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Isolation and Identification of Nonstarter Lactic Acid Bacteria from Traditional Baramily Cheese

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Abstract

Nonstarter lactic acid bacteria (NSLAB) have an important role in quality and safety of traditional Baramily cheese (Domiati cheese related type). Therefore, the objective of this study was to isolate and identify NSLAB with potential technological features from traditional Baramily cheese. Thirty-three samples of Baramily cheese randomly collected from retails in Cairo metropolitan area. The samples were characterized by physiochemical, textural profile and microbiological analysis. Ninety presumptive NSLAB (30 *Lactobacillus spp*. and 35 *Enterococcus spp*.) strains were isolated on MRS and Kenner-Faecal (KF) Streptococci media; and were characterized for growth temperature, salt tolerance and milk coagulation. All presumptive NSLAB isolates were tolerant to 6.5 % NaCl. Of them, 40 isolates were tolerant to 10.0 % NaCl including 16 presumptive *Lactobacillus spp*., and 24 presumptive *Enterococcus spp*. isolates. based on the results, 11 representative isolates with potential technological features were selected for genetic identification using 16S rRNA technique, then were confirmed for growth and acidity development in skim milk within 48 h, and were tested for antimicrobial activity against some food spoilage and pathogenic microorganisms. The eleven isolates were identified as *Ent. durans* (1), *Ent. faecalis* (5), *Lb. paraplantarum* (1), *Lb. plantarum* (3), and *Lb. rhamnosus* (1). All isolated strains were confirmed active in skim milk, and some exhibited antimicrobial activity against tested food spoilage and pathogenic microorganisms. Both *Lb. rhamnosus* and *Lb. plantarum* were confirmed as the isolates with high activity in milk. *Ent. durans* and *Ent. faecalis* exhibited antimicrobial activity against *Enterobacer aerogenes* and *E. coli*. However, *Lb. plantarum* exhibited antimicrobial activity against both *Enterobacer aerogenes*, *E. coli* and *Ps. aeruginosa*. *Listeria. monocytogenes*, *S. typhimurium*, and *Campylobacter. jejuni* showed significant resistance to all tested NSLAB isolates. They can be concluded that the identified NSLAB isolated can be used to standardized and improve the quality and safety of Baramily cheese and other types related to Domiati cheese.

Keywords: Baramily; Cheese; NSLAB; Isolation; Identification.

1 Introduction

Lactic acid bacteria (LAB) have an essential role in the quality and safety of cheese and fermented food (Bylund 1995). They are used in cheese production as starter cultures, and adjunct cultures. They may present in cheese as contaminants from processing equipment environment and personnel. Nonstarter lactic acid bacteria (NSLAB) include four main

groups of bacteria: mesophilic lactobacilli, Pediococci, Enterococci and *Leuconostoc*, which are not part of cheese cultures (Casey et al 2006). Most artisanal and traditional cheeses contain at least one of these four groups, depending on cheese variety, processing steps and ripening conditions (Beresford 2003). NSLAB can dominate the cheese microflora and contribute to the quality and safety of the ripened cheese. Also, NSLAB havean important protective role against food pathogens and spoilage bacteria through the production of different metabolites including bacteriocins, organic acids, acetaldehyde, diacetyl and hydrogen peroxide (Essid et al 2009). The facultatively heterofermentative species of Lactobacilli including *Lb. plantarum*, *Lb. casei*, *L.paracasei*, *Lb. rhamnosus* and *Lb. pentosus* are frequently isolated from cheese Settanni and Moschetti (2010). Also, the genus *Enterococcus*, especially *Ent. durans, Ent. faecium* and *Ent. faecalis* were frequently isolated and identified from many types of traditional cheese manufactured from raw and pasteurized milk Cogan et al (2007).

Domiati cheese is a popular Egyptian soft white cheese. Sodium chloride is normally added to the milk before renneting in variable large quantities depending on milk quality and season. Tallaga (refrigerated in Arabic), Baramily (barrel in Arabic) and Double Cream are cheese types closely related to Domiati cheese. They mainly matured and stored in metal tins without brine under refrigeration for up to 3 months before consumption. These cheese types are manufactured with lower salt, rennet and renneting temperature lower than those of traditional Domiati cheese. The ripened cheeses have clean acid flavor and smooth creamy body and texture. NSLAB have been isolated and identified from Domiati cheese including *Ent. faecalis, Ent. faecium and Lb. plantarum* (El-Zayat et al 1995). During the late aging stages of Domiati cheese, *Lactobacillus spp*. become dominant (Shehata et al 1984). The isolated cocci strains from ripened *Domiati* cheese are usually salt-tolerant Enterococci (Hematiet al 1998).

