Egyptian Journal of Medical Microbiology Volume 27 / No.4 / October 2018 123-132 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Characterization of Some Probiotic Bacteria Isolated from Different Dairy Products at Assiut Governorate

[1]Sherein G. Elgendy*, [1]Wegdan A. Mohamad, [2]Amany I. Ali, [1] Ismael S. Mohamad
[1]Department of Medical Microbiology & Immunology, Faculty of Medicine, Assiut University,
[2]The general Administration for Feeding, Assiut University, Egypt

Key words:
Probiotics, Lactobacillus, Bifidobacterium, PCR, Enzymatic activity

Corresponding Author:
Sherein G. Elgendy
Department of Medical Microbiology and Immunology
Faculty of Medicine , Assiut University, Egypt
Tel.: 20-01021887728
Shereinelgendy@yahoo.com

ABSTRACT

Background: *Probiotics are health promoting microorganisms incorporated into various kinds of food. Members of Lactobacillus and Bifidobacterium are the most commonly used.* ***Objectives:*** *This study aimed to isolate Lactobacillus and Bifidobacterium from different products at Assiut Governorate, to detect its antibacterial activities against pathogenic bacteria, to evaluate probiotic, haemolytic and enzymatic activates, to compare between conventional methods for identification and PCR as gold standard.* ***Methodology:*** *Isolation and identification of Lactobacillus and Bifidobacterium by conventional methods. Probiotic properties were determined by growth at different temperatures, different NaCl concentrations, pH and bile tolerance. Antibacterial activity examined against three indicator pathogenic organisms. PCR was used for identification of Lactobacillus and Bifidobacterium species. Also haemolytic and enzymatic activities were determined.* ***Result :*** *The identified Lactobacillus spp. were L. acidophilus 54(29.5%), L. fermentum 50(27.3%), L. rhamnosus 34 (18.6%), L. plantarum 27(14.8%), L . paracasei 13(7.1%) and L.GG 5(2.7%) .The Bifidobacterium species were B. Breve 59(31.7%), B. dentium 42 (22.6 %) , B . bifidum 53 (28.5%) , B . subtile 15(8%) B. longum 6(3.2%), B . animalis 7 (3.7%) and B. infantis 4 (2.2%). All the isolates tolerated low pH and bile salts, non heamolytic and had good antibacterial activity. The sensitivity and specificity of the conventional methods was (94.7%, 95.97%) and (93.86%, 94.97%) in Lactobacillus and Bifidobacterium respectively. Isolated strains produced β-galactosidase which is beneficial for lactose intolerance and did not produce carcinogenic enzymes.* ***Conclusion:*** *Rayeb, yogurt, milk powder and cereals can be used as potential source of probiotics because they tolerate acidic media, bile salts, have good antibacterial activity. Also, they have beneficial enzymatic activities. PCR was a rapid method for identification of probiotic strains.*

INTRODUCTION

Probiotics are microorganisms when administrated in adequate amount provide health benefits, the most common types of microbes used as probiotics are Lactic acid bacteria (LAB) and *Bifidobacteria* [1].

Several factors must be considered when using probiotic bacteria in fermented products .Primarily, the probiotics must be non- pathogenic, non- toxic, viable, capable of remaining viable for periods under storage conditions, and present in high count at time of consumption to achieve the desired benefits [2].

The beneficial effects of probiotics include treatment of antibiotic associated diarrhoea, inhibition of pathogenic microorganisms, anticarcinogenic and antimutagenic activities**,** lactose intolerance, allergies, hypocholesterolemic properties, hepatic disease and *Helicobacter pylori* infections [3].

This study aimed to isolate *Lactobacillus* and *Bifidobacterium* from different dairy products at Assiut Governorate, to detect its growth inhibition activities against pathogenic bacteria, to evaluate probiotic, haemolytic and enzymatic activites. Also to compare between conventional methods for identification and PCR as gold standard.

