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Research Paper

Antibiotic resistance in lactic acid bacteria isolated from some pharmaceutical and dairy products

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Abstract

A total of 244 lactic acid bacteria (LAB) strains were isolated from 180 dairy and pharmaceutical products that were collected from different areas in Minia governorate, Egypt. LAB were identified phenotypically on basis of morphological, physiological and biochemical characteristics. *Lactobacillus* isolates were further confirmed using PCR-based assay. By combination of phenotypic with molecular identification *Lactobacillus* spp. were found to be the dominant genus (138, 76.7%) followed by *Streptococcus* spp. (65, 36.1%) and *Lactococcus* spp. (27, 15%). Some contaminant organisms such as (*Staphylococcus* spp., *Escherichia coli*, *Salmonella* spp., mould and yeast) were isolated from the collected dairy samples but pharmaceutical products were free of such contaminants. Susceptibility of LAB isolates to antibiotics representing all major classes was tested by agar dilution method. Generally, LAB were highly susceptible to Beta-lactams except penicillin. Lactobacilli were resistant to vancomycin, however lactococci and streptococci proved to be very susceptible. Most strains were susceptible to tetracycline and showed a wide range of streptomycin MICs. The MICs of erythromycin and clindamycin for most of the LAB were within the normal range of susceptibility. Sixteen *Lactobacillus*, 8 *Lactococcus* and 8 *Streptococcus* isolates including all tetracycline and/or erythromycin resistant strains were tested for the presence of tetracycline and/or erythromycin resistant genes [*tet*(M) and/or *erm*(B)]. PCR assays shows that some resistant strains harbor *tet*(M) and/or *erm*(B) resistance genes.

Key words: lactic acid bacteria, phenotypic and molecular identification, PCR assay, antibiotic resistance genes.

Introduction

The Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) defined a probiotic as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO/WHO, 2002). Lactic acid bacteria (LAB) have received considerable attention as probiotics over the past few years. This concept has grown from traditional dairy products to a profitable market of probiotic health supplements and functional foods. Extensive research is done on novel potential probiotic strains, with specific emphasis on their health benefits and mode of action (Dicks and Botes, 2010).

Probiotic strains of lactobacilli are used in different medical and health-related areas including the control of intestinal inflammation; treating infections during pregnancy; management of allergic diseases; control of antibiotic-related diarrhea and prevention of urinary tract infections (Bernardeau *et al.*, 2008). Also, LAB have a role in the treatment of people suffering with tumors and immunocompromised subjects. This may add many components to conventional therapies, which have relatively low toxicity compared to other treatments (Wood, 1992). Commensal LAB can be used to deliver vaccines and other biologically active material to the gastrointestinal tract. Their use in vaccine delivery is of special value in stimulating mucosal immunity that is protective at the site of pathogen entry (Hollmann *et al.*, 2010).

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Regarding the safety assurance of probiotic organisms in food, FAO/WHO (2002) guidelines suggest testing probiotic strains for antibiotic resistance patterns. Investigation of the antibiotic resistance profiles of LAB is motivated by three fundamental reasons. First is the possibility of exchange of resistance factors with other microorganisms, with the risk of transferring these genes to many pathogenic bacteria. Second, lactobacilli have been reported as the etiological agents in some cases of endocarditis that can be only controlled by antibiotic therapy (Salvana and Frank, 2006). Finally, the optimization of the use of probiotic lactobacilli in cases of gastrointestinal disorders requires the knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy (Salminen *et al.*, 1998).

Selective pressure of using antibiotic organisms in both human and animal treatment, and dissemination of antibiotic resistance bacteria has the possibility to aggravate acquisition and spread of resistant genes. In this context, probiotic organisms are considered to pool the resistant genes and transfer these to pathogenic bacteria. In order to eliminate this possibility, MIC of the most relevant antimicrobials for each strain used as a probiotic organism could be determined (Rabia and Shah, 2011). Phenotypic assays for characterizing LAB as being either susceptible or resistant to antibiotics have now been complemented by molecular methods, which directly screen for the presence of antibiotic resistance determinants (Perreten *et al.*, 2005). Two of the most commonly observed resistance genes found in LAB so far are *tet*(M) for tetracycline and *erm*(B) for erythromycin resistance (Cataloluk and Gogebakan, 2004).

