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CONTAMINATION OF FERMENTED FOODS IN EGYPT WITH UNDESIRABLE BACTERIA

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ABSTRACT: This study assessed the safety and quality of some fermented foods in Egypt. The fermented milk (yogurt), sour milk (Laban Rayeb) and flour fermented barley (Buza or Boza) samples were collected from Sharkia Governorate (Zagazig, Faqous, Abu Kebir and El Husseiniya District), Egypt. Total bacterial diversity, lactic acid bacteria, yeasts and molds, staphylococci and micrococci, coliforms, *Enterobacteriaceae*, pseudomonads and enterococci counts within each sample were determined. The identity of the isolates was established through microscopic, biochemical and molecular techniques. The total bacterial counts and lactic acid bacteria of analysed samples ranged from 6.70 to 9.23 log cfu/ml and 6.09 to 9.24 log cfu/ml, respectively. The overall results on the bacterial biota of the fermented foods revealed that total bacterial loads of yogurt, Laban Rayeb and Boza were 8.30, 9.23 and 8.19 log cfu/ml, respectively. Generally, results revealed that undesirable bacterial isolates from de Man, Rogosa, and Sharpe agar (MRS) and M17 selective media for lactic acid bacteria belonged to *Bacillus, Pseudomonas* and *Staphylococcus* genera and fungal isolates belonged to *Saccharomyces* and *Candida*. genera. The most dominant bacterial species identified by 16S rRNA sequencing analysis were *B. sporothermodurans, B. thuringiensis, B. safensis, B. sphaericus B. tequilens, B. licheniformis, B. vietnamensis, B. amyloliquefaciens, B. subtilis, P. psychrotolerans, S. cohnii* and *S. saprophyticus*. This study proved the presence of unusual bacteria contaminants (UBC) in the selected fermented foods. The UBSs could be pathogenic and/or toxigenic by producing various types of toxins.

Key words: Yogurt, laban rayeb, buza, unusual bacteria contaminants, *Bacillus*.

INTRODUCTION

Fermented foods are one of the major food groups in Africa, especially in Egypt. There is limited knowledge on the production of fermented foods in Egypt especially fermented-milk and cereals. Zabady (Yogurt) is considered to be the oldest fermented milks known in the world, especially in the area of Middle East. Zabady is the national type of yogurt manufactured in Egypt. Laban Zabady is produced locally by small traditional operates and are often produced on site by hand. Traditional yogurt made from buffalo's or cow's milk after pasteurization followed by adding starter cultures. Zabady is extremely smooth, white to off-white when prepared from cow milk, characteristic taste and full, pleasant, slightly sour aroma. Microbiological and chemical analyses of Zabady have been reported **(Abd El-Malek and Demerdash, 1970a; El-Gendy, 1983; Bassyouni *et al*., 2012).**

Egyptian farmers, mainly in Lower Egypt and partially in Upper Egypt put fresh milk in earthenware pots (Matared) and leave it undisturbed in a warm place until the cream rises and the lower partially skimmed milk coagulates. This is called "Laban Rayeb or Laban "Matared". The sour milk "Laban Rayeb" is either consumed as it or after conversion to a soft acid cheese "Karish cheese". Laban Rayeb is one of the most popular sour milk consumed

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in Egypt. During hot weather, it is stored on successive days in earthenware containers named "Zeer" for preparation of Laban Zeer used for the Kishk making. The sour milk in Egypt has been examined for chemical and microbiological by several authors **(Abd El-Malek and Demerdash, 1970b; El-Gendy, 1983; Bassyouni *et al.*, 2012).**

Regarding raw milk products, it is necessary to guarantee the following microbiological criteria: absence of *Listeria monocytogenes* at numbers exceeding 100 cfu/ml, the absence of *Salmonella* spp., the absence of staphylococcal enterotoxins and low numbers of hygiene indicators like coliforms. The label must clearly indicate "made with raw milk. The commercialization of raw milk and dairy products made from raw milk in some North African countries is not allowed in European countries for sanitary concerns **(EU, 2010).** Among other microbiological hazards, *Mycobacterium bovis*, which was found to infect 30% of dairy cattle and 40% of farm workers and represents a serious risk **(Hassanein *et al.*, 2009).** In a first comprehensive study regarding the prevalence and pathogenic potential of *Escherichia coli* in dairy products in Egypt, **Ombarak *et al.* (2016)** found that raw milk, Karish cheese, and Ras cheese are highly contaminated with *E. coli*, including potentially pathogenic strains that may pose a public health threat. Raw milk is considered at high risk of causing illnesses, based on the latest surveillance data published by the Center of Disease Control and Prevention **(Langer *et al.*, 2012).**