Isolation and screening of LAB from natural environments is are the most effective strategy to select LAB strains with potential features to improve cultures used in the production of cheese and fermented milk (Ibourahema et al 2008). Therefore, the objective of this research was to isolate, characterize and identify NSLAB with potential technological features from traditional *Baramily* cheese.

2 Materials and and Methods

2.1 Cheese sampling

Thirty-three samples of each ripened and fresh traditional *Baramily* cheese were randomly collected from retails in Cairo metropolitan area. The samples were handled and kept refrigerated (3^{o}C) until analysis.

2.2 Physicochemical analysis

The pH value of cheese samples was determined by mixing25 g cheese with distilled water at a ratio of 1:1 in stomacher (Steward 400, UK) before measuring by a pH meter (Rehman and Fox 2002). Fat content was determined using Gerber method as described by (IDF 1997) and protein content was determined using Kjeldahl method (Fox et al 2000). Salt content was determined using Mohr method (**IDF 1988**) and Total solids were determined according to AOAC (2012).

2.3 Texture profile analysis

Quadrilateral samples were prepared with dimensions of 10x10x10 mm. Cheese cubes were cut from a deep of 12 mm of surface to eliminate the effect of surface dryness. Textural profile including hardness, cohesiveness, springiness, adhesiveness, and chewiness were determined with a Texture Analyzer (model TMS-Pro, Food Tech. Co., USA). A two-bite penetration test was performed with TA60 degree cone and Perspex probe for cheese operated at a crosshead speed 50 mm/sec. Calculations described by Bourne (2002) were used to obtain the texture profile parameters.

2.4 Microbiological analysis

Using sterile cheese triers, 10 g cheese samples were cut under aseptic conditions, were decimally diluted in sodium citrate solution (2%), then, were homogenized in a stomacher (Steward 400, UK) at normal speed for 2 min. Further dilutions were decimally prepared with Maximum Recovery Dilutant (MRD: 8.5 g Na cl and 1g peptone / l). Dilutions were plated on specific media to count, detect and isolate target microorganisms. Lactobacilli and Enterococci were enumerated on MRS and KF Streptococci agar (Oxoid) with 2,3,5-Triphenyltetrazolium chloride 1% (TTC) as a supplement and incubated at 37°C and 42°C for 24-48 h, respectively (Pisano et al 2006). Total mesophilic bacterial counts were determined on plate count agar (Oxoid) incubated at 30°C for 72 h (ISO 4833-1:2013). Coliform bacteria were enumerated on Violet Red Bile Lactose (VRBL) agar (Oxoid) incubated at 37 °C for 22 to 26 h (ISO 4832, 2006). Yeast and Molds were counted on Oxytetracycline Glucose Yeast Extract Agar (Oxoid) with incubation at 25 °C for 5 to 7 days according to ISO 21527-1 :(2008).

According to the method described by ISO 6888-1 (2003), *Staphylococcus aureus* was enumerated on Baird–Parker agar (Oxoid) with incubation at 37 °C for 24 h. In accordance with ISO 6579-1 (2017), *Salmonella spp*., was detected using Rappaport–Vassiliadis (RVS) broth (Oxoid) with incubation for 24 h at 41.5 °C. Then, plates of Xylose Lysine Deoxycholate (XLD) agar (Oxoid) were streaked RVS incubated broth, then, incubated for 24 h at 37 °C.

2.5 NSLAB isolation and preservation

Presumptive Enterococci isolation was performed on KF Streptococci agar (Oxoid) at pH 7.1, which incubated for 48 h at 42°C. Isolation of lactobacilli was performed on MRS agar (Oxoid) atpH 5.4, which incubated for48 h at 37°C in a carbon dioxide incubator (5%) according to Pisano et al (2006).

Ninety Presumptive NSLAB isolates (30 *Lactobacillus spp*. and 35 *Enterococcus spp*.) confirmed as pure gram positive and catalase negative cultures were preserved in 20% glycerol media (MRS or KF Streprococci) in ultra-freezer (-80 °C).

