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Original Research Article

ANTIBIOTIC RESISTANCE AND ANTIMICROBIAL ACTIVITIES OF *Lactobacillus* SPECIES ISOLATED FROM SOME ARTISANAL EGYPTIAN DAIRY PRODUCTS

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Abstract: Among popular artisanal dairy products, kariesh cheese, Laban Rayeb and yoghurt are occupying advanced rank in the human consumers` interest in Egypt. The current study included the microbiological investigations of 25 each of such products primarily to isolate and discriminate staphylococci and different *lactobacillus* strains and secondly to search for some of this *lactobacillus* to be considered as a probiotic. via investigating several characteristics including, their ability to resist and survive gastrointestinal tract conditions represented in gastric acidity (pH 2.5-3) and duodenal bile acids, and at the same time, having safety or non-pathogenicity, which principally includes non-harboring of antibiotic resistance (AR) patterns or virulence factors. Finally, the isolated *lactobacillus* strains were tested as inhibitors for some pathogenic bacteria such as *Staphylococcus aureus* and *E. coli.*

Key words: drtisanal; dairy products; probiotics; safety; inhibitory effects

Introduction

Currently, an extensive trend has been arising towards healthy functional foods which combine both desirable aromatic characteristics and owning beneficial microorganisms (M.Os) having health-promoting effects; the so called "Probiotics" (1, 2). In this sense, yoghurt as well as various cheeses and fermented dairy products are largely occupying advanced rank in consumers' interest globally. In particular, within Egyptian rural areas, the raw milk is frequently incorporated in the manufacture of several artisanal dairy products. Kariesh cheese and Laban Rayeb are marked Egyptian examples for those products. They are mainly processed from raw milk without heat-treatment which depend on the fermentation process adopted by existed lactic acid bacteria (LAB). Among included LAB, various types of beneficial probiotic bacteria may exist (3, 4).

Probiotic as a term is describing a group of mainly LAB bacteria having beneficial effects for the host when sufficiently provided (5). The guidelines accredited by FAO/WHO for evaluating probiotics in foods impose that proper *in vitro* investigations should be applied to prove their efficacy and safety. Additionally, each strain has to go through all assigned tests solely, since a property of a strain may be not species specific (6). There are many LAB strains that had been reported generally regarded as safe' (GRAS) status and are used widely in commercial food products.

Accordingly, to be considered as a probiotic, several characteristics must be possessed by the bacterial strain including, their ability to resist and survive gastrointestinal tract (GIT) conditions represented in gastric acidity (pH 2.5-3) and duodenal bile acids (7), possessing pathogen antagonizing mechanisms possibly via the production of antimicrobial compounds such as lactic acid, acetic acid, hydrogen peroxide, bacteriocins and bacteriocin-like substances (8), and finally having adhesive ability to gut epithelium (9). Concurrently, safety or non-pathogenicity of probiotic strain is a non-debatable concept, which principally includes non-

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harboring of antibiotic resistance (AR) patterns or virulence factors (9,10).

Lactobacilli are widely found in food and within the intestinal microbiota of humans and most animals. They are autochthonous bacteria accompanied with the mammalian gastrointestinal tract. They can contaminate raw milk, multiply during fermentation, survive during food processing and persist in finished products. They form an integral part of the natural microflora in fermented dairy products (7). Lactobacilli constitute a main proportion of non-starter lactic acid bacteria "NSLAB" of raw milk cheeses and the most considerably encountered are facultatively heterofermentative lactobacilli belonging to the species: *Lb. plantarum*, *L. casei*, *L. paracasei*, *L. rhamnosus, L. brevis* and *L. curvatus* (6). Due to the great diversity of NSLAB, they may be used to enhance cheeses characteristics (11).

Isolation and screening of microorganisms from naturally occurring processes had been the most powerful means for obtaining useful cultures for commercial and scientific purposes. Some characteristics of LAB such as texture and flavor formation are very important to the food industry due to their applicability for a variety of products. The dairy industry used well-defined single strain or multiple strain starter cultures to obtain dairy products of constant and high quality. So, a continuous need exists for the isolation of new strains with superior natural qualities (7, 11,12).