The aim of this study was to accurately identify the dominant LAB that occur in pharmaceutical and dairy products. We use both phenotypic methods and genotypic methods. In addition, to assess the safety of the collected products by detection of contaminants, determination of the antibiotic resistance patterns of LAB strains and to identify the antibiotic resistance determinants through the use of PCR-based molecular methods.

Material and Methods

Bacterial strains

A total of 176 dairy and 4 pharmaceutical probiotic products, were collected during the study period from November 2009 to May 2010 from different markets and pharmacies in Minia governorate, Egypt. *Lactobacillus* and *Lactococcus* strains were isolated onto de Man Rogosa Sharpe (MRS) agar (Oxoid, UK), whereas streptococci were isolated onto M17 medium (Oxoid, UK). The isolated strains were stored on selective broth supplemented with 15% glycerol at -20 °C.

Identification of LAB

Phenotypic identification

The identification of LAB was performed according to their morphological, cultural and biochemical characteristics as described (Collins and Lyne, 1980).

Genotypic Identification of Lactobacillus isolates

Lactobacillus isolates were identified to the genus level using PCR-based assay:

a. Bacterial DNA extraction. Bacterial cells were grown in 10 mL MRS broth for 18 h at 37 °C. A 500-µL aliquot of each culture were mixed with 500 µL cetyltrimethyl ammonium bromide (CTAB) buffer (50 mM hexadecyltrimethyl ammonium bromide, 1.4 mol L^{-1} NaCl 100 mmol L^{-1} Tris-HCl at pH 8.0, 20 mmol L^{-1} EDTA, 0.2% β-mercaptoethanol), incubated at 65 °C for 30 min and then centrifuged at 12,000 *g* for 10 min. The supernatant was transferred to a new 1.5 mL Eppendorf tube, precipitated with one volume of isopropanol and centrifuged at 12,000 *g* for 10 min. After discarding the supernatant, the pellet was washed with 500 µL of 70% v/v ethanol before drying for 10 min. The pellet was dissolved in 100 µL Tris-EDTA buffer (10 mmol L^{-1} Tris-HCl at pH 8.0, 1 mmol L^{-1} EDTA) and stored at -18 °C (Picozzi *et al.*, 2006).

b. PCR for identification of the genus *Lactobacillus.* Identification of *Lactobacillus* strains was performed by genus-specific PCR primers targeted to the 16S/23S ribosomal RNA intergenic spacer region (Dubernet *et al.*, 2002). The sequences of the primers were taken from GenBank sequence database of the National Center for Biotechnology Information. 5' - CTC AAA ACT AAA CAA AGT TTC -3' was used as a forward primer and 5' -CTT GTA CAC ACC GCC CGT CA- 3' was used as a reverse primer. The primers were synthesized by the Midland Certified Reagent Company Inc. (Texas, USA).

The reaction mixture (50 µL) contains 2.5 µL of each forward and reverse primer (20 pmol of each), 100 ng (4 µL) of the extracted DNA, 25 µL of GoTaq® Green Master Mix (Promega) and 16 µL of distilled water. PCR amplification was performed in a DNA thermal cycler (UNO II Thermocycler; Biometra GmbH, Gottingen, Germany) with the following temperature program: initial denaturation at 95 °C for 5 min; 20 cycles of 95 °C for 30 s (denaturation), 55 °C for 30 s (annealing), and 72 °C for 30 s (extension); and a final extension step at 72 °C for 7 min.

Agarose gel electrophoresis was conducted using 1% agarose. The Gene Ruler 100 bp DNA ladder (Fermentas, USA) was used. The gel was stained with ethidium bromide 0.5 mg/mL and observed under UV transilluminator for the presence of DNA bands.

Isolation and identification of contaminant strains. Some contaminants were isolated from the previous 180

pharmaceutical and dairy samples. Identification of these strains was performed according to the procedures described (Benson, 2002).

Determination of antimicrobial susceptibilities of LAB. Minimum inhibitory concentrations (MICs) of 19 antibiotics (Oxoid, UK) were determined by the agar dilution method, according to the Clinical and Laboratory Standards Institute (CLSI) (2007).