METHODOLOGY

Rayeb milk and pasteurized yogurt were collected from supermarkets, milk based weaning cereals and milk powder were collected from pharmacies. The samples were immediately transported to the laboratory in an icebox.

Preparation of samples:

Pour plate method was used for *Lactobacillus* counting, tenfold serial dilutions up to 10^{-7} were prepared in peptone-physiological saline solution. For *Bifidobacteria* counting, tenfold serial dilutions up to 10^{-7} were prepared in ml peptone– physiological saline solution supplemented with L –cystine hydrochloride to provide anaerobic condition [4].

Isolation of *Lactobacillus* and *Bifidobacterium*:

De Man Rogosa Sharpe agar was used for *Lactobacilli* [5] and Bifidobacterium Media (BFM) for *Bifidobacterium*[6].

Identification of the isolates:

Morphological identification by Gram stain[4] and Motility test [7].

Biochemical identification was done by conventional methods including Indole production test [8], Catalase and oxidase test [9].

Biotyping was done by Carbohydrate fermentation for *Lactobacillus* [10] and for *Bifidobacteria*[11], (Table 1&2).

Table 1: Characterization of *Lactobacillus* strains by carbohydrate fermentation

Identified Species	*L.acidophilus*	*L. plantarum*	*L. paracasei*	*L. rhamnosi*	*L. fermentum*	*L. rhamnosus GG* (*L.GG*)
Arabinose	−	−	-	-	+	-
Fructose	+	+	+	+	+	+
Lactose	+	+	+	+	+	-
Mannose	+	+	+	+	-	+
Mannitol	-	+	+	-	-	+
D- Ribose	-	+	+	+	+	+
Sorbitol	-	+	−	−	-	+
Threhalose	-	−	+	+	-	-
Xylose	-	-	-	-	+	-

Table 2: Characterization of *Bifidobacterium* strains by carbohydrate fermentation

	Lactose	Sucrose	Mannitol	Sorbitol	Xylose	D-ribose	Maltose	Trehalose	Arabinose
B. bifidum	+	±	-	-	-	±	-	-	-
B. longum	+	+	-	-	±	+	+	-	+
B. infantis	+	+	-	-	±	+	+	-	-
B. breve	+	+	±	±	-	+	+	±	-
B. dentium	+	+	+	-	+	+	+	+	+
B. subtile	-	+	-	+	-	+	±	±	-
B. animalis	+	+	-	-	+	+	±	±	+

Probiotic properties of isolates:

1. ***Growth at Different Temperatures*[12]:** Overnight incubated cultures were inoculated into MRS tubes containing bromecresol purple indicator and incubated for 7 days at 15 °C and 45 °C. Growth was observed by the color change from purple to yellow.
2. ***Growth at Different NaCl Concentrations* [13]:** For this purpose 4% and 6.5% NaCl concentrations were selected.
3. ***Bile tolerance* [14]:** Freshly prepared cultures were inoculated into MRS broth with different concentrations of bile salts at 0, 0.1, 0.3, 0.5 and 0.7 % and incubated at 37°C for 24 h under anaerobic condition. Optical densities (OD) were spectro-photometrically measured at 620 nm after 0, 2, 6, and 24 h.
4. ***pH tolerance*[15]:** The isolated bacterial cultures were inoculated into MRS broth of varying pH 2, 3, 4, and 5 and incubated at 37°C for 48h then inoculated on MRS agar medium and incubated at 37°C for 48 hrs.
5. ***Haemolytic Activity*[16]:** Haemolysis was evaluated on columbia blood agar supplemented with 5% sheep blood.

Study of the antibacterial activity of the isolated probiotic strains on some pathogenic bacteria by well diffusion method[17&18]:

Three indicator strains were used; *Escherichia coli* (NCTC No. 12023), *Bacillus cereus* (DSM No. 351) and *Staph.aureus* (NCTC No. 7447). All strains were obtained from High Quality Media unit (HQM) in Animal Health Research Institute in Dokki, Egypt. Probiotic strains were grown anaerobically in MRS broth for 24 h at 37°C.