A quantitative risk assessment, carried out to determine consumer risk from *Staphylococcus aureus* and staphylococcal enterotoxin in raw milk in California. *S. aureus* levels above the 10^5 cfu/ml level are concern and enterotoxin production may represent a potential risk of staphylococcal enterotoxin intoxication in all consumer age groups **(Heidinger *et al.*, 2009).** Moreover, contamination of raw milk by enterococci of bovine fecal origin during the milking process or from environmental sources (such as milking equipment or contaminated water) was a relevant factor in the microbial contamination of raw milk products **(Poznanski *et al.*, 2004).** It is noteworthy that *Enterococcus* spp. were also predominant pathogens causing bovine mastitis, which affects udder health and milk quality **(Rysanek *et al.*, 2009).**

Consequently, contamination of fresh milk products with animal, personal or environmental enterococcal strains through different steps of processing are likely to occur. This depends on the sanitary practices involved throughout processing. Irrespective of the benefits derived from fermented foods, there are concerns about their safety because of the continuous and unpredictable pre- and post-processing contamination by pathogenic microorganisms some of which can be pathogenic and toxigenic bacteria that produce harmful secondary metabolites including toxins. Therefore, this research was conducted to assess the bacterial and fungal diversity in some Egyptian fermented foods to gain insight into their safety.

MATERIALS AND METHODS

Collection of Samples

Fermented food samples and their collection sites are shown in Table 1, sixty samples of traditional fermented milk and cereal were collected from individual shops in four districts from Sharkia Governorate (Zagazig, Faqous, Abu Kebir and El Husseiniya cities). About 100 ml of each sample was aseptically collected and transported to Microbiological Laboratory, Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University in a 4°C icebox. Subsequently, all of the samples were analyzed within a few hours.

Enumeration, Isolation and Phenotypic Characterization of Isolates

Ten milliliters of each sample was mixed with 90 ml of 0.85% (*W/V*) sterile physiological saline to make an initial dilution (10^{-1}). Serial dilutions were made for each sample and 1.0 ml of the appropriate dilutions (10^{-4}, 10^{-5}, $10^{-6}$and 10^{-7}) was poured plated according to type of bacterial group in triplicates on media. Media and growth conditions used for enumeration of the main groups of the culturable bacteria and fungi of fermented food samples are presented in Table 2. Plate count agar (PCA; Merck, 1.05463) was incubated at 30°C for 48 hr., for enumeration of the total bacterial count (TBC).

Table 1. Sample type, common name and code number collected from four different districts (Zagazig, Faqous, Abu Kebir and El Husseiniya) Sharkia Governorate, Egypt

Sample type	Common name	Code number	Total No. samples
Fermented milk (Yogurt)	Laban Zabady (Zabady)	Y1-Y24	24
Fermented milk (Sour milk)	Laban Rayeb (Laban Matrad)	R1-R24	24
Fermented cereal (Boza)	Boza (Buza)	B1-B12	12

Table 2. Media and growth conditions used for enumeration of the main groups of the culturable bacteria in fermented food samples

Growth media	References	Presumptive microorganisms	Growth conditions			
			Plate method	Incubation condition	Incubation time (hr.)	T°C
***PCA**	**APHA (1978)**	Mesophilic aerobes	Pour	Aerobiosis	48	30
MRS	**De Man *et al*. (1960)**	-Mesophilic lactobacilli	Pour	Microaerophilia	48-72	30 or 42
		-Thermophilic lactobacilli				
M17	**Terzaghi and Sandine (1975)**	-Mesophilic streptococci	Pour	Aerobiosis	48-72	30 or 42
	Aquilanti *et al*. (2012	- Thermophilic streptococci				
RBCA	**APHA (1978)**	Yeasts and molds	Spread	Aerobiosis	72-96	30
MSA	**Chapman (1945) Bannerman (2003)**	Micrococci and staphylococci	Spread	Aerobiosis	24-48	37
VRBA	**Maslen (1952)**	Total coliforms	Pour	Microaerophilia	24-48	37
VRBGA	**APHA (1978)**	Total coliforms	Pour	Microaerophilia	24-48	37
PAB	**Geftic *et al*. (1979)**	Pseudomonads	Spread	Aerobiosis	24-48	30
SBA	**Slanetz and Bartley (1957)**	Enterococci	Spread	Aerobiosis	24-48	37

*PCA, Plate count agar; MRS, de Man, Rogosa & Sharpe agar; RBCA, Rose Bengal Chloramphenicol Agar; MSA, Mannitol Salt Agar; VRBA, Violet Red Bile Agar; VRBGA, Violet Red Bile Glucose Agar; PAB, *Pseudomonas* Agar Base ;SBA, Slanetz and Bartley Agar

de Man, Rogosa and Sharpe agar (MRS; Difco Laboratories, Detroit, MI) and M17 (M17: Oxoid Ltd., Basingstoke, UK) plates were used with a double layer of the same medium for counting lactobacilli and lactococci at 30°C for 48 hrs, respectively. Colonies with distinct morphological differences (color, shape, and size) were selected and purified by streaking on the same medium.