PCR detection of *tet*(M) and *erm*(B) genes. Isolates that were resistant to tetracycline or erythromycin were subjected for PCR-based detection of *tet*(M) and *erm*(B) genes respectively. DNA extraction was performed according to the standard protocols mentioned earlier. Each PCR reaction (total volume, 50 μL) contained 2 μL of each primer (20 pmol of each), with 50 ng (4 μL) of the extracted DNA and 25 μL of GoTaq® Green Master Mix (Promega) and 17 μL of distilled water. The sequences of the primer used and their amplicon sizes are listed in Table 1. The primers were synthesized by the Midland Certified Reagent Company Inc. (Texas, USA).

PCR-based detection of the *tet*(M) gene was performed using the following thermal cycles: 95 °C for 5 min; 95 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s (25 cycles); and 72 °C for 7 min. While for *erm*(B) the thermal cycling program was as follows: 94 °C for 5 min; 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min (30 cycles); and 72 °C for 10 min. Amplification products were detected by electrophoresis on a 1% agarose and subsequent staining with ethidium bromide solution.

Results

Isolation and identification of LAB

A total of 244 LAB isolates were recovered from 180 dairy and pharmaceutical samples collected from different markets and pharmacies. Out of the 244 isolates, 152 were *Lactobacillus* spp., 27 were *Lactococcus* spp. and 65 were *Streptococcus* spp.

Physiological and biochemical tests for identification of LAB

The isolates were identified phenotypically as illustrated in Table 2. Based on these characters, the coccid LAB isolates were characterized as mesophilic homofermentative cocci, 27 isolates, and so they belonged to *Lactococcus* spp. and thermophilic homofermentative cocci; 65 isolates. All strains of second group promoted growth at thermophilic conditions, failed to grow in 4 and 6.5% NaCl concentration and so they belonged to *Strepto-*

Table 1 - Primers for PCR detection of *tet*(M) *and erm*(B) genes.

Primers	Sequence (5'-3')	Amplicon size (bp)	PCR type	Reference for primers
tet(M) forward	GGTGAACATCATAGACACGC	401	*tet*(M)	(Werner *et al*., 2003)
tet(M) reverse	CTTGTTCGAGTTCCAATGC			
erm(B) forward	CATTTAACGACGAAACTGGC	405	*erm*(B)	(Jensen *et al*., 1999)
erm(B) reverse	GGAACATCTGTGGTATGGCG			

Table 2 - Phenotypic characteristics of the isolated LAB strains.

	Lactobacillus spp.(152)	*Lactococcus* spp.(27)	*Streptococcus* spp. (65)
Gram straining	+	+	+
Cell shape and arrangement	Rods arranged in single, pairs and short, long chains	Cocci arranged in single, pairs and chains	Cocci arranged in pairs and long chains
Catalase test	–	–	–
CO_2 production from glucose	Homofermentative (-) (126) Heterofermentative (+) (26) *	Homofermentative (-)	Homofermentative (-)
Growth at 15 °C	–	+	–
Growth at 37 °C	+	+	+
Growth at 45 °C	+ (32)**	–	+
Growth in 4% NaCl	+	+(22)***	–
Growth in 6.5% NaCl	+	–	–
Citrate utilization	–	–	–

* 126 homofermentative, 26 heterofermentative lactobacilli (152).
** 32 thermophilic lactobacilli (152).
*** 22 lactococci tolerate 4% NaCl concentration (27).

coccus spp.. All the isolates lacked reduction of citrate. Lactobacilli bacteria (152 isolates) were represented by 3 groups; (i), mesophilic homofermentative lactobacilli, 94 isolates; (ii), thermophilic homofermentative lactobacilli, 32 isolates and (iii) mesophilic heterofermentative lactobacilli, 26 isolates. All strains grow in 4 and 6.5% NaCl concentration.

Genotypic identification of *Lactobacillus* spp.

Out of the 152 *Lactobacillus* isolates 138 were confirmed using PCR-based assay. When DNA from the *Lactobacillus* strains were used as a template, a 250 bp PCR product was obtained for all the tested strains (Figure 1).

So by combination of both phenotypic and genotypic identification it was found that the *Lactobacillus* isolates were the most dominant genus (138, 76.7%) followed by *Streptococcus* (65, 36.1%) and *Lactococcus* isolates (27, 15%). Table 3 illustrates the incidence of the isolated LAB in relation to the different samples.

Incidence of contaminant strains in pharmaceutical and dairy samples. Out of the 180 pharmaceutical and dairy samples, 123 (68.3%) contaminant strains were isolated and identified. Out of the 123 isolates, 48 were *Staphylococcus* spp. (39%), 16 were *E.coli* (13%), 8 were *Salmonella* spp. (6.5%) and 51 were mould and yeast (41.5%). No contamination was observed in pharmaceutical samples.