2.6 NSLAB characterization

The ninety presumptive NSLAB isolates were preliminary characterized for ability to coagulate milk, grow at different temperatures (10°C and 45°C) and salt tolerance (6.5 and 10% NaCl) in MRS and KF Streptococci broth media (OXOID) according to Whittenbury (1964) and Yavuzdurmaz (2007).

2.7 NSLAB identification

Eleven selected isolates with potential technological role in cheese quality and safety were identified using 16S rRNA sequence analysis methods by genomic as following:

1) DNA extraction was performed using genomic DNA purification kit (Thermo K0721, Gene Jet™).
2) PCR was performed using Maxima Hot Start PCR Master Mix (Thermo K1051).
3) PCR was clean up to the PCR product using GeneJET™ PCR Purification Kit (Thermo K0701).

PCR product sequencing was performed by ABI 3730xl DNA sequencer with forward and reverse primers (GATC Co., Germany).

2.8 Activity of identified NSLAB isolates in milk

Identified NSLAB isolates were assessed for activity in skim milk (growth as log cfu/ ml and pH reduction) at 37°Cfor 48 h.

2.9 Antimicrobial activity of identified NSLAB isolates

Antimicrobial activity of the identified NSLAB isolates was assayed in Cell-Free Filtrates (CFF) using the paper disc diffusion

method against some food spoilage and pathogenic microorganisms including *Enterobacter aerogenes* EMCC 30053, *E. coli* NRRL 3008, *L. monocytogenes* EMCC 19116, *S. typhimurium.* ATCC 25566, *Ps. aeruginosa* ATCC 27853, *C. jejuni* EMCC 6214 and *Staph. aureus* EMCC 6538 according to Albano et al (2007) and Buntin et al (2008). All indicator strains were obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2.10 Statistical analysis

The statistical analysis of treatment effects was calculated through a General Linear Model (GLM), separating the three replicates via Duncan's Multiple Range Test (MRT) at P≤0.05 (SAS, 2009).

3 Results and Discussions

3.1 Physiochemical analysis

Data in **(Table 1)** show the physiochemical composition of *Baramily* cheese samples. Total solids were 42.34 ±1.98 in fresh cheese samples while it were 46.05 ±0.65 in ripened samples. These results were complying with the limits required by the Egyptian standards (EOS, 2005), which should be 40% at minimum.

Fresh cheese samples contained 19.50 ±0.67% fat and ripened cheese samples contained 21.60 ±0.22 as a result of decreased moisture and increased total solids due to whey syneresis during the ripening period. The Egyptian standards do not set a limit for fat content, but a limit for fat in dry matter (60% at minimum), which was exceeded according to the determined fat and solids contents. These results were higher than results reported by Idris (1989) who found that fat content of Domiati cheese ranged from 14.0 to 15.31 with a mean of 14.46%.

In the same trends with total solids and fat content, protein content was 14.33 ±0.46 in fresh cheese while it was 17.17 ±0.15 in ripened cheese samples. These results were remarkedly above the minimum protein content required by Egyptian standards (EOS 2005), which should be 10% at least and the results reported by Abou-Dawood et al (2005) found that total protein of Domiati cheese ranged from 5.35 to 12.5 with a mean of 7.89%. However, the results compared to those reported by Dabiza et al (1999) who reported that TP of soft white cheese ranged from 17.7 to 20.5%. The variation in protein content depended on several factors such as milk composition, process efficiency, moisture content, acidity development and the rate of whey syneresis (Idris 1989).

Table 1. Physiochemical analysis of *Baramily* cheese samples.

Physiochemical parameter	Mean±SE[1]		Egyptian Standard[2]
	Fresh	Ripened	Limits %
Moisture (%)	57.66 ±1.98 a	53.95 ±0.65 b	60
Total Solids (%)	42.34 ±1.98 b	46.05 ±0.65 a	40
Fat (%)	19.50 ±0.67 b	21.60 ±0.22 a	--
Protein (%)	14.33 ±0.46 b	17.17 ±0.15 a	10
Salt (%)	09.15 ±0.29 a	07.24 ±0.16 b	9
pH	05.63 ±0.07 a	04.45 ±0.02 b	--

[1] SE: Standard Error. [2]Egyptians Standard: 3- 1008/2005. Data is a mean of 33 samples. Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