Yeasts and moulds were determined on Rose Bengal Chloramphenicol Agar (RBCA: Lab M, 36 supplemented with chloramphenicol X009), incubated at 30°C for five days. Pseudomonads were counted on HiFluoro (™) *Pseudomonas* Agar Base (PAB; Lab M), incubated at 30°C for 48 hr. Staphylococci group was determined on Mannitol Salt Agar (MSA; Biolife, Italy), then the plates were incubated at 37°C for 48 hr. *S. aureus* was detected by examining the plates for typical colonies. *Enterobacteriaceae* and coliform bacteria were determined on Violet Red Bile Glucose Agar (VRBGA; Biolife, Italy) and Violet Red Bile Agar (VRBA; Biolife, Italy) with a double layer of the same medium and the plates were incubated at 37°C for 24 hr., respectively. Enterococci on Slanetz and Bartley

Agar (SBA: LAB M 106) were incubated at 37°C for 48 hrs.

The catalase and Gram reactions of all the isolates were assessed, and Gram-positive, catalase-negative isolates were preserved in milk containing 0.1% sodium glutamate and stored at –20°C. A representative isolate in each species was tested for gas production from glucose, salt tolerance (3.0% and 6.5% NaCl), growth at different temperatures (5, 10, 45, and 50°C) and pH (3.0, 3.5, 4.0, and 7.0) in MRS broth, as described by the method of **Kozaki *et al.* (1992).** Also, Gram-positive, catalase-positive isolates were preserved in nutrient agar at 4°C.

Molecular Identification of UBCs Isolates from MRS and M17 Media

DNA extraction and 16S rRNA gene sequencing

Total genomic DNA was extracted from overnight grown cultures by using the method reported by **Yu *et al*. (2009).** Purified DNA was diluted to a final concentration of 100 ng/µl for application. All Gram-positive, Gram-negative and catalase-positive bacterial isolates from MRS and M17 media were preliminary identified by determining the nucleotide sequences of 16S rRNA gene. For each strain, the genomic DNA was used as a template for PCR amplification of a segment of 16S rRNA gene on the automatic thermal cycler (PTC-200, MJ Research, Waltham, MA). Primers and reaction mixtures are listed in Table 3. The sequencing of purified PCR products was performed in CNR IPSP (UOS Bari and URT Larino) Istituto per la Protezione Sostenibile delle Piante, Bari, Italy. The sequences were analyzed and determined using the BLAST algorithm (http://www. ncbi.nlm. nih.gov/blast; (**Altschul *et al.*, 1997**) and were submitted to GenBank (http://www.blast. ncbi. nlm.nih.gov).

Consensus sequences were imported into MEGA version 4.0 software (http://www.mega software.net; **Tamura *et al*., 2007**), with which a sequence alignment and phylogenetic trees were created based on the neighbor-joining (NJ) method. The percentage of bootstrap confidence levels for internal branches, as defined by the MEGA program. Discrimination of closely related species and subspecies was done according to the sequences of 16S rRNA gene of the similar overseas strains of *Bacillus sporothermodurans, B. thuringiensis, B. safensis, B. sphaericus B. tequilens, B. licheniformis, B. vietnamensis, B. amyloliquefaciens, B. subtilis, Pseudomonas psychrotolerans, Staphylococcus cohnii* and *S. saprophyticus*.

RESULTS

Enumeration of Isolates and Phenotypic Properties

The culturable microbial counts of the total bacteria, lactobacilli, lactococci, total yeasts and molds, staphylococci and micrococci, coliform, *Enterobacterieceae*, pseudomonads and enterococci populations isolated from yogurt, Laban Rayeb and Boza samples from the four districts in Sharkia Governorate are shown in Tables 4, 5 and 6. Total bacterial counts in the sixty fermented food samples on PCA agar varied in their range from 6.70 to 9.23 log cfu/ml. The total bacterial counts in 24/24/12 yogurt, Laban Rayeb and Boza samples, respectively, ranged from 8.20 to 8.30, 8.19 to 9.23 and 6.70 to 8.19 log cfu/ml, respectively.

The total lactobacilli counts in 60 samples of yogurt, Laban Rayeb and Boza samples ranged from 6.09 to 9.14, 8.16 to 9.06 and 7.16 to 9.24 log cfu/ml, respectively. The total lactococci counts in yogurt, Laban Rayeb and Boza samples were ranged from 6.22 to 9.17, 7.07 to 9.14 and 7.15 to 9.16 log cfu/ml, respectively. The total staphylococci and micrococci counts in 60 samples were ranged from 5.92 to 7.17, 6.20 to 8.08 and 6.11 to 8.06 log cfu/ml, respectively.