Antibiotic susceptibility and determination of MICs. Tested strains of LAB demonstrated different profiles of antibiotic resistance. Table 4 shows the distributions of MICs of different antibiotics among LAB isolates. When resistance to Beta-lactams was tested, most LAB isolates showed more susceptibility to ampicillin and amoxicillin. The highest prevalence of penicillin resistance was shown among the isolates of *Lactobacillus* spp. (20.3%). Nearly high percentage of *Lactobacillus* isolates showed intermediate resistance to cephalexin and a low percentage were resistant to cefoperazone. All *Lactococcus* isolates were sensitive to cefoperazone.

Lactobacillus strains were highly resistant to vancomycin (40.6%) and streptomycin (17.4%). All *Lactococcus* and *Streptococcus* isolates were susceptible to vancomycin. High-level of resistance to nalidixic acid, ciprofloxacin and norfloxacin and low-level of resistance to chlormphenicol was detected in LAB isolates. All *Lactococcus* isolates were susceptible to chlormphenicol. Variations in the susceptibility of erythromycin against LAB were observed. Higher percentage of erythromycin

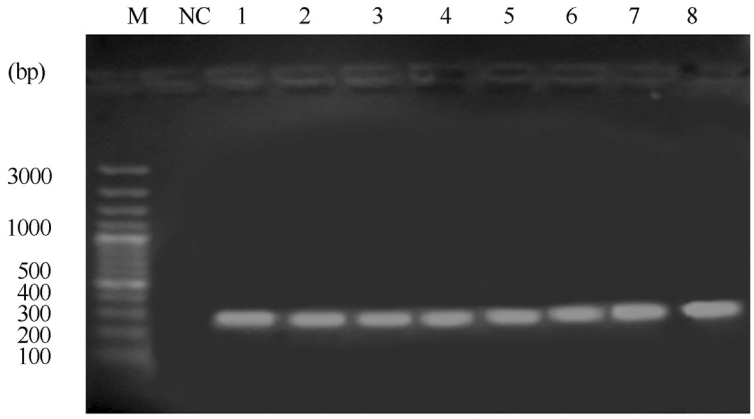

Figure 1 - PCR analysis of some *Lactobacillus* spp. Lane M: 100-bp marker, Lane NC: Negative control, Lane 1-8: 250-bp band of *Lactobacillus* spp.

Table 3 - Incidence of the isolated LAB in relation to the different types of samples.

Sample type	Number of samples (n = 180)	LAB isolates (n = 230)	*Lactobacillus* isolates (138)		*Lactococcus* isolates (27)		*Streptococcus* isolates (65)	
			No.	%*	No.	%*	No.	%*
Cheese	68	91	55	80.9	13	19.1	23	33.8
Yogurt	35	71	33	94.3	6	17.1	32	91.4
Fresh Milk	33	22	16	48.5	3	9.1	3	9.1
Fermented milk	30	34	25	83.3	4	13.3	5	16.7
Cream	10	8	5	50	1	10	2	20
Pharmaceutical products	4	4	4	100	0	0	0	0

*Percentage was correlated to the number of LAB isolates collected from each type of samples.

Table 4 - MIC distribution, antibiotic resistance and MIC_{90} of the isolated LAB (138 lactobacilli, 27 lactococci and 65 streptococci)