Overnight Culture was centrifuged at 5000 rpm at 4°C for 20 min to obtain a cell free supernatant. Muller-Hinton agar was seeded with overnight cultures of the indicator strains then 10 mm wells were made. 100 ul aliquots of sterile neutral supernatant were poured into the agar wells. This was done in duplicates for each test isolate.

The plates were kept at 4 °C for 2 h (to allow for prediffusion of antimicrobial substances) then incubated for 48 h at 37°C, the diameter of the inhibition zone was measured by calipers in millimeters.

PCR:

Reference strains: *L. acidophilus* (DSN 20079), *L. fermentum* (DMS 0049), *L. rhamnosi* (NCTC 6375), *B. dentium* (BDRR16SG), *B. bifidum* (DSN 20082) and *B.*

breve (ATCC 15700) were obtained from Cairo Mircen, Faculty of Agriculture, Ain Shams University, Egypt.

***DNA extraction using QIA amp kit* [19].**

DNA amplification: performed using Thermocycler T100 gradient system (BioRadT100, USA). Primer sequences of *Lactobacillus* spp and *Bifidobacterium* spp used for PCR listed in Table (3) and (4) respectively.

1% Agarose gel electrophoresis for detection of PCR amplification product.

Table 3: Primer sequences of *Lactobacillus* species used for PCR

Species	Primer	Oligonucleotide sequence (5′ → 3′)	Tem	Product size (bp)	References
L. acidophilus	***LA* (F)**	**5′** GATCGCATGATCAGCTTATA**′3**	56°C	124	**20**
	***LA* (R)**	**5′** AGTCTCTCAACTCGGCTATG **′3**			
L. fermentum	***LF* (F)**	**5′** AATACCGCATTACAACTTTG **′3**	57°C	337	**21**
	***LF* (R)**	**5′** GGTTAAATACCGTCAACGTA **′3**			
L. rhamnosi	***LR* (F)**	**5 ′**TGCTTGCATCTTGATTTAATTTTG **′3**	59°C	116	**22**

Table 4: Primer sequences of *Bifidobacteria* species used for PCR

Species	Primer	Oligonucleotide sequence (5′ → 3′)	Tem	Product size (bp)	References
B.bifidum	***BiBIF*-1 (F)**	**5′** CCACATGATCGCATGTGATTG **′3**	59°C	278	**23**
	***BiBIF*-2 (R)**	**5′** CCGAAGGCTTGCTCCCAAA **′3**			
B. breve	***BiBRE*-1 (F)**	**5′** CCGGATGCTCCATCACAC **′3**	55°C	288	**23**
	***BiBRE*-2 (R)**	**5′** ACAAAGTGCCTTGCTCCCT **′3**			
B.dentium	***BiDEN*-1 (F)**	**5′**ATCCCGGGGGTTCGCCT **′3**	57°C	387	**23**
	***BiDEN*-2 (R)**	**5′**GAAGGGCTTGCTTGCTCCCGA **′3**			

Analysis of Enzyme Activity of *lactobacillus* and *Bifidobacterium* isolates[24]:

By API ZYM kit (bio-Mérieux, France) according to manufacturer instructions, Table (5)**.**

Table 5: Reading of APIZYM Kit:

No	Enzymes assayed	Result	
		Positive	**Negative**
1	Control	**Colorless or color of the sample if it has an intense coloration**	
2	Alkaline phosphatase	Violet	Colorless or very pale yellow
3	Esterase	Violet	
4	Esterase Lipase	Violet	
5	Lipase	Violet	
6	Leucine arylamidase	Orange	
7	Valine arylamidase	Orange	
8	Cysteine arylamidase	Orange	
9	Trypsin	Orange	
10	α-chymotrpsin	Orange	
11	Acid phosphatase	Violet	
12	Naphthol-ASL-phosphohydrolase	Blue	
13	α- galactosidase	Violet	
14	β- galactosidase	Violet	
15	β-glucronidase	Blue	
16	α – glucosidase	Violet	
17	β- glucosidase	Violet	
18	N-acetyl-β- glucosaminidase	Brown	
19	α – mannosidase	Violet	
20	α – fucosidase	Violet	

The number of free n mole of hydrolyzed substrate was determined based on the color strengthen; negative reaction; 1, 5 nmol; 2, 10 nmol; 3, 20 nmol; 4, 30 nmol; 5, 40 nmol or higher.