The coliform counts in the samples were ranged from 6.02 to 6.95, 6.96 to 8.03 and 5.15 to 9.15 log cfu/ml, respectively. The total *Enterobacreriaceae* counts were ranged from 5.91 to 7.20, 6.27 to 7.13 and 5.15 to 9.15 log cfu/ml, respectively in the 60 samples. The total Pseudomonads counts were ranged from 5.99 to 7.00, 6.27 to 7.13 and 6.07 to 8.01 log cfu/ml, respectively in the collected samples. The total enterococci counts in the samples of yogurt, Laban Rayeb and Boza were ranged from 5.10 to 7.00, 6.15 to 7.09 and 5.87 to 6.96 log cfu/ml, respectively. The total yeasts and molds counts

Table 3. Oligonucleotide primers and PCR reactions used in this study

Primer	Oligonucleotide sequence (5'–3')	Composition of 50-µl reaction[1]	PCR cycle	Target (product size)	Reference
27F	AGAGTTTGGATCMTGGC TCAG	5.0 µl of 10× buffer, 2 µl of dNTP mix, 2 µl of each primer, 200 ng of DNA, 2.5 U of Taq	94°C/1 min; 58°C/1 min; 72°C/2 min; 30 cycles*	16S rRNA gene (1,500 bp	**Weisburg *et al.* (1991)**
1492R	CGGTTACCTTGTTACGA CTT				

1 Contained 10 p*M* each primer; 10 m*M* dNTP mix (Takara Bio-Company, Shiga, Japan), Taq DNA polymerase (Takara Bio-Company).
*Initial denaturation at 94°C for 3 min and final extension of the amplified product at 72°C for 10 min.

Table 4. Microbiological analyses of yogurt samples purchased from both different districts and shops at Sharkia Governorate

Sampling location	Sample numbers and types			Mean bacterial counts	SE
	*A (n=2)	B(n=2)	C(n=2)		
			Total bacterial count (PCA)		
Zagazig	8,00	8,19	8,41	8,20	0,17
Faqous	8,00	8,28	8,40	8,23	0,17
Abu Kebir	8,28	8,45	8,00	8,24	0,18
El Husseiniya	8,20	8,30	8,41	8,31	0,09
			Total lactobacilli (MRS)		
Zagazig	6,11	5,85	6,32	6,09	0,20
Faqous	8,08	8,20	8,28	8,19	0,08
Abu Kebir	7,48	8,08	8,31	7,95	0,35
El Husseiniya	8,78	9,23	9,40	9,14	0,26
			Total lactococci (M17)		
Zagazig	6,85	7,11	7,32	7,09	0,20
Faqous	6,28	6,08	6,30	6,22	0,10
Abu Kebir	8,95	9,20	9,35	9,17	0,16
El Husseiniya	8,00	7,60	8,28	7,96	0,28
			Total yeasts and molds (RBCA)		
Zagazig	5,65	5,75	5,77	5,72	0,05
Faqous	5,53	5,76	5,65	5,65	0,09
Abu Kebir	5,83	5,58	5,83	5,74	0,12
El Husseiniya	6,65	6,81	6,94	6,80	0,12
			Total micrococci and staphylococci (MSA)		
Zagazig	6,04	5,54	6,18	5,92	0,27
Faqous	7,20	7,23	7,30	7,25	0,04
Abu Kebir	5,78	6,04	6,20	6,01	0,18
El Husseiniya	7,00	7,20	7,30	7,17	0,13
			Total coliform (VRBA)		
Zagazig	5,82	6,26	6,00	6,02	0,18
Faqous	6,95	7,28	6,60	6,95	0,28
Abu Kebir	6,60	7,00	7,19	6,93	0,25
El Husseiniya	5,95	6,18	6,32	6,15	0,15
			Total enterobacteriaceae (VRBGA)		
Zagazig	5,98	6,23	6,30	6,17	0,14
Faqous	5,70	6,00	6,20	5,97	0,21
Abu Kebir	7,00	7,22	7,37	7,20	0,15
El Husseiniya	5,60	6,00	6,15	5,91	0,23
			Total pseudomonads (PAB)		
Zagazig	5,74	6,00	6,23	5,99	0,20
Faqous	6,78	7,05	7,19	7,00	0,17
Abu Kebir	5,70	6,20	6,30	6,07	0,26
El Husseiniya	6,70	7,00	7,08	6,92	0,16
			Total enterococci (SBA)		
Zagazig	7,00	6,60	7,28	6,96	0,28
Faqous	7,00	7,12	6,88	7,00	0,10
Abu Kebir	4,85	5,15	5,31	5,10	0,19
El Husseiniya	5,78	6,11	6,30	6,06	0,22

*A, B, C = replicates of samples, SE= Standard error.

