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Isolation and Identification of Antimicrobial Producing Lactic Acid Bacteria from Local Egyptian Dairy Products

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ABSTRACT

The aime of the present work is to evaluate the antimicrobial characteristics of lactic acid bacteria (LAB) being utilized as starter cultures in cheese and fermented dairy products. Morphological, biochemical and physiological features of the selected isolates were performed in their identification. A total of 226 isolates were recovered from 40 yogurt samples (23.45%), 30 Ras chesse samples (19.47%), 48 Kariesh cheese samples (33.18%) and 32 samples of Laban Rayeb (23.9%) that were randomly collected from local markets in Dakahlia governorate. The distribution of LAB isolates by the genus was as follows: *Lactobacillus* (33.63%), *Streptococcus* (17.69%), *Lactococcus* (8.85%) and *Enterococcus* (39.82%). Among these isolates, *E. faecalis* (55 isolates, of 24,34%), *S. thermophilus* (40 isolates, of 17.69%), *Lb.delbrueckii subsp. bulgaricus* (30 isolates, of 13.27%) *E. faecium* (25 isolates, of 11.06%), *Lb. helveticus* (16 isolates, of 7.1%), *Lb. salivarius* (15 isolates, of 6.64%), *Lc.lactis subsp. lactis* (15 isolates, of 6.64%), *Lb. acidophilus* (10 isolates, of 4.42%), *E. gallinarum* (7 isolates, of 3.1%), *Lc. Lactis subsp. cremoris* (5 isolates, of 2.21%) and *E. malodoratus* (3 isolates, of 1.33%). These 226 isolates were further examined for their capability to produce antimicrobial compounds. Only 16 out of 226 LAB isolates could inhibit the growth of the examined indicator organisms. Among these isolates, 8 LAB isolates (3 isolates of *Enterococcus fecalis*, 2 isolate of each *E.faecium*, *E. gallinarum* and *E. malodoratus*) were still capable to inhibit the indicator organisms by neutralization to pH 6.8. These isolates may produce bacteriocin like substances.

Keywords: Lactic acid bacteria, Isolation, Dairy products, Antimicrobial.

INTRODUCTION

The 'true lactic acid bacteria are Gram-positive, nonmotile, non-sporeforming, rod- and coccus-shaped organisms, which can ferment carbohydrates and higher alcohols, forming mainly lactic acid.

The true rod-shaped lactobacilli could be classified by Orla Jensen(1919) on the basis of their optimum growth temperature (mesophilic at 30^0 or thermophilic at 45^0C), and according the type of fermentation being done (homo- or hetero-fermentations), into 3divisions, namely: thermobacterium, streptobacterium and betabacterium. He also emphasized the impact of both of streptocooci and lactobacilli in milk and dairy products.

The main members of LAB in foods and dairy industries are mainly belonging to the genera: *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Vandamme *et al.,* 1996). According to the 16s rRNA data obtained by examining the LAB proposed new groupings of these bacteria. It could also reclassify lactobacilli, leuconostocs and pediococci as three major groups (Collins *et al.,* 1993b; Schleifer and Ludwig, 1995; Vandamme *et al.,* 1996).

The isolation of Lactic acid bacteria from traditional dairy products have received more importance as natural food preservative due to their antimicrobial activity against pathogens contaminating food, such as *Listeria monocytogene*s (Jamuna & Jeevaratnam, 2004). LAB are widely spread in the nature, they are included a many members of the spontaneous food fermentation. These LAB isolated have been extensively examined by Holzapfel *et al.,*(1995).

Furthermore, certain individuals of LAB characterized with their antimicrobial activity against pathogens, deleterious bacteria, yeasts, molds and viruses by different mechanisms presenting a potential application in food preservation (Deegan *et al.* 2006 ; Todorov *et al*. 2010). Lactic acid, acetic acid, hydrogen peroxide and diacetyl produced by LAB play an important role as an antimicrobial metabolites. Bacteriocins, on the other hand, have also the most instant prospective in food application as biopreservatives. These bacteriocins could be safely used into food industries (Cotter *et al.* 2005). Since lactic acid bacteria are generally regarded as safe (GRAS) according to the FDA, they could be used in food production and food biopreservation.