Statistical analysis:

Date entry and data analysis were done using SPSS version 22 (Statistical Package for Social Science). Data were presented as number, percentage, mean, standard deviation and median. Med-calc Program was used to calculate sensitivity, specificity. P-value considered statistically significant when $P < 0.05$.

RESULTS

This study was done from May 2015 to March 2017 in Department of Microbiology and Immunology, Assiut University at Research Laboratory and PCR Laboratory. The highest average count of *Lactobacillus* and *Bifidobcaterium* were found in rayeb samples (4.16×10^6 cfu/ml.) and (4.56×10^6 cfu/ml) respectively, Table (6).

Table 6: The mean counts of the isolated *Lactobacillus spp.* and *Bifidobacterium spp.*

	Lactobacillus	***Bifidobacterium***	**P-value**
Yogurt:			0.112
Mean ± SD	455,000 ± 185,068	936,000 ± 829,661	
Median (Range)	470,000 (220,000-800,000)	550,000 (340,000-3,000,000)	
Rayeb:			0.405
Mean ± SD	4,160,000 ± 1,165,428	4,560,000 ± 1,030,857	
Median (Range)	4000,000 (2,600,000-6,000,000)	4,700,000 (2300,000-6,000,000)	
Milk powder:			0.910
Mean ± SD	42,700 ± 13,483	43,000 ± 14,306	
Median (Range)	42,000 (24,000-62,000)	41,000 (24,000-66,000)	
Milk based weaning Cereals:			0.197
Mean ± SD	23,600 ± 8,289	19,200 ± 6,125	
Median (Range)	22,000 (12,000-40,000)	18,500 (12,000-32,000)	

The identified *Lactobacillus spp. and Bifidobacteriumspp* were illustrated in Table (7 &8).

Table 7: Distribution of *Lactobacillus spp*. in the examined samples

Lactobacilli spp.	**Pasteurized yogurt No = 200**		**Rayeb No = 100**		**Milk powder No = 40**		**Milk based weaning cereals No = 20**		**Total No = 360**	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
L.acidophilus	29	14.5%	19	19%	3	7.5%	3	15%	54	29.5%
L.fermentum	24	12%	24	24%	2	5%	0	0	50	27.3%
L. rhamnosi	19	9.5%	14	14%	1	2.5%	0	0	34	18.6%
L.plantarum	15	7.5%	7	7%	4	10%	1	5%	27	14.8%
L.paracasei	8	4%	3	3%	2	5%	0	0	13	7.1%
L.GG	4	2	0	0	1	2.5%	0	0	5	2.7%

Table 8: Distribution of *Bifidobacteria spp*. in the examined samples

Bifidobacteria spp.	**Pasteurized yogurt No = 200**		**Rayeb No = 100**		**Milk powder No = 40**		**Milk based weaning cereals No = 20**		**Total No = 360**	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
B. breve	37	18.5%	16	16%	5	12.5%	1	5%	59	31.7%
B. dentium	24	12%	15	15%	2	5%	1	5%	42	22.6%
B. bifidum	29	14.5%	17	17%	3	7.5%	4	20%	53	28.9%
B. subtile	8	4%	6	6%	1	2.5%	0	0	15	8%
B. longum	2	1%	3	3%	1	2.5%	0	0	6	3.2%
B. animalis	5	2.5	2	2%	0	0	0	0	7	3.7%
B. infantis	3	1.5%	1	1%	0	0	0	0	4	2.2

All the examined *Lactobacillus* strains resist to 4% NaCl, while only *L. fermentum* and *LGG* can grow in presence of 6.5% NaCl. All *Lactobacillus* strains grow at 15°C except *L. acidophilus* and *L. fermentum*. While all *Lactobacillus* strains grow at 45°C except *L. plantarum* and *L. paracasei*. All *Lactobacillus* strains grow on pH 2,3 ,4, 5 except *L. paracasei* and *LGG* do not grow at pH 2.