Table 5. Microbiological analyses of Rayeb milk samples purchased from both different districts and shops at Sharkia Governorate

Sampling location	Sample numbers and types			Mean bacterial counts	SE
	A (n=2)	B (n=2)	C (n=2)		
			Total bacterial count (PCA)		
Zagazig	7,95	8,20	8,40	8,19	0,18
Faqous	9,28	9,00	9,41	9,23	0,17
Abu Kebir	8,00	8,20	8,36	8,19	0,15
El Husseiniya	8,30	8,02	8,40	8,24	0,16
			Total lactobacilli (MRS)		
Zagazig	8,90	8,20	8,28	8,46	0,31
Faqous	8,19	8,26	8,36	8,27	0,07
Abu Kebir	9,03	9,15	9,00	9,06	0,06
El Husseiniya	8,02	8,16	8,29	8,16	0,11
			Total lactococci (M17)		
Zagazig	7,95	8,04	8,30	8,10	0,15
Faqous	7,28	7,11	7,32	7,24	0,09
Abu Kebir	6,85	7,04	7,31	7,07	0,19
El Husseiniya	9,00	9,13	9,28	9,14	0,11
			Total yeasts and molds (RBCA)		
Zagazig	5,88	5,75	5,94	5,86	0,08
Faqous	5,69	5,43	5,68	5,60	0,12
Abu Kebir	6,52	6,57	6,58	6,56	0,03
El Husseiniya	5,43	5,88	5,45	5,59	0,21
			Total micrococci and staphylococci (MSA)		
Zagazig	6,60	7,08	7,28	6,99	0,28
Faqous	7,15	6,95	7,18	7,09	0,10
Abu Kebir	7,95	8,02	8,28	8,08	0,14
El Husseiniya	6,23	6,08	6,30	6,20	0,09
			Total coliform (VRBA)		
Zagazig	7,78	8,00	8,30	8,03	0,21
Faqous	7,11	7,00	7,20	7,11	0,08
Abu Kebir	6,48	7,08	7,32	6,96	0,35
El Husseiniya	7,15	7,26	7,31	7,24	0,07
			Total enterobacteriaceae (VRBGA)		
Zagazig	6,70	7,00	7,28	6,99	0,24
Faqous	6,29	6,26	6,28	6,27	0,01
Abu Kebir	6,90	7,15	7,30	7,12	0,16
El Husseiniya	7,21	7,00	7,18	7,13	0,09
			Total pseudomonads (PAB)		
Zagazig	5,85	6,11	6,30	6,09	0,19
Faqous	7,15	6,00	6,11	6,42	0,52
Abu Kebir	5,95	6,15	6,30	6,13	0,14
El Husseiniya	6,24	6,23	6,40	6,29	0,08
			Total enterococci (SBA)		
Zagazig	5,95	6,20	6,30	6,15	0,15
Faqous	6,20	6,01	6,23	6,15	0,10
Abu Kebir	6,74	7,20	7,32	7,09	0,25
El Husseiniya	6,26	6,15	6,36	6,25	0,09

*A, B, C = replicates of samples , SE= Standard error.

Table 6. Microbiological analyses of Boza samples purchased from both different districts and shops at Sharkia Governorate

Sampling location	Sample numbers and types			Mean bacterial counts	SE
	A (n=1)	B (n=1)	C (n=1)		
			Total bacterial count (PCA)		
Zagazig	8,28	8,00	8,31	8,20	0,14
Faqous	7,48	7,28	7,30	7,35	0,09
Abu Kebir	7,30	6,43	6,38	6,70	0,42
El Husseiniya	7,20	7,04	7,45	7,23	0,17
			Total lactobacilli (MRS)		
Zagazig	8,04	8,20	8,32	8,19	0,12
Faqous	8,30	8,28	8,40	8,33	0,05
Abu Kebir	9,18	9,30	9,26	9,24	0,05
El Husseiniya	7,08	7,24	7,16	7,16	0,07
			Total lactococci (M17)		
Zagazig	9,18	9,00	9,30	9,16	0,12
Faqous	8,30	8,11	7,98	8,13	0,13
Abu Kebir	7,95	8,18	8,32	8,15	0,15
El Husseiniya	6,94	7,11	7,41	7,16	0,19
			Total yeasts and molds (RBCA)		
Zagazig	5,57	5,86	5,59	5,67	0,13
Faqous	5,80	5,57	5,58	5,65	0,11
Abu Kebir	5,41	5,68	5,59	5,56	0,11
El Husseiniya	5,86	5,92	5,97	5,92	0,04
			Total micrococci and staphylococci (MSA)		
Zagazig	6,91	7,11	7,32	7,11	0,17
Faqous	6,11	5,95	6,26	6,11	0,12
Abu Kebir	6,78	7,02	5,90	6,57	0,48
El Husseiniya	7,95	7,85	8,38	8,06	0,23
			Total coliform (VRBA)		
Zagazig	5,85	6,08	6,30	6,08	0,19
Faqous	5,25	4,90	5,30	5,15	0,18
Abu Kebir	7,01	6,95	7,30	7,09	0,15
El Husseiniya	9,08	8,95	9,41	9,15	0,19
			Total enterobacteriaceae (VRBGA)		
Zagazig	7,70	7,95	7,96	7,87	0,12
Faqous	6,96	6,76	7,30	7,01	0,22
Abu Kebir	5,60	6,00	6,18	5,93	0,24
El Husseiniya	6,91	7,18	7,34	7,14	0,18
			Total pseudomonads (PAB)		
Zagazig	7,60	8,11	8,32	8,01	0,30
Faqous	6,95	7,30	7,15	7,13	0,14
Abu Kebir	5,70	6,11	6,30	6,04	0,25
El Husseiniya	5,88	6,00	6,32	6,07	0,19
			Total enterococci (SBA)		
Zagazig	5,54	5,95	6,11	5,87	0,24
Faqous	6,60	7,00	7,28	6,96	0,28
Abu Kebir	6,60	7,00	7,28	6,96	0,28
El Husseiniya	5,56	5,94	6,20	5,90	0,27