The aim of this work is, therefore to evaluate antimicrobial activity of some LAB isolates being recovered from the Egyptian traditional dairy products such as yogurt, Ras cheese, Kariesh cheese and laban Rayeb.

MATERIALS AND METHODS

Collection of dairy product samples

One hundred fifty samples of local Egyptian dairy products were randomly collected from the markets in Dakahlia governorate. These samples involved 40 yogurt samples, 30 Ras cheese samples, 48 samples of Kariesh cheese and 32 samples of Laban Rayeb.

Isolation and identification of lactic acid bacteria (LAB) from dairy product samples

Ten grams of each sample was added to 90 ml of sterile saline solution (0.85% NaCl) followed by preparing serial dilutions of the resultant sample suspension in the same sterile solution. Suitable dilutions were plated onto MRS and M17 agar (Oxoid, Basingstoke, UK), followed by incubation at 30 and 37°C for 24 and 48 h,respectively. Suspected colonies were picked and maintained on MRS agar for further examinations.Isolates were maintained as frozen cultures in MRS broth with 50% glycerol at -20°C.

Single colonies from each plate were examined Gram stained (Pollack *et al*. 2005),and tested for catalase production (Macfaddin 1977) and milk coagulation(Sharpe 1979).

Catalase –negative, Gram-positive and milk coagulation positive isolates were selected for further tests. They were characterized by conventional and physiological tests growth at 10 and 45 °C, growth at 6.5% salt, pH = 9.6 (Abd-El-Malek and Gibson 1948),growth in

SF broth (Sharpe 1979), KAA medium and aesculin hydrolysis.

Confirmed *Enterococcus* isolates were further identified to the species level by biochemical tests such as growth at 50°C, survival at 60 °C for 30 min (Sharpe 1979), glycerol utilization(Facklam 1972), pyruvate utilization (Gross *et al*. 1975), motility and hydrogen sulfide (H_2S) production (Harrigan and McCance 1966).

Confirmatory examination of *Lactococcus* and *Streptococcus thermophilus* isolates were done by carrying out the carbohydrate fermentation such as maltose and sucrose (Bergey's manual., 1986), their ability to grow at 39.5°C, pH 9.6 and in the presence of 4% Nacl (Sharp *et al.,* 1979).

Lactobacillus isolates were also examined for carbohydrate fermentation such as sucrose, lactose, maltose, galactose and sorbitol as sole carbon sources in MRS broth (Bergey's manual., 1986) ,their ability to grow at 15 – 45°C (Sharp *et al.,* 1979) and production of CO_2 from glucose in MRS broth (with Durham tubes) (De Man *et al.* 1960).

Determinating of antimicrobial activity of lactic acid bacteria recovered from conventional dairy products

A 24 h culture of each suspected *Enterococcus* isolates grown in tryptone Soya Broth (TSB) broth (oxoid) at 37°C were examined were. Cells free extarets were harvested by centrifugation (10,000 rpm, 20 min, 4°C). The supernatant fluids were adjusted to pH 7 with sterilized 1M NaOH to get rid the inhibitory activity of acid (Daba *et al.*1991). The supernatants were filtered through a 0.45-mm filter (Millipore, Bedford, MA, USA) to get the cell-free supernatants (CFSs). These CFSs were examined for antimicrobial activity against *Staphylococcus aureus* MSD-7447, *Escherichia coli* MSD -10418, *Salmonella typhi* MSD -9331, *Bacillus cereus*, *Enterococcus faecalis* as indicator strains. All of these strains were obtained from Fermentation Biotechnology and Applied Microbiology (FERM-BAM) Centre, Al-Azhar University, Cairo, Egypt. Antimicrobial activities of CFSs from *Enterococcus* isolates was tested by the agar well diffusion assay (AWDA) as described by Ennahar *et al.* (2000). Petri dishes containing 20 ml of tryptone soya agar (TSA) (Oxoid) were prepared formerly and inoculated with 0.5 ml of 24 hr broth culture of pathogenic bacteria. Once the medium solidified in the dishes , wells (8mm diameter) were done and a sterile pasteur from the indicator-seeded agar and 100 µl of cell free supernatant fluid (CFS) was poured in to each well. The dishes were stored for 4 hrs in a refrigerator to allow the radial diffusion of the inhibitor agents, followed by incubation upside down at 37°C for 24 h . The diameter of the inhibition zone was then measured with calipers in mm and counted for the numbers of *Enterococcus* colonies.The antimicrobial activity was determined by measuring the clear zone around the wells. The diameter of zone of inhibition against indicator organism in (mm) is performed using the following formula:

Z (mm) = diameter of inhibition zone obtained (mm) – diameter wells (8mm).