Because the screening of microbial population in raw milk and artisanal dairy products is considerably neglected in Egypt and as a consequence of the potential role of lactobacilli in dairy industry, the aims of current study were: (1) Isolation and typing of *Lactobacillus* spp. from some artisanal dairy products marketed in Egypt such as Kariesh cheese, Laban Rayeb and yoghurt (2) Assessment of bacteriological quality of such products via isolation and identification of *Staphylococcus* spp., (3) Selection of some *lactobacillus* spp. isolates and confirming their typing; using 16S rRNA gene sequencing, to be incorporated as potential probiotic bacteria, (4) Testing of probiotic properties for the selected *lactobacillus* spp. strains based on both resistance to gastrointestinal conditions and Human safety conditions.

Material and methods

Collection of samples

A total of 75 samples of artisanal Laban Rayeb, Kariesh cheese and yoghurt (25 samples of each) were aseptically sampled from Zagazig City's markets, Sharkia Governorate, Egypt. After being transferred to the Laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University, microbiological investigation was performed without any delay.

Preparation of samples for Microbiological investigations

Samples were adequately prepared and serially diluted as previously described (13,14). Accordingly, for Kariesh cheese and yoghurt, 11 grams or ml of each sample were homogenized and thoroughly-mashed in 99 ml of 0.1% sterile peptone water (40°C) inside a clean sterile mortar under sterile conditions. After the mixture become homogenous, 1 ml from the solution was used for the preparation of decimal dilution.

Microbiological Examination

Enumeration, isolation and identification of lactobacillus spp.

Following the serial dilution, the preliminary enumeration and isolation of *Lactobacillus* was done by plating on De Man, Rogosa and Sharp (MRS) agar medium (Difco Labs, Detroit, MI) adjusted to pH of 5.5 (15). MRS plates were incubated anaerobically (BBL Gas pak plus Anaerobic Sys.) at 30°C for 48 hrs. After enumeration of total *Lactobacillus*, 3-5 colonies with distinct morphological characters were selected and further purified by re-streaking two successive times on fresh MRS plates. All isolates were coded and maintained in glycerol stock (50%) at -20 °C (16).

The isolates were identified primarily based on Gram staining followed by biochemical identification based on Catalase, Kliger's Iron Agar, pH tolerance, NaCl tolerance, Phenol tolerance, sugar fermentation (Glucose, fructose, sucrose, xylose and lactose) and Casein digestion tests.

Identification of lactobacillus spp. isolates based on 16S rRNA GS

The different steps for PCR amplification and the sequencing based on 16S rRNA gene followed by comparative identification within Blast

tool in the GenBank were done according to standard protocols previously described (17, 18). The steps are illustrated briefly in Figure 1.

Amplification and sequencing of 16S rRNA gene

A fragment of the 16S rRNA gene was amplified by PCR using the universal primer pair p8FPL (Forward: 5'-AGTTTGATCCTGGCTCAG-3') and p806R (Reverse: 5'-GGACTACCAGGGTATCTAAT-3') (19). Genes' amplification was done using thermal Cycler (Mycycler, Bio-Rad, USA) following (20). The results of PCR have been visualized accurately using Chemi-doc™ MP imaging system (BioRad).

Prior to sequencing, the PCR products were purified using the "EXOSAP-IT" Kit (GE Healthcare, Uppsala, Sweden) to remove excess primers and nucleotides. Direct sequencing was performed using the "Big Dye Terminator v3.1" Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and the same primers used for PCR were also used for the sequencing of both strands of the PCR products. The sequencing reactions were analysed in an automatic sequencing system (ABI 3730XL DNA-Analyser, Applied Biosystems) coupled with the POP-7 system.

Phylogenetic analysis and construction of phylogenetic tree

Analysis of gene sequences were computed by Chromas (Griffith University, Australia) after alignment with Clustal-X software (21). Next, these sequences were identified by sequence homology alignment among published reference sequences in GenBank using BLAST (NCBI). Phylogenetic analysis and construction of phylogenetic tree were conducted using MEGA 5.0 software (22) and the neighbour-joining method (23).