Contrary with solids, fat and protein, salt content was 09.15 ±0.29 in fresh samples while it was 07.24 ±0.16 in ripened samples. The ripened cheese complied with the salt limits required by the Egyptian standards (EOS 2005), which should not exceed 9%. These results were higher than the results reported by Abou-Dawood et al (2005) who found that salt content of *Domiati* cheese ranged from 3.0 to 6.0 with a mean of 4.2%, while they were lower than the results as reported by Hofi et al (1975) who found that salt content of Domiati cheese ranged from 16.26 to 20.97 with a mean of 18.22%. The early high salting level in Domiati cheese and related types acted as a preventive control for potential high microbial counts in milk and processing environment.

The mean value of pH in fresh cheese samples was 05.63 ±0.07, which was compared to the results reported by Idris (1989) who found that pH values of Domiati cheese ranged from 5.6 to 5.8 with a mean of 5.72. In the ripened cheese samples, pH fell to 04.45 ±0.02 due to the developed acidity by lactic microflora. This acidic pH was reported by Hofi et al (1975) who found that pH values of Domiati cheese ranged from 4.5 to 4.8 with a mean of 4.6. The relatively decreased pH of ripened cheese samples is attributed to cheese manufacturing from raw milk with high level of natural microflora.

3.2 Texture Profile analysis

The data in **(Table 2)** presents the texture profile analysis of fresh and ripened Baramily cheese samples. There were significant differences between fresh and Baramily cheese in all parameters including hardness (N), springiness (mm), adhesiveness (mj), cohesiveness and chewiness (mj). hardness (N), adhesiveness (mj) and cohesiveness significantly increased after cheese ripening. Contrary, both springiness (mm) and chewiness (mj) significantly decreased. The increase of hardness in ripened cheese may have been related to acid development and whey syneresis concomitant with the decrease in moisture during storage (Souza and Saad, 2009). Ahmed et al (2005) and Souza and Saad (2009) reported a gradual decrease in the adhesiveness values of traditional soft cheese during the storage period. They suggested these results were attributed to high fat value in cheese which was not sufficiently retained in the protein matrix. Contrary, the decrease in chewiness may have been related to proteolysis and lipolysis.

Table 2. Texture profile analysis of *Baramily* cheese

Texture Profile Parameters	Mean±SE[1]	
	Fresh	Ripened
Hardness (N)	10.95±0.87 b	15.93±0.1.01 a
Springiness (mm)	11.19±0.42 a	05.14±0.34 b
Adhesiveness (mj)	01.17±0.10 b	01.73±0.21 a
Cohesiveness	00.64±0.03 b	00.97±0.04 a
Chewiness (mj)	78.61±2.26 a	48.81±2.03 b

[1] SE: Standard Error. Data is a mean of 33 samples.
Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

3.3 Microbiological analysis

Traditional Domiati cheese and *Baramily* are still widely made of raw milk lacking compliance with Egyptian standards (EOS 2005), which require milk pasteurization before processing and distribution. As shown in **(Table 3)**, both fresh and ripened samples were highly populated with NSLAB, despite of a significant decrease was observed in ripened cheese. The high counts of NSLAB may be attributed to the half level salt added in *Baramily* cheese compared to Domiat cheese. The hygiene indicator microorganisms including total bacteria, coliform bacteria, and yeast & moulds counts were significantly high in fresh samples. Significant decreases were determined for all hygiene indicator microorganisms in ripened cheese samples. Moreover, coliform bacteria were eliminated. Regarding food pathogens, both *Staph. aureus* and *S. typhimurium* did not survive in ripened cheese samples. These results agreed to the results reported by Sayed et al (2011), who examined

Table 3. Microbial counts (log cfu/g) in *Baramily* cheese samples

Microorganisms	Mean±SE: log cfu/g [1]		Egyptian Standards Limits cfu/g or 25 g [2]
	Fresh	Ripened	
Lactobacillus. spp.	07.38 ±0.43 a	6.08b ±0.14 b	--
Enterococcus spp.	05.46 ±0.30 a	4.59±0.21 b	--
TBC	07.80 ±0.43 a	6.34 ±0.14 b	--
Total Coliform	04.98 ±0.35 a	0.00 b	10 cfu/g
Yeast and Moulds	04.27 ±0.50 a	2.85 ±0.16 b	400 cfu/g
Staph. aureus	02.39 ±0.01 a	0.00	0 cfu/g
Salmonella spp.	+	-	0 cfu/25g

[1] SE: Standard Error. [2] Egyptian Standards: 3- 1008/2005. Data is a mean of 33 samples.
Means in the same row with the same superscript letters are insignificantly different (P ≤0.05).