RESULTS AND DISCUSSION

Isolation and identification of antimicrobial producing lactic acid bacteria from local Egyptian dairy products:

One hundred fifty samples of local Egyptian dairy products were randomly collected from the markets in Dakahlia governorate. These samples involved 40 yogurt, 30 Ras cheese, 48 Kariesh cheese and 32 of Laban Rayeb (Table 1). The original samples were serially diluted and plated onto MRS and M17 agar. A total of 438 suspected Lactic acid bacteria isolates were recovered from the previous by mentioned samples, and subjected to rapid preliminary identification including catalase test, Gram-staining and milk coagulation test.

Table 1. Isolation and rapid preliminary identification of LAB from local Egyptian dairy products.

Samples	Samples Number	Number of Suspected LAB Isolates	The Potential LAB Isolates					
			Cocci		Lactobacilli		Total	
			No	%	No	%	No	%
Yogurt	40	104	33	14.60	20	8.85	53	23.45
Ras cheese	30	96	28	12.39	16	7.08	44	19.47
Kariesh cheese	48	125	62	27.43	13	5.75	75	33.18
Laban Rayeb	32	113	27	11.95	27	11.95	54	23.9
Total	150	438	150	66.37	76	33.63	**226**	**100**

Catalase –negative, Gram-positive and milk coagulation positive isolates were assessed as potential LAB (Table1). Out of 438 suspected isolates, 76 and 150 isolates were characterized as potential LAB lactobacilli, cocci respectively (Table 1). Potential LAB lactobacilli and cocci were recovered from all of the examined samples. The present results could reflect the composition of LAB starters and non LAB starters (NLAB) being used for making of these products.

Potential isolates of LAB were, furthermore, subjected to physiological tests as indicated in Table 2. 40 potential cocci and all of the 76 lactobacilli could not be able to grow at pH 9.6, 10°C and in the presence of 6.5% NaCl. However, 90 potential cocci could grow at 45°C, 10°C and at pH 9.6 and in the presence of 6.5% NaCl. These results revealed that these 90 cocci could be classified as *Enterococcus* species (Sharpe *et al.,* 1979 and Hardie & Whiley 1995). Another 20 cocci could grow at 10°C,but they showed negative results with the other tests. These isolates were, therefore, considered as potential *Lactococcus* sp. (Hardie & Whiley 1995) (Table 2). Further biochemical examinations of 150 lactic acid cocci showed the capability of 90 isolates to grow in the SF medium, producing black colonies on the BEA medium, and producing black colonies surrounded by brown or black zone on Kanamycin Asculin Azid (KAA) agar (Table 2). However, the other 60 cocci isolates were not able to grow in the SF medium, and could not produce

black colonies on BEA, and they could be classified as *Streptococcus* sp. or *Lactococcus* sp. (Table 2).

The above results highlighted the diversety of lactic acid bacteria isolates present in the traditional types of Egyption dairy products, which were also confirmed in other studies, for instance, LAB isolates belonging to the genera *Enterococcus, Lactobacillus, Streptococcus, Lactococcus* and *pediococcus* from different dairy products (Ras cheese, Domiatti cheese, Mish, Zabady and Laban Rayeb (Ayad *et al.,* 2004 & 2006).

Table 2. Physiological confirmation of potential LAB isolates recovered from conventional Egyptian dairy products.

Potential LAB Isolates	Growth At 10°C	Growth At 45°C	Growth at pH 9.6	Growth in 6.5% NaCl	Growth in SF broth	Growth on BEA medium	Growth on KAA medium	Results of Identification (No. of Isolates)
Lactobacilli (76)	-	+	-	-	NE*	NE	NE	*Lactobacillus* sp. No. (76) % (33.63)
Cocci (150)	-	+	-	-	-	-	-	*Streptococcus* sp. No. (40) % (17.69)
	+	-	-	-	-	-	-	*Lactococcus* sp. No. (20) % (8.85)
	+	+	+	+	+	+	+	*Enterococcus* sp. No. (90) % (39.82)

*** NE: Not Examined**

It could also be obvious that *Enterococcus* strains were the most frequently isolated Lactic acid bacteria from the conventional dairy products. which is in agreement with Süßmuth (1995).