Isolation and Identification of staphylococci

Staphylococci were isolated from previously prepared decimal serial dilutions and plated on Baird Parker agar (Oxoid). Characteristic colonies (Black with and without opaque halo) were picked up, and each one was purified by re- streaking for twosuccessive times on fresh Baird Parker agar me dium plates. After the second pu-ri fication step, all isolates were coded and kept at -20°C, for additional investigations when required. The identification of staphylococci recovered from the

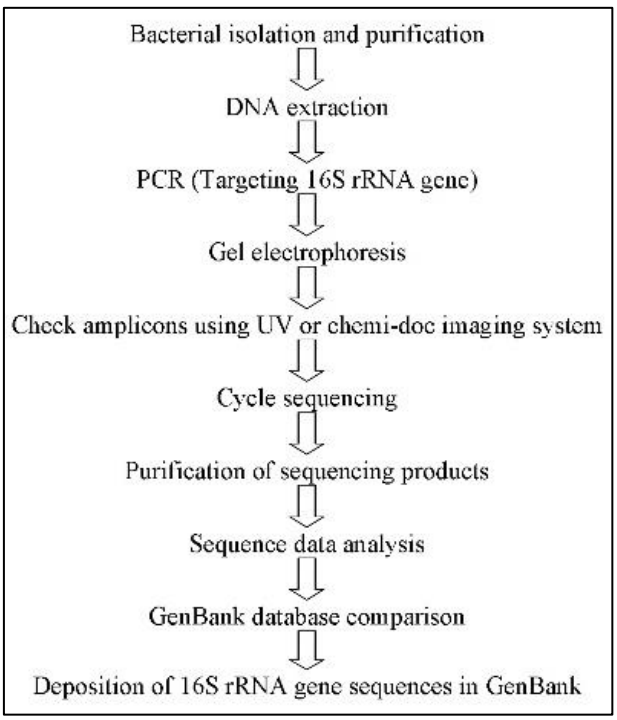

Figure 1: Schematic diagram for PCR and 16S rRNA GS-Based identification

examined samples was based on testing of Coagulase activity, Gram-Staining (Microscopic examination), motility test and biochemically based on Catalase, Oxidation fermentation, thermostable nuclease, mannitol test and nitrate reduction.

Testing of Probiotic properties for selected *lactobacillus* spp. *isolates* (24, 25).

The following tests were applied to each isolate to confirm the probiotic properties: Acid tolerance (Tolerance to low pH)**,** Bile Tolerance**,** acidifying ability and Salt resistance: MRS broth tubes containing 4% sodium chloride were inoculated with a loopful of 18 hrs active culture. The tubes were incubated at 37°C and the growth was observed after 48 hrs. The examination of Safety-related properties for selected *lactobacillus* spp. isolates was assessed as previously described (6, 11, 17, 21, 24-27).

Antibiotic Resistance of selected Lactobacilli

The isolates were inoculated into MRS broth individually and incubated at 37°C for 24 hrs. MRS agar were inoculated by the cultures of lactobacilli isolates (10^6 CFU/ml), mixed well, poured into sterile Petri plates and allowed to solidify. Different antimicrobials discs were placed

and pressed on the top of the Mueller Hinton agar (MHA) plates. The plates were incubated at 37ºC overnight. The absence of a growth inhibition zone around discs indicated resistance.

Blood haemolysis of selected Lactobacilli

Lactobacillus isolates were evaluated for haemolysis on Columbia agar plates (Oxoid) supplemented with 5% sheep blood.

β-Galactosidase Production of selected Lactobacilli

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

Testing of antibacterial *activity of selected lactobacillus spp. isolates against S. aureus and E. coli isolates*

The preserved frozen cultures of *S. aureus* and *E. coli* were subjected to refreshment process into nutrient broth and incubated for 24 hrs at 37ºC to be used as indicator organisms for antibacterial activity of lactobacilli isolates. The agar well diffusion method was used to detect the antibacterial property of the *Lactobacillus* isolates as previously described (24, 25, 28).