At 0.3 % bile salt concentration, the concentration of bile in the small intestine, all strains were resistant. *L. acidophilus* and *B. breve* were the most resistant with percentage of 94.92% and 93.46% respectively (Figure1and2).

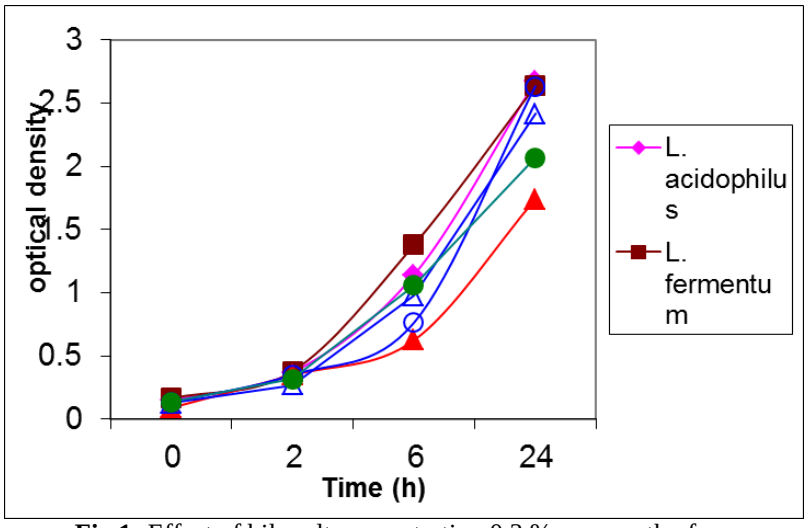


Fig 1: Effect of bile salt concentration 0.3 % on growth of *Lactobacillus* strains at 37°C and optical density of 620 nm.

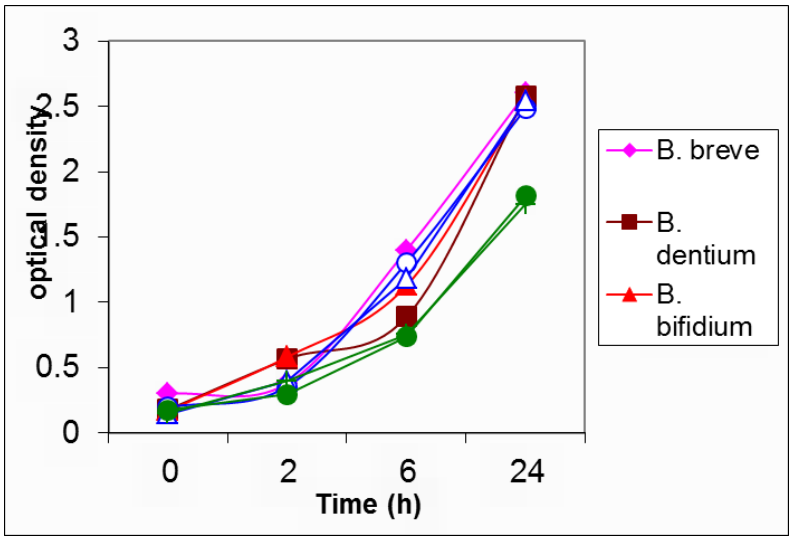


Fig. 2: Effect of bile salt concentration 0.3 % on growth of *Bifidobacterium* strains at 37°C and optical density of 620 nm.

Most isolated strains of *Lactobacillus* and *Bifidobacterium* had antibacterial effect on the examined indicator microorganism,(Figure3and4).