This also might be attributed to the high ability of *Enterococcus* strains to survive stress environmental conditions of high heat treatment, high NaCl concentrations and low pH in conventional Egyptian dairy products (Jokovic *et al.,* 2008).

Identification of *Enterococcus* isolates recovered from the local dairy products:

Further examinations of the above mentioned isolates were performed for specification within the genus *Enterococcus.* Several biochemical tests were performed.These included the examination of theis capability to utilize glycerol, pyruvate, the ability to survive at 60°C for 30 min, and to grow at 50°C. It could be seen in Table (3) that 55 out of the 90 *Enterococcus* isolates were identified as *E. faecalis*. These isolates could utilize glycerol and pyruvate. They could survive the exposure to 60 °C for 30 min, but could not be able to grow at 50 °C, and were not able to motile, and could not produce H_2S (Jones *et al.,* 1972). Another 25 isolates were able to grow at 50°C, and survived exposure to 60°C for 30 min, but gave negative results with the remaining examinations (Table 3). On the other hand, 3 cocci produced H_2S and survived exposure to 60 °C and utilize glycerol, but were not capable to grow in the other testing examination (Table 3) (Day et al. 2001). They were, therefore, considered as potential *E. malodoratus*. The remaining 7 isolates could also survive exposure to 60°C for 30 min, grew at 50°C and were able to motile, but could not utilize glycerol or pyruvate and could not produce H_2S. These isolates were, therefore, identified as *E. gallinarum* (Day *et al.* 2001)

Table 3. Biochemical identification of *Enterococcus* Isolates recovered from local Egyptian dairy products.

Numbers of Isolates	Physiological tests						Identification
	Glycerol utilization	Pyruvate utilization	H_2S	Motility	Survival at 60 °C	Growth at 50°C	
55 (61.1%)	+	+	-	-	+	-	*E. faecalis*
25 (27.8%)	-	-	-	-	+	+	*E. faecium*
3 (3.33%)	+	-	+	-	+	-	*E. malodoratus*
7 (7.8%)	-	-	-	+	+	+	*E. gallinarum*

Physiological and biochemical examination of *Lactococcus* Isolates and the confirmatory tests of *Streptococcus thermophilus* cultures:

Further physiological and biochemical identification were also performed to characterize the previous Lactococci cultures being isolated from the Egyptian dairy products to the species level and to confirm the presumptive characterization of some cultures as *S. thermophilus.* These tests involved the ability to grow in the presence of 4% NaCl, at pH 9.2, at 39.5 °C, and the abilety to ferment of sucrose and maltose (Sharpe *et al.* 1979 and Schleifer *et al.,* 1985). Results in Table 4, show that 15 out of 20 *Lactococcus* isolates were identified as *Lc. Lactis* subsp *lactis*. These strains were capable to grow in 4% NaCl, at pH 9.2 and at 39.5°C. They were also found to utilize maltose, but not sucrose. The other 5 lactococci were unable to grow in 4% NaCl, at pH 9.2, at 39.5°C,and utilize sucrose or maltose. These cultures were identified as *Lc. Lactis subsp cremoris*. Forty potential *S. thermophilus* cultures were able to grow at 39.5°C, but gave negative results with other identification tests,which

assured their previous presumptive characterization as *S.thermophilus*.

Table 4. Physiological and biochemical speciation of *Lactococcus* Isolates and the confirmatory test of *Streptococcus thermophilus* cultures.