Results and discussion

A wide variety of fermented milks and cheeses are produced in rural areas of Egypt without addition of any starter as the fermentation process is mainly dependent on wild flora which present in the surrounding environment (4, 12, 29-32). The nature of locally produced; particularly artisanal traditional dairy products, often varied between different regions depending on the local indigenous microflora, particularly LAB.

The composition of LAB in the previously studied dairy products in certain region differed from similar styles of same products produced elsewhere, pointing to the importance of natural contamination by inhabitant flora from specific geographical localities (16). The evaluation of LAB diversity obtained by many researchers showed the potential effect of using raw milk on the microbial ecology of the fermented milk products and cheeses. Interestingly, strict European food safety law had resulted in lowering flexibility in food production and would finally lead to the disappearance of a number of geographical and artisanal dairy products and their related indigenous MOs (16, 33).

In the present study, 25 samples of each of Laban Rayeb, yoghurt and Kariesh cheese were randomly collected from different regions in Sharkia Governorate, Egypt. The microbiological assays for the isolation and identification of LAB and Staphylococci in artisanal Egyptian dairy products were done. The results of *Lactobacillus* and Staphylococci counts/ml in the examined samples were illustrated in detail in Table 1. The Kariesh cheese samples showed to harbor the highest *Lactobacillus* count ($1.8\times10^7\pm0.33\times10^7$), meanwhile the highest staphylococci count ($7.2\text{x}10^7 \pm 5.2\text{x}10^5$) was reported in Laban Rayeb samples.

On the other hand, the discrimination of such groups based on biochemical tests was illustrated in tables 2 and 3. *L. acidophilus* predominated among *Lactobacillus* isolates in Laban Rayeb, while *L. casei* was the predominant isolate in Kariesh cheese (Table 2). The discrimination of staphylococci isolates from the examined samples and their identification based on biochemical characteristics showed that *S. aureus* was the predominant species in Kariesh cheese samples. On the other hand, *S. succinus* showed to be the most-frequently isolate in yoghurt samples. Our results were similar to previously published studies (12,16, 32, 34, 35).

Discrimination of some selected Lactobacilli isolates based on 16S rRNA GS-based identification

Recently, genotypic identification had been emerged as an alternative or a complement to established phenotypic method within dairy diagnostics providing more accuracy, less labor and time saving. The 16S rRNA gene sequencing had been applied extensively within food safety diagnostic labs proving powerful identification and discrimination potentials (18, 35, 36).

The partial 16S rRNA gene sequences obtained from the 10 selected strains were compared to 16 S rRNA gene sequences in the GenBank database and the sequence similarities were determined using the BLAST tool (http://blast.ncbi.nlm.nih. gov/).

Table 1: *Lactobacillus* and Staphylococci counts/ml or gm in examined locally produced Egyptian dairy products

Samples	No. of samples	*Lactobacilli*					*Staphylococci*				
		Positive		*Lactobacillus* count/ml or gm			Positive		Staphylococci count/ml or gm		
		No.	%	Min.	Max.	Mean ±S.E.M.	No.	%	Min.	Max.	Mean ±S.E.M.
Kariesh cheese	25	25	100.00	6.5×10^5	4.6×10^8	$1.8\times10^7\pm0.33\times10^7$	19	76.00	$7.4x10^3$	$6.5x10^7$	$9.1x10^5 \pm 3.3x10^3$
Laban Rayeb	25	25	100.00	1.3×10^3	4.8×10^7	$4.7\times10^6\pm2.5\times10^6$	13	52.00	$8.9x10^5$	$7.4x10^7$	$4.7x10^7 \pm 6.3x10^5$
Yoghurt	25	-	-	-	-	-	13	52.00	$4.7x10^4$	$6.8x10^7$	$1.3x10^6 \pm 4.2x10^4$

Table 2: Incidence of isolated Lactobacilli from locally-produced Egyptian dairy products

L. sp. Isolates	Kariesh cheese		Laban Rayeb	
	No	%	No	%
L. plantarum	5.00	11.11	12.00	23.08
L. acidophillus	2.00	4.44	14.00	26.92
L. casei	17.00	37.78	11.00	21.15
L. brevis	3.00	6.67	3.00	5.77
L. rhamnosus	7.00	15.56	6.00	11.54
L. fermentum	11.00	24.44	6.00	11.54
Total	45.00	100.00	52.00	100.00