Antibiotic	Genus	Break point* µg/mL	MIC (µg/mL) **												MIC_{90}***	Resistant	%****
			≤ 0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512			
Penicillin G	Lb	4	36	20	19	14	21	14	12	2					8	28	20.3
	Lc	4		20	4	0	1	2							1	2	7.4
	St	4	10	22	20	10	2	1							2	1	1.5
Ampicillin	Lb	4	40	31	32	29	4		2						2	2	1.4
	Lc	4	3	9	8	7									2	0	0
	St	4	17	24	12	11		1							2	1	1.5
Amoxycillin	Lb	4	42	35	20	22	14	3	2						4	5	3.6
	Lc	4	5	8	6	5	2	1							2	1	3.7
	St	4	16	18	20	7	2	2							2	2	3.1
Amp/Sul*****	Lb	8	49	43	22	21	2		1						2	1	0.7
	Lc	8	11	10	4	2									1	0	0
	St	8	32	15	9	8	1								2	0	0
Amox/Clav*****	Lb	4	66	34	13	16	5	2	2						2	4	2.9
	Lc	4	8	10	4	4	1								2	0	0
	St	4	12	23	23	5	1		1						1	1	1.5
Cephalexin	Lb	16		4	29	26	25	13	12	18	7	4			32	29	21
	Lc	16	1	5	8	4	2	1	3	3					16	3	11.1
	St	16			15	15	12	10	6	5	2				16	7	10.8
Cefuroxime	Lb	8	36	23	24	20	15	10	4	3	3				8	10	7.2
	Lc	8	2	2	6	7	4	3		2	1				8	3	11.1
	St	8		13	17	14	13	5		3					8	3	4.6
Cefoperazone	Lb	16	38	24	21	20	11	9	7	5	3				16	8	5.8
	Lc	16	9	5	2	3	4	2							4	0	0
	St	16	18	11	12	3	7	6	5		3				16	3	4.6
Vancomycin	Lb	4	18	28	23	8	5				6	16	21	13	256	56	40.6
	Lc	4	10	8	6	3									1	0	0
	St	4	16	18	13	13	5								2	0	0
Gentamicin	Lb	8	25	26	11	19	20	16	12		8	1			16	21	15.2
	Lc	8		7	8	6		2	2	1	1				16	4	14.8
	St	8	11	31			10	5	3	4		1			16	8	12.3
Streptomycin	Lb	16		11	14	12	31	24	22	7	7	2	6	2	64	24	17.4

Table 4 (cont.)

Antibiotic	Genus	Break point* µg/mL	MIC (µg/mL) **											MIC_{90}***	Resistant	%****
			≤ 0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512			
	Lc	16			2	3	9	6	4	1	2				16	3	11.1
	St	16		13	12	10	9	8	8	2	2	1			16	5	7.7
Nalidixic acid	Lb	4		23	30	23	16	3	12	15	5	7	2	2	64	46	33.3
	Lc	4	4	7	8		2		1	3	2				32	6	22.2
	St	4	4	25	9	11	5	3	4	1	3				16	11	16.9
Ciprofloxacin	Lb	4	39	26	17	16	9	10	7	9	5				16	31	22.5
	Lc	4	6	7	6	4	1	1	1	1					4	3	11.1
	St	4	14	3	15	18	9	5	1						4	6	9.2
Norfloxacin	Lb	4	21	27	29	14	12	12	4	4	13	2			64	35	25.4
	Lc	4	5		8	3	7		3		1				16	4	14.8
	St	4		13	17	20	9	2	2	2					4	6	9.2
Erythromycin	Lb	4	31	37	32	21	9		1	2	1		3	1	4	8	5.8
	Lc	4	6	12	5	2			4		1		1		2	6	22.2
	St	4	11	20	12	10	6	2							4	2	3.1
Clindamycin	Lb	4	20	24	35	32	11	2	4	3	4	2		1	8	16	11.6
	Lc	4	5	5	8	7	2								2	0	0
	St	4	10	22	12	11	8			1			1		4	2	3.1
Tetracycline	Lb	8	11	26	31	33	11	10			8	3	3	2	64	16	11.6
	Lc	4	9	4	3		3			2	4	2			64	8	29.6
	St	4	2	8	11	19	17			5	1		1	1	32	8	12.3
Chloramphenicol	Lb	4		31	44	39	19	3	2						4	5	3.6
	Lc	8			8	13	5	1							4	0	0
	St	8	8	5	17	25	10	7		1					4	1	1.5
Rifampicin	Lb	4	41	33	20	12	16	2	6	6	2				8	16	11.6
	Lc	4	16	2	6		1	1		1					1	2	7.4
	St	4	23	5	5	12	13		7						4	7	10.7

*Break points of different antibiotics according to (CLSI, 2007).
**MIC: minimum inhibitory concentration.
***MIC_{90}: minimum inhibitory concentration for 90% of isolates.
****Percents were correlated to the number of isolates of each microorganism.
Lb: *Lactobacillus* spp., Lc: *Lactococcus* spp., St: *Streptococcus* spp.

resistant strains was observed among lactococci (22.2%). *Lactococcus* isolates showed high resistance to tetracycline (29.6%) followed by *Streptococcus* (12.3%) and *Lactobacillus* isolates (11.6%).