Isolates numbers	Physiological tests					Identification
	Growth in 4% NaCl	Growth at pH 9.2	Growth at 39.5°C	Maltose	Sucrose	
40	-	-	+	-	+	*Streptococcus thermophilus*
15	+	+	+	+	-	*Lc. Lactis* subsp. *lactis*
5	-	-	-	-	-	*Lc. Lactis* subsp. *cremoris*

Characterization of *Lactobacillus* isolates recovered from the examined local dairy products

Attempts to analyze at the phenotypic level of lactobacilli isolates to accurately identify the examined species, the following tests: CO_2 production, growth at45°C or 15°C and fermentation of lactose, galactose, sorbitol, sucrose and maltose. As seen in Table 5, 30 out of 76 *Lactobacillus* isolates were identified as *Lactobacillus delbrueckii* subsp. *bulgaricus*. These isolates could grow at 45°C, but could not grow at 15°C. They previous isolates could not produce CO_2 or ferment sucrose, maltose or sorbitol, whereas they fermented galactose and lactose (Kandler& Weiss, 1986). Sixteen other isolates characterized with similar results, but they fermented maltose (Table 5). They were, thus, considered as belonging to *Lb.helveticus.* Ten other isolates gave similar results to *Lb. delbrueckii* subsp. *bulgaricus,* but could utilize sucrose and maltose. They were, thus, identified as *Lb. acidophilus* (Table 5). Fifteen other isolates were classified as *Lb. salivarius,* since they grew at 45°C and utilized all of the tested sugars, but they were not capable of producing CO_2, or grow at 15°C (Table 5). The remaining five isolates showed good growth ability at 15°C, variably grew at 45°C and produce CO_2. They fermented all tested sugars except sorbitol. These cultures were considered as *Lactobacillus* sp. (Table 5). (Kandler, 1983&1984).

Table 5. Characterization of *Lactobacills* cultures recovered from Egyptian dairy products to the species level.

Isolates Numbers	Physiological tests								Identification
	CO_2 production	Growth at 15°C	Growth at 45°C	sucrose	Lactose	Maltose	Galactose	Sorbitol	
16	-	-	+	-	+	+	+	-	*Lb. helveticus*
10	-	-	+	+	+	+	+	-	*Lb. acidophilus*
15	-	-	+	+	+	+	+	+	*Lb. salivarius*
30	-	-	+	-	+	-	+	-	*Lb.delbrueckii* subsp. *Bulgaricus*
5	+	±	-	+	+	+	+	-	*Lactobacillus* sp.

Screening of antimicrobial activity of lactic acid bacteria recovered from conventional dairy products

The antimicrobial activity of the characterized 226 cultures of lactic acid bacteria (LAB) isolated from local dairy products was detected. This was performed by using cell free filtrates (CFFs) of overnight cultures of isolates and assessing their effect against Gram- negative bacteria (*Esherichia coli* and *Salmonella typhi*), Gram- positive bacteria (*Staphylococcus aureus* and *Enterococcus feacalis*) and spore forming bacteria (*Bacillus cereus*). Results in Table (6) , show the presence or absence of an inhibitory zone around the wells filled with CFFs from the examined isolates. It could be noticed that only 16 out of 226 LAB isolates could inhibit the growth of indicator organisms. These isolates included 3 isolates of *Enterococcus fecalis*, 2 isolate of each of *Lc.lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris*, *E.faecium* and *E. gallinarum*, 1 isolate of each *Lb. acidophilus, Lb. delbrueckii* subsp. *Bulgaricus*, *Streptococcus thermophilus* and *E. malodoratus* (Table 6). It could also be noticed that *B.cereus* was resistance to all antimicrobial agents produced from the fifteen examined LAB strains. The CFFs from *Enterococcus faecium* 14, *Enterococcus faecalis* 52, *Streptococcus thermophilus*102 displayed inhibition against Gram negative indicator bacteria. They were not able to inhibit Gram positive indicator bacteria and spore forming indicator bacteria. The other CFFs from *Enterococcus faecalis* 41, *Lc.lactis* subsp *lactis, Lactococcus lactis* subsp *cremoris* presented inhibitory effect against Gram positive indicator bacteria. However they were not of inhibitory effect toward Gram negative indicator bacteria (Table 6). The *Lactobacillus acidophilus* and *Lb delbrueckii* subsp *bulgaricus* were of the most inhibitory against *Lb. acidophilus* and *Lc.Lactis* subsp. *cremoris*. The inhibitory effect of other isolates varied within different Gram-negative isolates or Gram- positive isolates (Table 6).

It could also be found that a number of LAB isolates had an inhibitory effect against the examined Gram-negative or Gram- positive organisms. Several work on traditional Egyptian dairy products also presented that these products included LAB isolates displaying inhibitory effect toward foodborne pathogens. (Ayad *et al.,* (2004), Abdel-Kader 2005 and El-Backary 2005).