Domiati, Bramily, Fayomi and Tallaga cheese samples for aerobic plate count, thermoduric, psychrotrophs, *Enterococci*, coliforms, fecal coliforms, *E. coli*, *Staph. aureus*, yeasts and molds and anaerobes. They reported that microbiological counts exceeded the maximum limits set by the Egyptian Standards. These results suggested improving hygiene practices along milk production and processing chain with a cheese ripening period for 90 day as a control measure to reduce microbial food pathogens to acceptable levels.

3.4 NSLAB isolation and characterization

Ninety presumptive NSLAB isolates were confirmed as a gram-positive and catalase negative strains (35 presumptive *Lactobacillus spp*. and 55presumptive *Enterococcus spp*. isolates) as shown in **(Table 4)**.

All presumptive NSLAB isolates were tolerant to 6.5 % NaCl salt in MRS and KF Streptococci broth media. Among the presumptive NSLAB, 40 isolates were tolerant to 10.0 % NaCl salt including 16 presumptive *Lactobacillus spp*., and 24 presumptive *Enterococcus spp*. isolates.

NSLAB isolates exhibited variable ability to grow and develop acidity in milk. The results indicated that presumptive lactobacilli were fast acid producer (within 6 h) than presumptive Enterococci. With continued incubation to 24 h, the different isolates exhibited acidity development in milk. These results are in agreement with previous studies reported that isolated cocci strains from mature Domiati cheese were mainly salt-tolerant enterococci (Hemati et al 1998), but other microorganisms that have been found in the product including *Ent. faecalis, Ent. faecium and Lb. plantarum* (El-Zayat et al 1995).

3.5 NSLAB identification

Table 5 shows molecular identification of 11 NSLAB isolates at the species level based on partial sequence of 16S rRNA gene. They were *Ent.durans* (NSLAB 1), *Ent.faecalis* (NSLAB 2-6), *Lb. paraplantarum* (NSLAB 7), *Lb. plantarum* (NSLAB 8-10) and *Lb.rhamnosus* (NSLAB 11).

Isolated *Enterococcus* and *Lactobacillus* from cheese samples can grow in various environments such as acid, aw, salt and temperature. Therefore, it is logic to consider that NSLAB isolated from *Baramily* cheese is well adapted to cheese processing and ripening conditions. Subsequently, they dominant in final cheese product.

Enterococci, particularly *Ent. faecalis* and *Ent. Faecium* often found as NSLAB in different types of traditional cheeses manufactured from raw and pasteurized milk (Cogan et al 2007). The occurrence of *Enterococci* in cheese may be ascribed to inefficient pasteurization and post pasteurization contamination from unhygienic contact surfaces and environments (Robinson 1990). Food contamination

Table 4. Preliminary Characterization of presumptive NSLAB isolates from *Baramily* cheese

Presumptive NSLAB Isolates			Growth in 6.5 % Salt		Growth in 10 % Salt		Milk coagulation time (h)						Growth Temp. °C			
							6		12		24		10		45	
Media	Shape	No	No	%	No	%	No	%	No	%	No	%	No	%	No	%
MRS	Roads	35	35	100	16	40.45	7	20.00	14	40.00	14	40.00	7	20.00	2	0.06
KF Streptococci	Cocci	55	55	100	24	43.64	0	00.00	19	34.55	21	38.18	55	100	55	100

Table 5. Identification of NSLAB isolated from *Baramily* Cheese by 16S rRNA sequencing

NSLAB No.	Identification	Accession Number	Ref. Strain
1	*Ent. durans*	NR_113257	JCM 8725
2-6	*Ent. faecalis*	NR_113901	NBRC 100480
7	*Lb. paraplantarum*	NR_025447	DSM 10667
8-10	*Lb. plantarum*	NR_104573	CIP 103151
11	*Lb. rhamnosus*	NR_113332	NBRC 3425

with enterococci usually attributed to poor microbiological quality of ingredients and unhygienic environment (Franz et al 1999) despite of it has been proposed to use *Enterococci* as a culture for some cheese types (Garde et al 1997).