PCR detection of *tet*(M) and *erm*(B) resistance genes

Eighteen *Lactobacillus* spp. isolates, 9 *Lactococcus* spp. isolates and 9 *Streptococcus* spp. isolates including all tetracycline and/or erythromycin resistant strains were tested for the presence of *tet*(M) and *erm*(B) antibiotic resistance genes corresponding to their resistance phenotypes. Some strains were to be positive for one or both genes, giving a 401-bp band for *tet*(M), and a 405-bp band for *erm*(B) genes (Table 5 and Figures 2, 3).

Discussion

LAB are regarded as a major group of probiotic bacteria (Bernardeau *et al.*, 2008). In the present work, the incidence of LAB was studied. *Lactobacillus* was the most prevalent genus isolated from dairy and pharmaceutical products (76.7% of total samples). This consistent with the finding of Raquib *et al.* (2003). On the other hand, the results of Harun-ur-Rashid *et al.* (2007) contradict with our finding. They isolated a total of 266 strains of LAB from 28 Dahi samples with *Streptococcus* (50%) as the most dominant genus followed by *Lactobacillus* (27%), and *Lactococcus* (5%).

LAB were initially identified phenotypically on basis of morphological, physiological and biochemical characteristics. PCR analysis was then used for identification of *Lactobacillus* isolates. Out of the 152 isolates identified phenotypically, 138 were confirmed as *Lactobacillus* representing only 56.6% of total LAB isolates. We observed some false positive results after phenotypic characterization compared with molecular identification of genus *Lactobacillus* that could be attributed to the experimental conditions used in isolation and identification. Also Wang *et al.* (2008) agreed with us in the necessity of combination of conventional identification with molecular techniques in order to obtain more exact results.

In order to examine the safety of collected products, the percentage of contamination was determined in the collected samples. We found that 123 contaminant strains were recovered from the dairy samples, whereas pharmaceutical products were contaminant free. These results can be explained by the fact that the methods of production of the various traditional foods are usually primitive and the major risk enhancing factors such as the use of contaminated raw materials, lack of pasteurization and inadequate fermentation and storage conditions. These conditions with the antibiotic resistances among LAB require more attention to be focused on the usage and safety of these beneficial strains in dairy samples. Similar results were obtained by Soomro and Masud (2007) who isolated some contaminants such as *Staphylococcus, Micrococcus* and *Saccharomyces* spp. from randomly collected market dahi samples from Rawalpindi, Pakistan.

Our study revealed high susceptibility of LAB isolates to ampicillin and amoxicillin and more resistance to cephalosporins, also, high vancomycin resistance rate was observed. This is corroborated by data from other groups (Ammor *et al.*, 2007). The strains tested in this study showed also a high susceptibility toward erythromycin and tetracycline. These observations confirmed the data reported by Danielsen and Wind (2003). In contrast to our results Hoque *et al.* (2010) found that the *Lactobacillus* spp. were sensitive to clindamycin and highly resistant to tetracycline. Our results agreed with those of Ammor *et al.*

Table 5 - *tet*(M) and *erm*(B) antibiotic resistance genes detected in LAB isolates.

Genus	Number of examined strains	Relevant phenotype	Genes detected by PCR
Lactobacillus spp.	3	Tet^r, Er^r	tet(M), *erm*(B)
	3	Tet^r, Er^r	*tet*(M)
	9	Tet^r	*tet*(M)
	1	Tet^r	——
	2	Er^r	*erm*(B)
Lactococcus spp.	1	Tet^r, Er^r	*tet*(M), *erm*(B)
	4	Tet^r, Er^r	*tet*(M)
	1	Tet^r	*tet*(M)
	2	Tet^r	——
	1	Er^r	*erm*(B)
Streptococcus spp.	1	Tet^r, Er^r	*tet*(M)
	3	Tet^r	*tet*(M)
	4	Tet^r	——-
	1	Er^r	——-