The inhibitory effect of lactic acid bacteria could be attributed to diacetyl, organic acids, reuterin, acetaldehyde, bacteriocins and caron dioxide (Clark & Takacs, 1980; Hurst, 1981; Jay, 1982, Dahl *et al.,* 1989; Piard & Desmazeaud, 1992 and Kao & Frazier, 1996). To determine whether diacetyl, bacteriocn, acidity or H_2O_2 participated to the inhibitory effect against indicator organisms by lactic acid bacteria isolates, preparation of cell free filtrates (CFFs) from the previous 16 LAB isolates was carried out and neutralized to pH 6.8 to eliminate the inhibitory effect of acidity.

Table 6. Inhibitory effect of LAB strains isolated from conventional dairy products against indicator organisms.

Isolates No.	LAB isolates	Indicator organisms								
		Lc. lactis subsp *cremoris*	*Lb. acidophilus*	*Lc.lactis* subsp *lactis*	*S. thermophilus*	*Staph. aureus*	*E. feacalis*	*S. typhi*	*E. coli*	*B. cereus*
2	*Lc.lactis* subsp *lactis,*	+	_	+	-	+	+	_	_	_
6	*Lc.lactis* subsp *lactis,*	+	_	+	+	-	+	-	_	_
9	*Lc. lactis* subsp *cremoris*	+	+	+	+	+	+	_	_	_
10	*Lc. lactis* subsp *cremoris*	+	+	+	_	_	+	_	_	_
14	*E. faecium*	+	+	+	+	_	_	+	+	_
24	*E. faecium*	+	-	+	-	_	_	+	+	_
41	*E. faecalis*	+	+	+	+	+	+	_	_	_
44	*E. faecalis*	+	+	+	+	_	_	_	_	_
52	*E. faecalis*	+	+	+	+	_	_	_	_	_
60	*E. gallinarum*,	+	+	+	+	+	+	_	_	_
69	*E. gallinarum*,	+	+	+	+	+	+	_	_	_
82	*Lb. acidophilus*	_	+	_	+	+	_	_	_	_
89	*Lb. acidophilus*	+	+	_	_	_	_	_	_	_
101	*Lb. delbrueckii* subsp. *Bulgaricus*	+	+	_	_	+	_	_	_	_
102	*S. thermophilus*	+	+	+	+	_	_	_	_	_
72	*E.malodoratus*	+	+	+	+	_	+	+	_	_

Table 7 shows the inhibition of indicator organisms by CFFs were produced from 16 lactic acid bacteria isolates. It could be noticed that CFFs prepared from 3 isolates of *Enterococcus fecalis*, 2 isolate of each *E.faecium* and *E. gallinarum* and 1 isolate of *E. malodoratus* were still capable to inhibit indicator organisms in spite of neutralization to pH 6.8. While, neutralizing CFFs of 2 isolate of each *Lc. Lactis* subsp *lactis*, *Lc. Lactis* subsp *creomirs* and , *Lb. acidophilus*. 1 isolate of each *S.thermophilus* and *Lb.delbrueckii* subsp. *bulgaricus* resulted in a sharp reduction of the antibacterial zone of inhibition, which were less than 2 mm- this is indicated by the sign minus (-) in Table 7. This suggests that acidity played a essential role in preventing the growth of indicator organisms by 2 isolate of each *Lc. Lactis* subsp *lactis*, *Lc. Lactis* subsp *creomirs* and *Lb. acidophilus*. 1 isolate of each *S.thermophilus,* and *Lb.delbrueckii* subsp. *bulgaricus* compared with 3 isolates of *Enterococcus fecalis*, 2 isolate of each *E.faecium* and *E. gallinarum* and 1 isolate of *E.malodoratus* (Table 7).