Table 3: Incidence of *Staphylococcus* spp. isolated from examined samples and their identification based on bio-chemical characteristics

Isolates	Kariesh cheese		Laban Rayeb		Yoghurt	
	No	%	No	%	No	%
S. aureus	4	21.05	0	0.00	0	0.00
S. epidermidis	2	10.52	2	15.38	1	7.69
S. simulans	2	10.52	2	15.38	2	15.38
S. xylosus	2	10.52	2	15.38	2	15.38
S. saprophyticus	0	0.00	1	7.69	0	0.00
S. capitis	0	0.00	0	0.00	0	0.00
S. chromogenes	0	0.00	0	0.00	0	0.00
S. equorum	2	10.52	2	15.38	2	15.38
S. haemolyticus	0	0.00	0	0.00	0	0.00
S. lentus	2	10.52	1	7.69	2	15.38
S. succinus	3	15.78	1	7.69	3	23.07
S. succinus subsp. *succinus*	0	0.00	0	0.00	0	0.00
S. succinus subsp. *casei*	0	0.00	0	0.00	0	0.00
Staphylococcus spp.	2	14.28	2	15.38	1	7.69
Total	19	100.0	13	100	13	100.0

Additionally, the partial 16S rRNA gene sequencing of six other out-group strains of reliably genetically-near species (*Streptococcus, Lactococcus and Leuconostic* and *Enterococcus*) were kindly provided by colleagues (16, 18, 37), were used in the phylogenetic analysis using MEGA 6.0 software.

As shown in Figure 2, the phylogenetic dendrogram based on 16S rRNA GS has elucidated an efficient discrimination between different examined groups. By the following phylogenetic order: *L. plantarum* (A), *L. brieves* (B), *L. acidophillus* (C), *L. rhamnosus* (D), *L. casei* (E), *Lact. garviae* (F), while for out group the species *Leuc. mesentroides* (G), *Enterococcus* sp. (H) and *Streptococcus spp.* (I) were effectively reproduced (all exceeded 50% bootstrap). Accordingly, 16S rRNA GS proved to be an efficient tool in discrimination between dairy originated *Lactobacillus*, *Streptococcus*, *Leuconostic*, *Enterococcus* and *Lactococcus*. Our results coincide with other studies that revealed the precise discrimination between the same species considered in the current study based on 16s rRNA GS (16, 35, 38). Also, previous studies showed that all *Lactobacillus* isolates could be identified based on 16s rRNA GS as *Lactococci*, *Enterococci*, *Streptococci*, *Lactobacilli*, *Leuconostoc* and *Pediococci* (16, 39).

Testing of Probiotic properties of Selected Lactobacilli

Resistance towards gastrointestinal conditions (Acidity and Bile Conditions)

Potential probiotic bacteria should resist stressful conditions of the stomach to reach to the small intestine (25,27). The resistance to the acidic condition of stomach is usually determined *in vitro* by the detection of resistance to pH 3 for a 3 hrs time; nearly equal to that consumed by stomach for digestion (24). The results presented in Figure (3) showed that selected *Lactobacillus* strains survived the test period of 3 hrs at pH 3 without a critical decrease in survival percentage. *L. rhamnosus* and *L. plantarum* showed the highest tolerance. While other strains showed variable survival percentages. Nearly similar results were reported by Kamal et al. (25) who observed the ability of *L. plantarum* and *L. rhamnosus* strains to survive gastric acidity. Also, a study by Maragkoudakis *et al* (40) showed that all the tested *Lactobacillus* strains of dairy origin were resistant to pH 3 during 3 hrs period.