*A, B, C = replicates of samples, SE= Standard error.

in 24 yogurt, 24 Laban Rayeb and 12 Boza samples were ranged from 5.65 to 6.80, 5.59 to 6.56 and 5.56 to 5.92 log cfu/ml, respectively.

Gram-positive and catalase-positive bacteria grown on MRS and M17 agar were considered as presumptive non-lactic acid bacteria. In total, 31 presumptive non-lactic acid bacteria were isolated from 60 fermented food samples. The majority of isolated strains were rod-shaped bacteria and cocci bacteria were 31 isolates. Twenty Five isolates in total, with 20 isolates were from MRS and 5 from M17 medium and the remainders were cocci and short rods (6 isolates in total with 4 from MRS and 2 from M17 medium). All the isolates were non-lactose fermented rods or cocci, and these representative strains showed some different characteristics at the different growth conditions (data not shown).

16S rRNA gene sequences analysis

The partial 16S rRNA gene sequences (1,500 bp) of all the strains (n= 31) were determined. Then, the sequences were compared with related bacteria in GenBank and sequence similarities were determined using the BLAST program. According to the 16S rRNA gene sequences, all strains were divided into three groups: *Bacillus*, *Staphylococcus* and *Pseudomonas*. The GenBank accession numbers for the 16S rRNA gene sequences of 25 strains of *Bacillus* were *Bacillus* sp., (22.58%) *B. sporothermodurans* (19.35%), *B. subtilis* (9.68%), *B. amyloliquefaciens* (9.68%), *B. safensis* (3.23%), *B. tequilensis* (3.23%), *B. sphaericus* (3.23%), *B. thuringiensis*(3.23%), *B. vietnamensis* (3.23%) and *B. licheniformis* (3.23%) . The second group was 4 *Pseudomonas* sp. (12.90%) and the third group was 2 *Staphylococcus, S. saprophyticus* (3.23%) and *S. cohnii* (3.23%). The similarity of these strains with the accession number of the same strains in GenBank were KT350416.1 (94%), KY243891.1 (99%), KX214616.1(98%), AB813716.1 (93%), KM823957.1 (99%), EU024822.1 (96%), KJ210650.1 (81%), JF414770.1 (97%), DQ860128.1 (100%), NR_026010.1 (83%), KJ009395.1 (89%), KF597277.1 (98), KU922160.1 (77%), KX681800.1(85%), KY499462.1 (89%), HM107808.1 (95%), NR_026010.1 (88%), KU291379.1 (100%), NR_026010.1 (85%), KF597277.1 (98%), KF597277.1 (97%), KF597277.1 (97%), DQ402043.1 (88%), KT008284.1 (96%), NR_026010.1 (81%), EU430991.1 (92%), MF590152.1 (96%), HM195191.1 (99%), AY960700.1 (79%), KX982769.1 (80%), NR_026010.1 (81%), respectively (Table 7).

DISCUSSION

The fermented-milk and cereal samples analyzed in this study were produced using traditional and ancient methods of natural fermentation. These products vary depending on their quality, taste, ingredients, and production methods, which are unique among regions, cities, and even families. Therefore, various beneficial microorganisms are present in different fermented milk products. Sixty samples were analyzed to determine their non-lactic acid bacteria composition, with the aim of improving our understanding of specific characteristics of fermented milk products in Egypt.

Slight variations of the viable counts of total bacteria and lactic acid bacteria were observed among fermented products of milk and cereals. Also, there is a limited variation of the staphylococci and micrococci (MSA), *Enterobacteriaceae* (VRBGA), coliforms (VRBA), pseudomonads (PABA) and enterococci (SBA) counts were observed among fermented products.

The mean numbers of total bacterial and lactic acid bacteria counts in fermented milk were almost identical and were lower than those in Boza samples but these numbers in yogurt were slightly lower than those in Laban Rayeb samples. These findings are consistent with the results of previous studies. **Ombarak *et al.* (2016)** found that raw milk, Karish cheese, and Ras cheese are highly contaminated with *E. coli*, including potentially pathogenic strains which may pose a public health threat. Raw milk was considered at high risk of causing illnesses, based on the latest surveillance data published by the Center of Disease Control and Prevention **(Langer *et al*., 2012).** Notably, differences in the timing of milk ripening and transport or differences in sampling regions could lead to variations in all tested bacterial counts. M17 and MRS media are used routinely for the isolation and counting of lactic acid bacteria from most fermented food products.