Cogan et al (2007) reported that dominant NSLAB in raw or pasteurized cheese are frequently facultative homofermentative lactobacilli, which mainly produce lactic acid from lactose. They mainly include *Lb. plantarum and Lb.casei*. Luiz et al (2016) isolated and identified *Lactobacillus*, *Enterococcus*, *Pediococcus*, and *Lactococcus* from Brazilian Minas artisanal cheese. Samelis et al (2010) investigated the secondary microflora of Graviera cheese and they reported that identified LAB species were *Lb. casei Lb. paracasei*, *Lb. plantarum, Ent. faecium, S. thermophilus*, and *Lc. Lactis.* Also, Wassie and Wassie (2016) identified a total of 83 LAB isolates belonged to six genera including *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Pediococcus* and *Entrococcus.*

3.6 Activity of NSLAB isolates in skim milk

Fig 1 illustrates the growth and acidity development (pH reduction) of *Ent. faecalis* and *Ent. durans* isolates in skim milk at 37°C for 48h. The results indicated that *Ent. faecalis* had higher and rapid growth and pH reduction than *Ent. durans*. *Ent. faecalis* had achieved a maximum count (9.89 log cfu/ml) at 24 h with reduced pH to 4.71. Declined growth associated with a final pH reduction to 4.58 was determined after 48h of incubation.

As illustrated in **(Fig 2)**, the maximum growth among all *Lactobacillus* isolates in skim milk at 32°C for 48 h was determined for *Lb. rhamnosus* within 12 h of incubation **(Fig 2- C)**. Also, the same isolate exhibited maximum ability to develop acidity and reduce skim milk pH to the minimum compared to other *L.* isolates. Also, *Lb. plantarum* proved a good ability to grow in skim milk and coagulate the milk after 12 h of incubation and had attained the stationary phase after 18h of incubation at 32°C. Whereas, *Lb. paraplantarum*

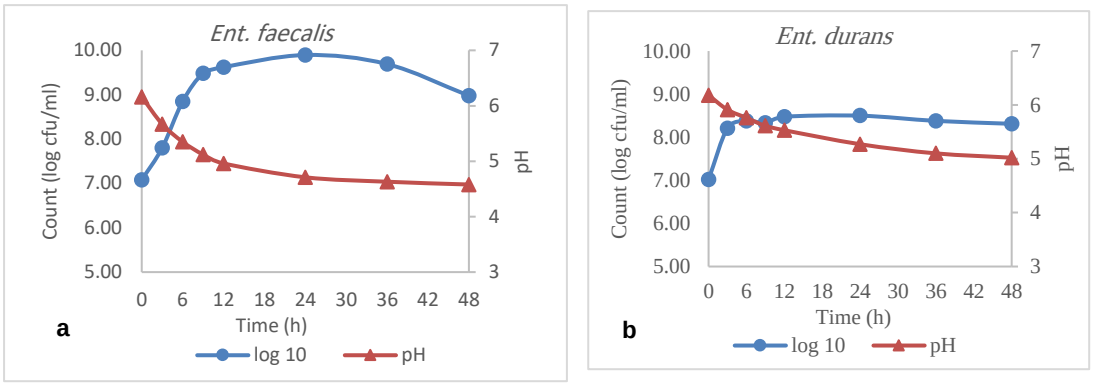

Fig 1. Growth and pH reduction of *Ent. faecalis* (a) and *Ent. durans* (b) isolates in skim milk at 37°C for 48h.