The mean bile concentration in the small intestine was suggested to be 0.3% (w/v) and the staying time of food was believed to be 4 hrs (24). In turn,

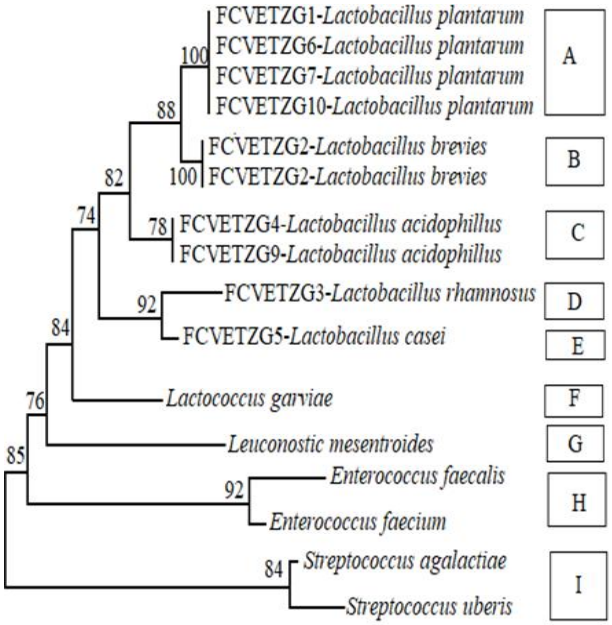

Figure 2: Phylogenetic dendrogram of some isolated lactobacillus sp. Strains based on 16S rRNA GS

these conditions were adopted in this study to explore the ability of strains to resist intestinal conditions. Like resistance to pH 3, the
results presented in Figure (3) showed that selected *Lactobacillus* strains survived test period of 3hrs at pH 3 without a critical decrease in survival percentage. *L. rhamnosus* and *L. plantarum* showed the highest tolerance.While other strains showed variable survival percentages. Similar to our results, all of the tested Lactobacillus strains of dairy origin were resistant to 0.3% bile salts concentration in 4hrs (40).

Testing of safety-related concerns in selected lactobacilli

Antibiotic susceptibility of Lactobacillus isolates

The absence of antibiotic resistance is a crucial issue when selecting a probiotic strain because bacteria which resist antibiotics may transfer such feature to gut micro biome (17, 27, 40). When the previously mentioned 10 *Lactobacillus* strains were tested for antibiotic resistance, as shown in table (4), only three Lactobacillus strains (2 *L. plantarum* and *L. rhamnosus*) were found to be sensitive to all tested antibiotics. Therefore, they could be chosen as a potential probiotic for further examination to be used in the production of different types of artisanal cheeses and fermented dairy products. Various literatures that examined *Lactobacillus* isolated from different food samples for antibiotic resistance concluded that the results of antibiotic resistance vary from study to study.