Table 7. *Bacillus, Staphylococcus* and *Pseudomonas* strains isolated from fermented food samples

Source	ID sample	Name of strain	Accessions number	Identity (%)
	18ACZAB080	*Bacillus* sp. MJAU D0006	KT350416.1	94
	18ACZAB096	*Bacillus* sp. DP4-80-110	KY243891.1	99
	18ACZAB107	*Bacillus* sp. Y-6	KX214616.1	98
	18ACZAB100	*Bacillus amyloliquefaciens*	AB813716.1	93
	18ACZAB111	*Bacillus amyloliquefaciens* IC1 .	KM823957.1	99
	18ACZAB104	*Bacillus subtilis* strain MJP1	EU024822.1	96
Yogurt	18ACZAB085	*Bacillus safensis* WB-260	KJ210650.1	81
	18ACZAB087	*Bacillus tequilensis* BPRIST017	JF414770.1	97
	`18ACZAB120	*Bacillus sphaericus* Bs13b	DQ860128.1	100
	18ACZAB097	*Bacillus sporothermodurans* strain M215	NR_026010.1	83
	18ACZAB093	*Staphylococcus saprophyticus*	KJ009395.1	89
	18ACZAB051	*Pseudomonas* sp. Snoq 117.2	KF597277.1	98
	18ACZAB117	*Staphylococcus cohnii*	KU922160.1	77
	18ACZAB114	*Bacillus* sp. CEB10	KX681800.1	85
	18ACZAB123	*Bacillus* sp. j5	KY499462.1	89
	18ACZAB122	*Bacillus amyloliquefaciens* 15	HM107808.1	95
Rayeb milk	18ACZAB092	*Bacillus sporothermodurans* M215	NR_026010.1	88
	18ACZAB047	*Bacillus thuringiensis* YJB4	KU291379.1	100
	18ACZAB082	*Bacillus sporothermodurans* strain M215	NR_026010.1	85
	18ACZAB038	*Pseudomonas* sp. Snoq 117.2	KF597277.1	98
	18ACZAB042	*Pseudomonas* sp. Snoq 117.2	KF597277.1	97
	18ACZAB048	*Pseudomonas* sp. Snoq 117.2	KF597277.1	97
	18ACZAB108	*Bacillus subtilis* strain EJH-1	DQ402043.1	88
	18ACZAB110	*Bacillus* sp. S113	KT008284.1	96
	18ACZAB079	*Bacillus sporothermodurans* M215	NR_026010.1	81
	18ACZAB081	*Bacillus sporothermodurans* 19	EU430991.1	92
Boza	18ACZAB105	*Bacillus subtilis* strain AU04	MF590152.1	96
	18ACZAB106	*Bacillus subtilis* KC3	HM195191.1	99
	18ACZAB089	*Bacillus licheniformis* GA1-17	AY960700.1	79
	18ACZAB099	*Bacillus vietnamensis* UMBR 3071	KX982769.1	80
	18ACZAB094	*Bacillus sporothermodurans* M215	NR_026010.1	92

Although described as a medium for the cultivation of lactobacilli, MRS has exhibited poor selectivity for lactobacilli **(Ouadghiri *et al.*, 2005).** The MRS and M17 media used in this study allowed the isolation of identified species found in fermented dairy products tested, such as *Bacillus* sp. *B. sporothermodurans, B. thuringiensis, B. safensis, B.sphaericus B. tequilens, B. licheniformis, B. vietnamensis, B. amyloliquefaciens, B. subtilis, Pseudomonas psychrotolerans, Staphylococcus cohnii* and *S. saprophyticus*. **Yu *et al.* (2011)** reported that more lactic acid bacteria isolates were obtained from MRS media (59.6%) than from M17 agar (40.4%), and the majority of lactobacilli (77.1%) were isolated from MRS media, whereas the majority of cocci (58.9%) were isolated from M17 media. Similar to this experimental results, a prior study of LAB in yogurt demonstrated MRS as the medium of choice for differential counting of lactobacilli and M17 as the preferred medium for counting cocci **(Coeuret *et al.*, 2003).**

In addition, a few other isolates were classified as *P. psychrotolerans, S. cohnii* and *S.saprophyticus*. Raw milk and cheese made from raw milk can be a major source of potentially harmful bacteria to human, such as pathogenic *E. coli* **(Oliver *et al.*, 2005)**. Consequently, foodborne disease outbreaks from consumption of raw milk and raw-milk products resulted in public health hazard over all the world **(De Buyser *et al.*, 2001; Oliver *et al.*, 2005).** *P. fluorescens* and *P. fluorescent* group-related strains predominated (*ca* 86%) in the Gram-negative psychrotrophic microflora were found in fermented foods **(Giacometti *et al.*, 2012).** The presence of *Pseudomonas* spp., *Staphylococcus* spp. and *Bacillus* spp. with such variation of strains are not surprising because other factors may contribute to the variability of strains, such as different production methods, recipes, and raw materials. Furthermore, the environmental temperature and location differences may also cause some variation in strains.