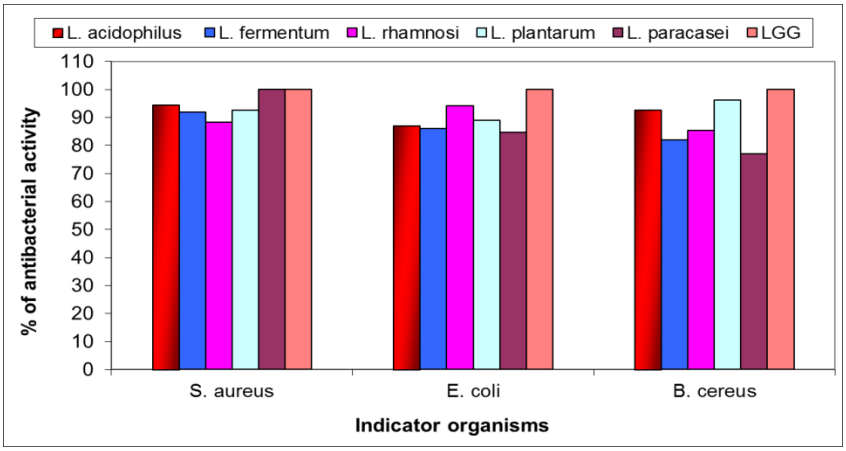


Fig. 3: Antibacterial activity of the isolated *Lactobacilli* strains against reference strain of *Staph. aureus, E. coli and B. cereus* .

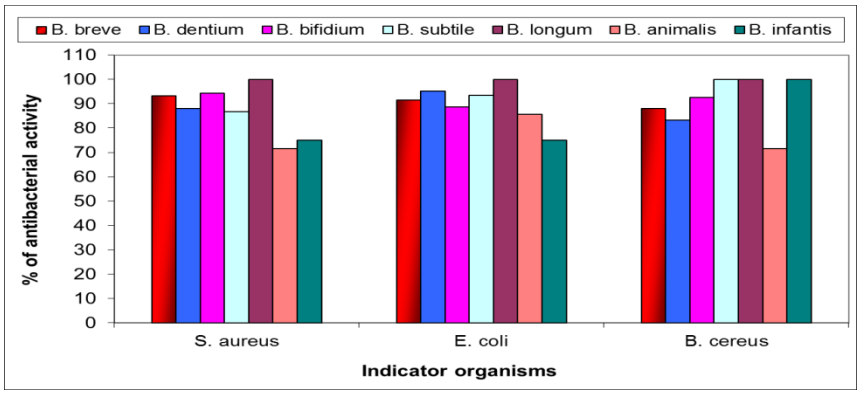
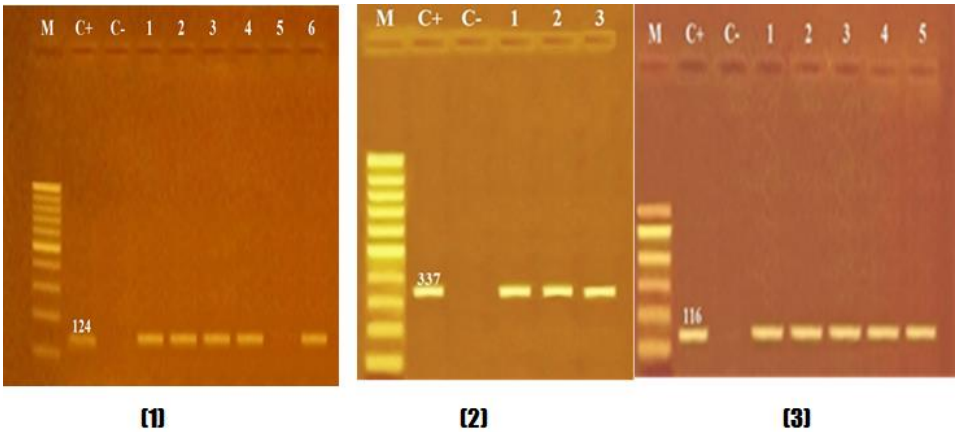


