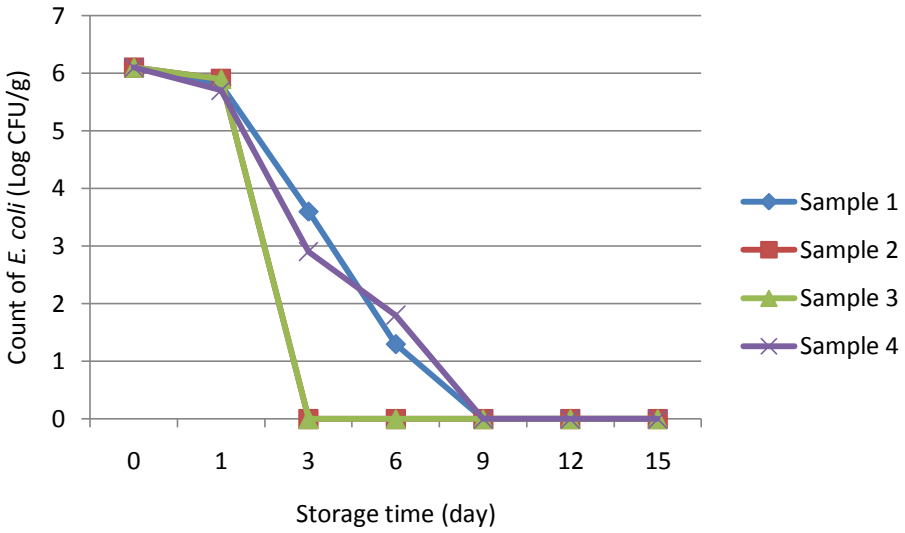

Fig. 2. Survival of *E. coli* ATTCC 25922 in *Laban Zeer* during storge time.

3.5. Survival of *Staph. aureus* ATTCC 25923 and *E. coli* ATTCC 25922 in *Laban Zeer* during refragerated storge period

The effects of *Laban Zeer* invronmente on the behaviour of *Staph*. *aureus* ATTCC 25923 and *E. coli* ATTCC 25922 during refragerated storage are showen in Figuer 1 and 2, respectivly. The number of both *Staph*. *aureus* and *E. coli* significantly decreased (*P*>0.05) during storage time. Counts of *Staph. aureus* decreased from initial inoculum level of 4.9 to 4.7, 4.8, 4.1 and 4.3 log cfu/g in product 1, 2, 3, and 4 within 24 hrs of storage period. During storage time the cells decreased markedly in all tested samples. The count of *Staph. aureus* in *Laban Zeer* numbers 2 and 3 lower significantly (*P*>0.05) than the count in samples numbers 1 and 4 after 3, 6 and 9 days of storage. Both samples number 2 and 3 completely inhibited the survival of *Staph. aureus* efter 12 days of storage, whereas, the cells were not detected after 15 days in samples number 1 and 4. Regarded to *E. coli*, slightly decreasing in count of cells was detected after 24 hrs of storage period in all tested samples. After 3 days, sharp decreasing in cells count to undetectable level was observed by *Laban Zeer* numbers 2 and 3. *E. coli* count continues decreasing in sample No. 1 and 4 until ninth day, when the cells were not detected.

In general, the strain of *E. coli* used in this study was more sensitive to the *Laban Zeer* environment than *Staph. aureus* as it survived for the shortest period in all tested sample. Samples of *Laban Zeer* number 2 and 3 have a stronger inhibition activity than numbers 1 and 4.

From our results, *Laban Zeer* is not suitable environment for growth and activity of such pathogen and would eliminate the pathogen early enough before the products were made ready for consumption.

The loss of viability of *Staph*. *aureus* ATTCC 25923 and *E. coli* ATTCC 25922 in *Laban Zeer* samples may be largely due to the seven LAB which identified in *Laban Zeer,* (Table 3) that may secret a lot of antibacterial substances such as organic acid, ethanol, hydrogen peroxide, diathetyl, low molecular weight proteins (bacteriocins) and NaCl added into the *Laban Zeer* may inhibit the survival of these pathogens. Huang et al., (2001) demonstrated that the combined effect of ethanol and NaCl decreased the viability of both *E. coli* and *Staph*. *aureus* depending on the concentration.

The inhibitory mechanism of fermented milk was investigated and was shown to be dependent on the lowering of the pH of the medium and the production of organic acids, in particular lactic and acetic acid (Makras and De Vuyst, 2006). Ouwehand and Vesterlud, (2004), Cizeikiene et al (2013), and Gao et al., (2013) found many antimicrobial components produced by lactic acid bacteria including organic acids, hydrogen peroxide, carbon dioxie, diacetyl, bacteriocins and low molecular weight antimicrobial substances. Thus, pathogens such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Shigella flexneri Salmonella* spp., *Salmonella enterritifis* were not able to grow in traditional fermented milk in different regions of the world (Daglioglu, et al., 2002; Tsegaye, et al., 2004; Tsegaye and Ashenafi, 2005 and Mufandaedza et al., 2006).

Our results are an agreement with Daglioglu et al., (2002) they observed that the count of *E. coils* O157:H7 not detected after the fifth day of fermentation of *Tarhana* (Turkish yoghurt-cereal mixture like Egyptian *Kishk*) inoculated with 8×10^4 cfu/g, and the count of *Staph. aureus* decreased markedly after first day of fermentation and were 10^2 cfu/g at the end of fermentation (the initial concentration 5×10^4). Tasegaye and Ashenafi, (2005) found that the inoculation of *E. coli* O157:H7 in Ergo (traditional Ethiopian fermented milk)at an initial level of 3 log cfu/ml, resulted in complete elimination of test organisms at 6 hr at ambient temperature storage, but they were recovered until 72 hr at refrigerated storage. Similar results were obtained by Tsegaye et al., (2004); Tsegaye and Ashenafi (2005) and Suganya et al., (2013).

4. Conclusion

Spontaneous microorganism's presents in *Laban Zeer* were identified. The domination of *Leuconostoc mesenteroides* subsp. *cremoris* and *Lactobacillus rhamnosus* among the associated LAB was established. Other strains occurring in relatively high numbers were identified as representatives of *Lactobacillus plantarum, Lactobacillus paracasei* subsp *paracasei, Lactobacillus delbercii* subsp *bulgaricus, Lactobacillus curvatus* subsp *curvatus* and *Lactobacillus acidophilus.* The predominance of *Sccharomyces cervisiae* and *Candida kefyr* observed in our study may play an important role in flavor and aroma together with LAB of *Laban Zeer*. In spite of the spontaneously during fermentation of *Laban Zeer*, it free from *Enterobacteriaceae.* The study of the antibacterial activity of *Laban Zeer* supernatant revealed that the major pathogenic either gram negative or positive bacteria were inhibited to different extents by tested samples, with *Listeria monocytogenes* being the most susceptible and *Staphylococcus aureus* ATCC 25923 was more resistance. Neither *Staph*. *aureus* nor *E. coli* could growth and survival in *Laban Zeer* medium. To obtain fermented milk with flavor characteristics similar to those of artisanal product, the isolated microorganism strains should first be tested in mixed cultures. Based on these results future works will be dedicated to the elaboration of industrial products using traditional starters and to the identification of aromatic compounds presents in *Laban Zeer.*

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