Table 7. The effect of neutralizing on the inhibitory effect of CFFs prepared from lactic acid bacteria isolates

Isolates No.	LAB isolates	Indicator organisms								
		Lc. lactis subsp *cremoris*	*Lb. acidophilus*	*Lc.lactis* subsp *lactis*	*S. thermophilus*	*Staph. aureus*	*E. feacalis*	*S. typhi*	*E. coli*	*B. cereus*
2	*Lc.lactis* subsp *lactis,*	_	_	_	_	_	_	_	_	_
6	*Lc.lactis* subsp *lactis,*	_	_	_	_	_	_	_	_	_
9	*Lc. lactis* subsp *cremoris*	_	_	_	_	_	_	_	_	_
10	*Lc. lactis* subsp *cremoris*	_	_	_	_	_	_	_	_	_
14	*E. faecium*	+	+	+	+	_	_	+	+	_
24	*E. faecium*	+	-	+	-	_	_	+	+	_
41	*E. faecalis*	+	+	+	+	+	+	_	_	_
44	*E. faecalis*	+	+	+	+	_	_	_	_	_
52	*E. faecalis*	+	+	+	+	_	_	_	_	_
60	*E. gallinarum*,	+	+	+	+	+	+	_	_	_
69	*E. gallinarum*,	+	+	+	+	+	+	_	_	_
72	*E.malodoratus*	+	+	+	+	_	+	+	_	_
82	*Lb. acidophilus*	_	_	_	_	_	_	_	_	_
89	*Lb. acidophilus*	_	_	_	_	_	_	_	_	_
101	*Lb. delbrueckii* subsp. *Bulgaricus*	_	_	_	_	_	_	_	_	_
102	*S. thermophilus*	_	_	_	_	_	_	_	_	_

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عزل وتعريف بكتريا حامض اللاكتيك المنتجه للمواد المضاده للبكتريا من منتجات الالبان المصرية المحلية

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تهدف الدراسة الحالية لتقدير خصائص المواد المضادة للميكروبات للبكتريا حامض اللاكتيك للأستفادة منها كبادئات للجبن وتحضيرات منتجات الألبان المتخمرة .الخصائص الفسيولوجية والحيوية والمورفولوجية للعزلات المختبرة استخدمت للتعريفها .تم عزل ٢٢٦ مزرعة من ٤٠ عينة زبادى (٢٣.٤٥ %) و٣٠ عينة جبن راس(١٩.٤٧ %) و ٤٨ عينة جبن قريش(٣٣.١٨ %) و ٣٢ عينة لبن رايب(٢٣.٩ %) حيث تم تجميعهم عشوائيا من الأسواق المحلية فى محافظة الدقهلية .توزيع مزارع بكتريا حامض اللاكتيك عن طريق الاجناس كانت كالأتى *Lactobacillus* (٣٣.٦٣%) و *Streptococcus* (١٧.٦٩%) و *Lactococcus* (٨.٨٥%) و *Enterococcus* (٣٩.٨٢%) .من بين هذه العزلات *E. faecalis* (٥٥ عزلة حوالى ٢٤.٣٤%) و *S. thermophilus* (٤٠ عزلة حوالى 69 .17 %) و *Lb.delbrueckii subsp. Bulgaricus* (٣٠ عزلة حوالى ٢٧ . ١٣%) و *E. faecium* (٢٥ عزلة حوالى ٠٦ . ١١%) و *Lb. helveticus* (١٦ عزلة حوالى ١ . ٧%) و *Lb. salivarius* (١٥ عزلة حوالى ٦٤ . ٦%) و *Lc.lactis subsp. Lactis* (١٥ عزلة حوالى ٦٤ . ٦%) و *Lb. acidophilus* (١٠ عزلات حوالى ٤٢ . ٤%) و*E. gallinarum* (٧ عزلات حوالى ١ . ٣%) و *Lc. Lactis subsp. Cremoris* (٥ عزلات حوالى ٢١ . ٢%) و *E. malodoratus* (٣ عزلات حوالى ٣٣ . ١%) . تم إختبار ٢٢٦ مزرعة لمقدرتها على إنتاج المركبات المضادة للبكتريا .١٦ عزلة فقط من ٢٢٦ مزرعة من بكتريا حامض اللاكتيك تستطيع تثبيط نمو العزلات المختبرة ضدها . بينما أظهرت ٧ مزارع مختارة من بكتريا حامض اللاكتيك تضمنت ٣ عزلات من *Enterococcus fecalis* و٢ عزلة من كلا من *E.faecium* و *E. gallinarum* و *E.malodoratus* مازالوا قادرين على تثبيط نمو العزلات المختبرة ضدها بالرغم من معادلة pH الى ٦.٨ هذه العزلات ربما تستطيع أنتاج المواد المشابهة للبكتريوسين .