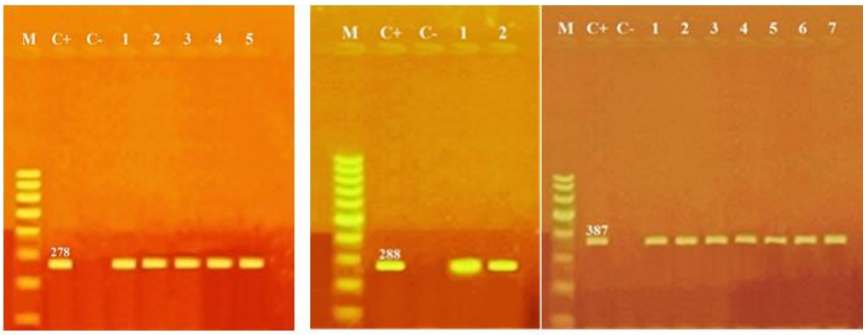
Fig. 4: Antibacterial activity of the isolated *Bifidobacterium* strains against reference strain of *Staph. aureus, E. coli* and *B. cereus* .

The positive PCR samples for *Lactobacillus* species were 130; *L. acidophilus*51 (39.2%), *L. fermentum* 49 (37.7%) and *L. rhamnosi* 30 (23.1%), Photograph (1,2&3) .



Photograph (1,2&3): Agarose gel electrophoresis of PCR of 16SrDNA using LA primer specific for *L. acidophilus*(124 bp), LF primer specific for *L. fermentum* (337 bp) and LR primer specific for *L. rhamnosi* (116 bp) .
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: positive Control of *L. acidophilus*, *L. fermentum*, and *L. rhamnosi*.
Lane C-: negative Control.

The positive PCR samples for *Bifidobacterium* species were 149; *B. berve* 59 (39.6%), *B. dentium* 40 (26.8%) and B. *bifidum* 50 (33.6%), Photograph (4,5&6).



Photograph (4,5&6): Agarose gel electrophoresis of PCR of 16SrDNA using *BiDEN* primer specific for of *B. dentium* (387 bp), *BiBIF* primer specific for *B. bifidum* (278 bp) and *BiBRE* primer specific for *B. breve* (288 bp).
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: positive Control of *B. dentium* , *B. bifidum* and *B. breve*.
Lane C-: negative Control.

Table 9: Frequency of detection of *Lactobacillus* strains in different samples using different laboratory techniques:

		Yogurt (n= 173)		Rayeb (n= 90)		Milk (n= 33)		Cereals (n= 19)	
		No.	%	No.	%	No.	%	No.	%
Conventional methods	**+ve**	72	41.6	57	63.3	6	18.2	3	15.8
	-ve	101	58.4	33	36.7	27	81.8	16	84.2
PCR	**+ve**	71	41.0	50	55.56	6	18.2	3	15.8
	-ve	102	59.0	40	44.44	27	81.8	16	84.2

Table 10: Frequency of detection of *Bifidobacterium* strains in different samples using different laboratory techniques:

		Yogurt (n= 182)		Rayeb (n= 88)		Milk (n= 38)		Cereals (n= 20)	
		No.	%	No.	%	No.	%	No.	%
Conventional method	**+ve**	90	49.5	48	54.5	10	26.3	6	30.0
	-ve	92	50.5	40	45.5	28	73.7	14	70.0
PCR	**+ve**	89	48.9	45	51.1	10	26.3	5	25.0
	-ve	93	51.1	43	48.9	28	73.7	15	75.0

Taking PCR as gold standard, the sensitivity and specificity of the conventional methods was 94.7% and 93.86 respectively in *Lactobacillus*. In *Bifidobacterium spp.* the sensitivity and specificity of the conventional methods was 95.97% and 94.79% respectively.