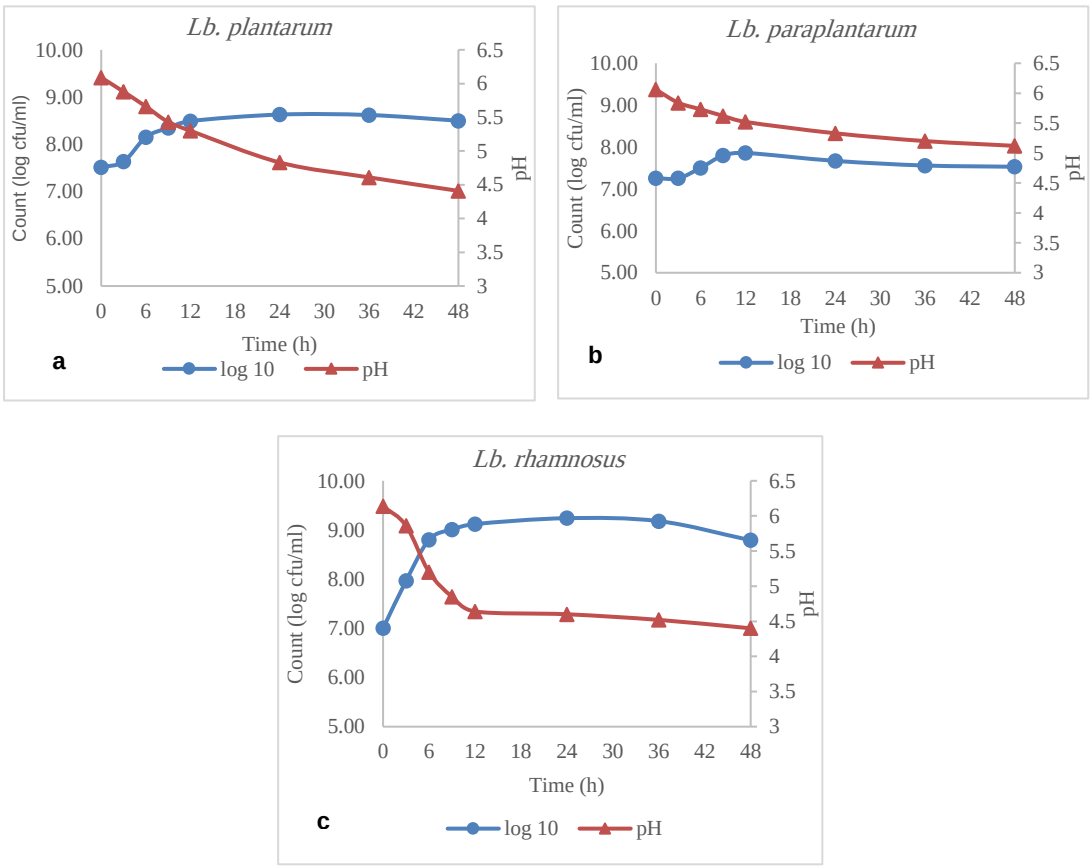

Fig 2. Growth and pH reduction of *Lb. plantarum* (a), *Lb. paraplantarum*(b) *and Lb rhamnosus*(c) isolates in skim milk at 32°C for 48h.

Table 6. Antimicrobial activity of identified NSLAB isolates against some hygiene indicator and pathogenic microorganisms

NSLAB Isolates	Inhibition Indicator Microorganisms						
	***Enterobacer aerogenes* DSM 30053**	***L. monocytogenes* ATCC 19116**	***S. typhimurium* ATCC 25566**	***E. coli* NRRL 3008**	***Ps. aeruginosa* ATCC 27853**	***Staph. aureus* ATCC 6538**	***C. jejuni* CCM 6214**
	Inhibition Zone						
Ent. Durans	+	ND[1]	ND	+	ND	ND	ND
Ent. Faecalis	+	ND	ND	+	ND	ND	ND
Lb. paraplantarum	ND	ND	ND	ND	ND	ND	ND
Lb. plantarum	+	ND	ND	+	+	ND	ND
Lb. rhamnosus	ND	ND	ND	+	+	+	ND

[1]ND: Non-Detected inhibition (zone dimeter less than 1 mm).

shows weak growth in skim milk. These results suggested that both *Lb. rhamnosus* and *Lb. plantarum* isolates had a relatively high activity in milk.

These results confirm that both *Enterococcus* and *Lactobacillus* may possibly affect the cheese quality and safety through associated growth and activities with the different microflora in cheese. This may partially result from different metabolic activities on nutrients in cheese due to different resistance to intrinsic factors including acidity, salt content, moisture and antimicrobial compounds. It is reported that using of *Ent. faecium* and a combination of mesophilic and thermophilic lactobacilli developed the characteristic flavor of Domiati cheese made from pasteurized milk (El-Soda and Abd El-Salam 2002).