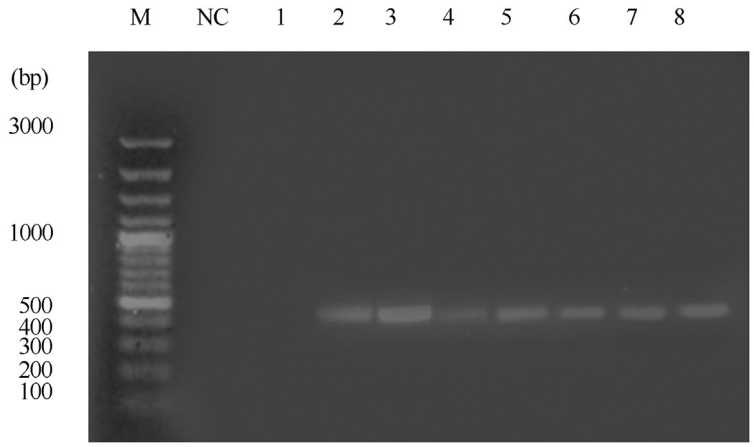

Figure 2 - PCR detection of tet(M) resistance gene in some *Lactobacillus* spp. Lane M: 100-bp marker, lane NC: Negative control, lane 2-8: 401-bp band of *tet*(M) gene, lane 1: no bands with DNA [no *tet*(M) gene].

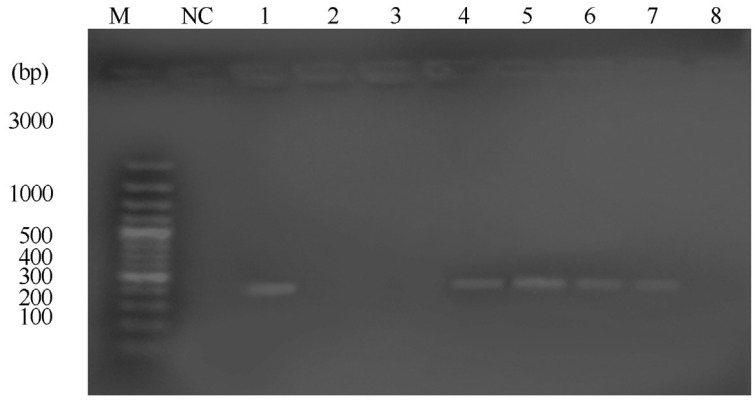

Figure 3 - PCR detection of *ermB* resistance gene in *Lactobacillus* spp. Lane M: 100-bp marker, Lane NC: Negative control, Lane 1, 4, 5, 6 and 7: 405-bp band of *ermB* gene, Lane 2, 3 and 8: no bands with DNA (no *erm*B gene).

(2007) who reported that the resistance of many *Lactobacillus* species toward vancomycin has been often described as intrinsic. However, Lim *et al.* (1995) found that isolated *Lactobacillus* spp. were susceptible to vancomycin but resistant to gentamicin and streptomycin.

Phenotypic assays that used to determine the antibiotic susceptible/resistant patterns have been complemented by molecular methods in which bacterial strains are directly screened for the presence of antibiotic resistance determinants. We use PCR for detection of *tet*(M) and *erm*(B) resistance genes in LAB. It was found that some strains harbor *tet*(M) and/or *erm*(B) genes and others that were previously showed tetracycline or erythromycin resistant patterns, were found to be negative for *tet*(M) or *erm*(B) genes respectively. These false results can be explained by the fact that there is currently no standard method for antibiotic susceptibility testing of LAB, although several microdilution methods have been used. Also, many factors may affect the susceptibility results such as the inoculum size, the incubation time, the incubation temperature, the composition of the atmosphere and the growth medium. An increased inoculum size and an extended incubation time resulted in elevated antibiotic MICs for some species (Egervärn *et al.*, 2007).

Nawaz *et al.* (2011) reported that out of 84 LAB strains, *erm*(B) gene was detected in eight *Lactobacillus* strains and one *Streptococcus thermophilus* strain. The *tet* genes were identified in 12 strains of lactobacilli from traditional foods which is consistent with our results. Also, detection of *tet*(M) and *erm*(B) resistance genes have been previously investigated (Devirgiliis *et al.*, 2010; Toomey *et al.*, 2010).

Conclusion

This study had established that wide variety of LAB are present in the Egyptian products and lactobacilli are considered to be one of the most important potential probiotics. Accurate characterization and identification of LAB and the precise screening for the presence of antibiotic resistance determinants requires the combined use of phenotypic properties and molecular methods since, conventional methods are time-consuming and not fully reli-

able. Also, isolated and identified LAB from pharmaceutical products show higher safety properties regarding contamination and antibiotic resistance if compared with commercial dairy products in this study. This is attributed to the more strict quality control measures and the proper characterization and maintenance of starter culture strains during the production of pharmaceutical products.

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