Traditional microbiological assays are often time-consuming and can yield variable results for the identification of closely related lactic acid bacteria and non-lactic acid bacteria **(Ercolini, 2004).** The sequencing and analysis of 16S rRNA genes is considered a cornerstone of modern microbial taxonomy **(Ventura and Zink, 2002).** For this reason, all isolates from MRS and M17 media as well as catalase-positive in this study were grouped and preliminarily identified based on their 16S rRNA gene sequences. The results indicated that 16S rRNA gene sequences analysis could discriminate them at the species level in the *B. sporothermodurans, B. thuringiensis, B. safensis, B. sphaericus B. tequilens, B. licheniformis, B. vietnamensis, B. amyloliquefaciens* and *B. subtilis*. In previous studies, the fermentation of melon, African oil bean seed and locust bean into ogiri , ugbaand iru, respectively is predominantly by *Bacillus* species **(Olasupo *et al.*, 2016).** Other bacteria species that can be isolated during the fermentation process are *Staphylococcus*, *Escherichia*, *Micrococcus*, *Leuconostoc*, *Pseudomonas* and *Corynebacterium* **(Achi *et al.*, 2007).**

This study describes a microbiological approach for detailed identification of non-lactic acid bacteria involved in natural fermented milk and cereals. *Staphylococcus*, *Bacillus, Enterococcus, Escherichia* and *Pseudomonas* are the dominant contaminated genera in fermented products in Egypt. Other fungal genera, including *Saccharomyces* and *Candida* were identified at lower frequencies. The genera distribution depends on the manufacturing processes, as well as on the specific ecological locality where the milk products were manufactured.

Conclusion

The results obtained in this study indicate that fermented food such as yogurt, Laban Rayeb and Boza may be contaminated with zoonotic strains of pathogenic bacteria. The presence of high populations of staphylococci and micrococci, *Enterobacteriaceae*, coliform, pseudomonads, enterococci counts in these food products points out a potential risk of human infection in Egypt. Continued surveillance of emerging pathogens is required for fermented foods. The results obtained within the frame of this study indicate the need for adoption of hygienic practices by food handlers to minimize the risks of transmission of foodborne pathogens through fermented foods. Efforts must be

employed to ensure that this kind of foods does not become contaminated before final packaging. The expiration dates of the products must accurately reflect the shelf life of products and refrigeration storage temperatures should be taken into account.

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تلوث الأغــذية المخمرة في مصــر ببكتيريا غيــر مرغـوبة

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قيمت هذه الدراسة سلامة وجودة بعض الأغذية المخمرة في مصر، فقد تم تجميع أغذية مخمرة مختلفة: اللبن المخمر (الزبادي)، اللبن الحامض (لبن رايب)، دقيق الشعير المخمر (بوزا أو بوازا) من مدن محافظة الشرقية (الزقازيق، فاقوس، أبو كبير والحسينية)، مصر، تم تحديد مجموع التنوع البكتيري ليشمل بكتيريا حمض اللاكتيك، الخمائر والفطريات، المكورات العنقودية والمكورات الدقيقة، القولون، البكتيريا المعوية، الكاذبة والعدوى المعوية داخل كل عينة كذلك تم تحديد هوية العزلات السائدة من خلال التقنيات المجهرية والبيوكيميائية والجزيئية، كانت تتراوح أعدد البكتيريا الكلية وبكتيريا حامض اللاكتيك في العينات التي تم تحليلها من ٦,٧٠ إلى ٩,٢٣ لوغارتم مستعمرة/مل و ٦,٠٩ إلى ٩,٢٤ لوغارتم مستعمرة/مل على التوالي، وقد أظهرت النتائج أن أعلي الاحمال في أعداد البكتيريا الكلية في الزبادي واللبن الرايب و البوزا كانت ٨,٣٠، ٩,٢٣ و٨,١٩ لوغارتم مستعمره/مل على التوالي، وعموما كانت العزلات البكتيرية غير المرغوبة والمعزولة من علي بيئات بكتيريا حامض الاكتيك تنتمي إلى البكتيريا العصوية الطويلة والمكورات العنقودية وبسيودوموناس العصوية القصيرة وكانت أجناس الخمائر تنتمي إلى الساركارواميسيس والكنديدا، وكانت الأنواع البكتيرية الأبرز التي تم تحديدها من خلال تحليل ال 16S rRNA هي: *B. sporothermodurans, B. thuringiensis, B. safensis, B. sphaericus, B. tequilens, B. licheniformis, B. vietnamensis, B. amyloliquefaciens , B. subtilis, P. psychrotolerans,* *S. cohnii* and *S. saprophyticus*. وأظهرت هذه الدراسة وجود البكتيريا غير المرغوبة والملوثة في الأغذية المخمرة ويمكن أن تكون هذه البكتيريا مسببة للأمراض أو توكسجينيك عن طريق إنتاج أنواع مختلفة من السموم.

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