3.7 Antimicrobial activity of NSALAB isolates

Table 6 shows that both *Ent. durans* and *Ent. faecalis* exhibited antimicrobial activity against tested *Enterobacer aerogenes* and *E. coli*, while, there was no antimicrobial activity against *Listeria monocytogenes*, *S. typhimurium*, *Ps. aeruginosa*, *Staph aureus* or *Campylobacter. jejuni*. Among the identified *Lactobacillus* isolates, *Lb. paraplantarum* did not show any antibacterial activity against all tested microorganisms, but *Lb. plantarum* exhibited antimicrobial activity against both *Ps. aeruginosa*, *Enterobacer aerogenes* and *E. coli*. Also, *Lb. rhamnosus* had antimicrobial activity against *Ps. aeruginosa*, *E. coli*, and *Staph. aureus*. Finally, *Listeria monocytogenes*, *S. typhimurium*, and *Campylobacter jejuni* showed resistance to all tested NSLAB isolates.

Our findings agreed to the results reported by many studies which confirmed the inhibiting activities of LAB against food pathogens and spoilage bacteria. Enterococci produce bacteriocins that inhibit some food pathogens including *Listeria monocytogenes, Staph. aureus, V. cholerae, Clostridium spp.* and *Bacillus spp.* (Giraffa 2003). The genus *Lactobacillus* exhibited a wide range of antimicrobial activity that associated with antimicrobial metabolites (Essid et al 2009). Therefore, it is reported that some *Lactobacillus* species can be used as adjunct cultures in cheese and fermented dairy products (Amin et al 2009).

4 Conclusions

Traditional *Baramily* cheese samples collected and characterized in this study complied with the Egyptian standards regarding the chemical composition. To ensure cheese

safety, it is suggested to control raw milk *Baramily* cheese by the 90-day ripening rule with hygiene control in milk production and cheese processing. Eleven isolates were identified as *Ent. Durans* (1), *Ent. faecalis* (5), *L. paraplantarum* (1), *L. plantarum* (3), and *L. rhamnosus* (1), whichallwere confirmed active in skim milk, and some exhibited antimicrobial activity against tested food spoilage and pathogenic microorganisms. Finally, our findings suggested that the identified NSLAB isolated can be utilized as adjunct cultures to standardized and improve the quality and safety of Baramily cheese and other related Domiati cheese types.

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عزل وتعريف بكتريا غير بادئ حمض اللاكتيك من الجبن البراميلي التقليدي

[23]

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الموجـــــــــز

تلعب بكتيريا غير بادئ حمض اللاكتيك (NSLAB) دورًا هاما في جودة وسلامة الجبن البراميلي التقليدي وهو من اصناف الجبن الدمياطى. لذلك كان الهدف من هذه الدراسة هو عزل وتعريفبكتيريا غير بادئ حمض اللاكتيكذات المميزات التكنولوجية المحتملة من الجبن البراميلي التقليدي حيث تم تجميع عدد 33عينة من الجبن البراميلى عشوائياً من متاجر التجزئة بمنطقة القاهرة الكبرى. وتم توصيف العينات بالتحليل الفيزيوكيميائي، والبنائى والميكروبيولوجي. وتم عزل عدد 90 سلالة محتملة لبكتيريا غير الموجودة ببادىء بكتريا حمض اللاكتيك (30 *Lactobacillus spp*. و 35 *Enterococcus spp*.) وذلك على بيئات MRS و KF Streptococci المتخصصة وتم تقييمها من حيث النمو على درجات حرارة مختلفة، وتحمل الملح، والقدرة على تجبن اللبن. وبناءاً على النتائج، تم تعريف 11 عزلة، ذات مميزات تكنولوجية محتملة، وراثيا باستخدام تقنية 16s rRNA، ثم تم تأكيد قدرتها على النمو وتكوين الحموضة في اللبن الفرز خلال 48 ساعة. وتم تعريف الـ 11 عزلة على أنها *Ent. durans*(1), *Ent. faecalis* (5), *Lb. paraplantarum* (1), *Lb. plantarum* (3), and *Lb. rhamnosus* (1), والتي ثبت نشاطها جميعًا في اللبن الفرز وأظهرت نشاطا مضادا لبعض الميكروبات المسببة لفساد وأمراض الغذاء. لذلك تقترح هذه النتائج استخدام عزلات NSLAB المعرفة في هذه الدراسة لتوحيد وتحسين جودة وسلامة الجبن البراميلي والاصناف الفرعية الأخرى ذات الصلة بالجبن الدمياطى.