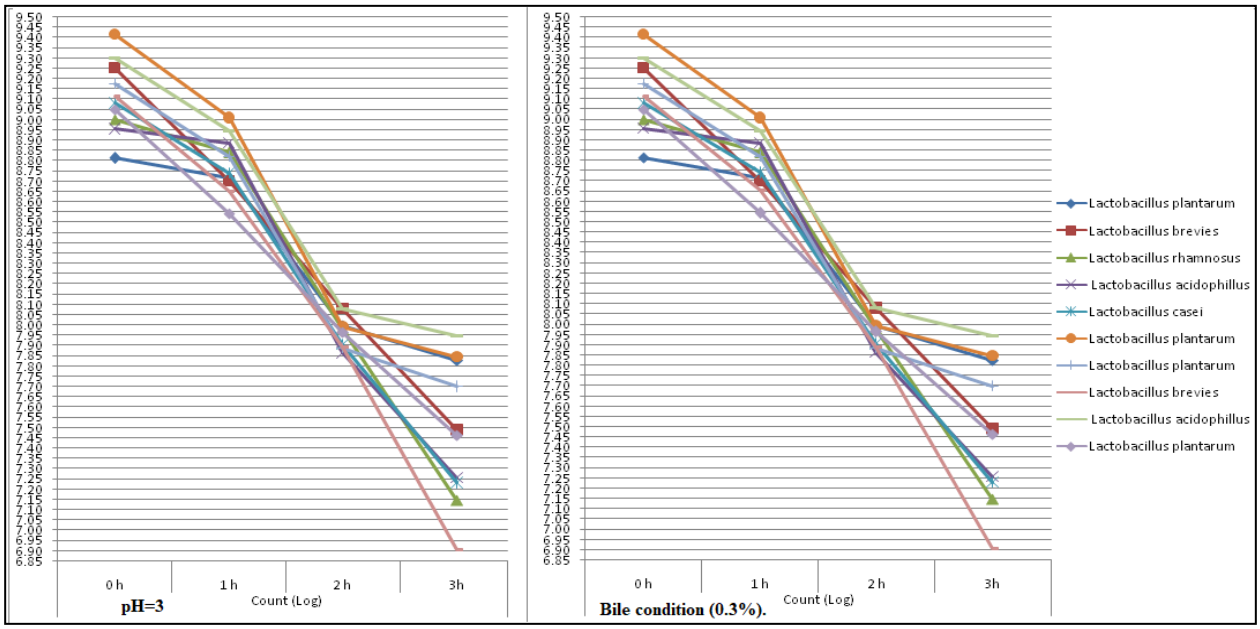

Figure 3: Resistance of selected *Lactobacillus* isolates towards acidic (pH 3) and bile condition (0.3%)

Table 4: Sensitivity/ resistance of examined *Lactobacillus* isolates against selected antimicrobials

Lactobacillus Strains	AMP	C	E	CN	S	TE	VA
L. plantarum	S	S	S	S	S	S	S
L. brevies	R	R	R	R	R	R	R
L. rhamnosus	S	S	S	S	S	S	S
L. acidophillus	S	R	R	R	R	R	R
L. casei	S	S	S	S	S	R	R
L. plantarum	R	R	R	R	R	R	R
L. plantarum	S	S	S	S	S	S	S
L. brevies	R	R	R	R	R	R	R
L. acidophillus	S	R	R	R	R	R	R
L. plantarum	S	R	R	R	R	R	R

R: resistant, S: susceptible**,** AMP: Ampicillin, C: Chloramphenicol, E: Erythromycin, CN: Gentamycin, S: Streptomycin, TE: Tetracyclin, VA: Vancomycin

Table 5: Blood haemolysis, acidifying ability, NaCL 4% tolerance and β- Galactosidase production of identified selected 10 *Lactobacillus* strains

Lactobacillus strains	Blood haemolysis	Acidifying ability	NaCl 4% tolerance	β-Galactosidase production intensity
L. plantarum	γ-hemolysis	Medium	+	++
L. brevies	γ-hemolysis	Fast	+	+
L. rhamnosus	γ-hemolysis	Fast	+	++
L. acidophillus	γ-hemolysis	Fast	+	++
L. casei	γ-hemolysis	Fast	+	++
L. plantarum	γ-hemolysis	Fast	+	+
L. plantarum	γ-hemolysis	Fast	+	+
L. brevies	γ-hemolysis	Fast	+	+
L. acidophillus	γ-hemolysis	Fast	+	++
L. plantarum	γ-hemolysis	Fast	+	++

Table 6: Antibacterial activity of neutralized *Lactobacillus* isolates' supernatant

Isolate	Code	Source of isolation	Diameter of inhibition zone (mm)	
			S. aureus	*E. coli*
L. plantarum	FCVETZG1	Kariesh cheese	--	14
L. brevies	FCVETZG2	Laban Rayeb	9	13
L. rhamnosus	FCVETZG3	Laban Rayeb	--	9
L. acidophillus	FCVETZG4	Laban Rayeb	9	--
L. casei	FCVETZG5	Laban Rayeb	9	9
L. plantarum	FCVETZG6	Laban Rayeb	8	--
L. plantarum	FCVETZG7	Kariesh cheese	8	7
L. brevies	FCVETZG8	Laban Rayeb	9	7
L. acidophillus	FCVETZG9	Kariesh cheese	9	11
L. plantarum	FCVETZG10	Kariesh cheese	11	8

Blood haemolysis, acidifying ability, NaCl tolerance and β-Galactosidase production intensity

Hemolysis on blood agar is one of the safety tests that ensure safe administration and non-hemolytic activity is considered as a safety prerequisite for the selection of a probiotic strain (41). Hemolysis was evaluated using Columbia blood agar plates containing 5% (v/v) sheep blood, and incubated at 37°C for 48 h. Characteristics of haemolysis on blood agar were shown as β-, α-, and γ-hemolysis. Our results presented in Table (5) showed that all the examined strains exhibit γ-hemolysis (no hemolysis). Thus, these isolates could not exhibit any pathogenicity and regarded as safe organisms due to their non-hemolytic activity.

A rapid decrease in pH during the initial step of cheese preparation is of definitive importance in the manufacture of cheese because it is very essential for coagulation and prevention or reduction of the growth of adventitious microflora (27, 42). The fast-acidifying strains are good candidate in the dairy fermentation process as primary starter organisms. Whereas, the poor acidifier strains can be used as adjunct cultures based on their other important properties, e.g., proteolytic and autolytic activity (8). With respect to the acidifying activities of the identified 10 *Lactobacillus* strains in our study (Table 5), most Lactobacillus strains showed a fast acidification activity with exception to one *L. plantarum* that exhibited medium acidification activity. Similar results were previously obtained (25, 43).

Because addition of salt in cheese manufacture is essential, thus probiotic strains should possess the ability to grow in the presence of 4-6.5% NaCl to ensure their application in dairy industry as primary starter cultures, or as adjunct cultures based on other technological properties. Salt tolerance of strains was measured as positive growth after 48hrs, incubation periods in liquid MRS medium containing NaCl 4%. In our study, All *Lactobacillus* strains grew well at NaCl 4% as shown in table (5). These results are similar to those reported by Sumathy *et al* (44).

β-galactosidase had been widely used for industrial as well as medical applications. In dairy industries, β-galactosidase had been used to prevent crystallization of lactose, improve sweetness and increase the solubility, flavor and digestibility of the milk products (26). Enzymatic hydrolysis of lactose by *β*-galactosidase is one of the most popular technologies to produce lactose reduced milk and related dairy products for consumption by lactose intolerant people (26, 44). In our study, the 10 *Lactobacillus* trains were screened for their β-galactosidase activity with X- gal and colonies with blue color were regarded as bacteria containing β-galactosidase enzyme as shown in table 5.

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

LAB compose the largest group of probiotic microorganisms; a successful potential probiotic strain is expected to have a number of desirable properties. The most important properties are

acid and bile tolerance. Besides, other safety related properties such as AR and blood hemolysis should be considered as essential characters for selection of potential probiotic strains (6, 11, 17, 24-27). The antibacterial effect of *Lactobacillus* isolates was tested against two previously isolates of food-safety concerns; *S. aureus* and *E. coli* (17, 45).

The antibacterial effect of LAB could be due to organic acids (lactic and acetic acid), hydrogen peroxide, acetaldehyde, diacetyl, reuterin, carbon dioxide, bacteriocins and bacteriocin-like substances (8, 24, 27). To determine whether acidity, bacteriocins and/ or H_2O_2 contributed to the inhibition effect by *lactobacillus* isolates, CFS were prepared from the previous *Lactobacillus* isolates showing antibacterial activities against *S. aureus* and/or *E. coli* and then were neutralized to pH 6.5 to exclude the antibacterial effect of acidity. Neutralized supernatants were examined for antibacterial effect as the previous method. Table (6) shows the diameters of the inhibition zones around wells previously filled with CFS from the examined *Lactobacillus*. spp. isolates, only 10 strains displayed the highest inhibition against at least one of the selected indicator pathogens after neutralization compared with other *Lactobacillus* isolates. It could be seen that only 6 out of 10 *Lactobacillus* strains could inhibit the growth of the examined *S. aureus* and *E. coli*. The inhibitory effect of other *Lactobacilli* varied with different *S. aureus* and *E. coli* and appeared to be strain-dependent.

Conclusion

From the obtained results, it can be concluded that Egyptian artisanal dairy products represent good sources for the detection of beneficial LAB strains. However, a thorough testing regarding strain safety and applicability should be performed prior to legal usage in food and dairy industry.

The authors declare that they have no competing interests.

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