API ZYM kit showed that *Lactobacillus* and *Bifidobacterium spp*. produced beneficial enzymes as β-galactosidase which is beneficial for lactose intolerance. All the species did not produce β-glucuronidase which has carcinogenic effect.

DISCUSSION

This study included 360 samples from different sources (200 yogurts, 100 rayeb, 40 milk powder and 20 milk based weaning cereals), *Lactobacillus* count in rayeb was 4.16×10^{6} cfu/ ml, in yogurt was 4.55×10^{5} cfu/gm, in milk powder was 4.27×10^{4} cfu/gm and in milk based weaning cereals was 23.6×10^{3} cfu/gm.

According to IDF (1992)[25] the probiotic products should contain 10^{6}-10^{7}cfu/g at time of consumption, the *Lactobacillus* count of rayeb milk samples was in agreement with these results.

Bifidobacterium count in rayeb was 4.56×10^6 cfu/ml, in yogurt was 9.36×10^5 cfu/gm, in milk powder was 4.3×10^4 cfu/gm and in milk based weaning cereals was 19.2×10^3 cfu/gm. According to IDF (1992)[25] *Bifidobacterial* count of rayeb milk samples was considered functional according to the number of *Bifidobacteria.*

From 200 pasteurized yogurt samples the predominantly isolated species were *L .acidophilus* 29 isolates (14.5%), then *L. fermentum* 24 isolates (12%). While *L. rhamnosi, L. plantarum, L. paracasie and L. rhamnosi GG* were present in (9.5 %, 7.5%, 4% and 2%) respectively. Table (7) shows that from 100 rayeb milk samples, only (67%) of samples contained *Lactobacilli spp.* Thirteen samples (32.5%) only of milk powder were positive for *Lactobacillus*. In milk based weaning cereals samples, 4 samples (20 %) only were positive.

Viability of probiotic bacteria depends on species of the probiotics, incubation time, temperature, pH of the final product, and the length of time from inoculation to consumption [26].

Table (8) shows that from all yogurt samples, the most predominantly isolated *Bifidobacteria* were *B. breve* 37 isolates (18.5%), *B. bifidum* 29 (14.5%) and *B. dentium* 24 (12%).In the examined milk powder and milk based weaning cereals, 30% of milk powder and milk based weaning cereals samples were positive for *Bifidobacteria* . The most commonly isolated species in milk powder was *B. breve* 5(12.5%).

Bifidobacteria , in particular , were more susceptible to this kind of stress, requiring strict anaerobic condition[27].

Growth at different temperatures showed that *L. rhamnosi , L. plantarum, L. paracasei* and *L. GG* could grow at 15°C, while *L. acidophilus* and *L. fermentum* could not grow . All species could grow at 45°C except *L. plantarum , L. paracasei* could not grow . These results are in agreement with Abbas and Mahasneh (2014)[28].

Growth at different NaCl concentrations showed that all of the *Lactobacillus* isolates had the ability to grow at 4% NaCl concentration. Only *L. fermentum* and *L.GG* grew at 6.5% NaCl concentration this is in agreement with Pundir *et al* .(2013)[29]. So the isolated *Lactobacillus* strains have good probiotic properties.

All *Lactobacilli* and *Bifidobacteria* isolates were tested for haemolytic activity and gave negative result. This result was agreed with Sandra *et al.* [30].

High tolerance to acid and bile helps probiotic bacteria survive the harsh physical-chemical conditions of GI tract and thus, is a perquisite for bacteria to be used as probiotics[31].

All *Lactobacilli spp.* were resistant to PH 3. While 90.16 % were resistant to PH 2 in accordance with previous results[13].All *Bifidobacteria spp* were resistant to PH 3 but not resistant to PH in accordance with AL-Saadi[32].

The exopolysaccharides produced by bacteria are thought to play a role in the protection of microbial cells against low pH and bile salts[33].

The production of antimicrobial compounds by a probiotic organism provides a competitive edge to their survival and proliferation in GI tract and can also help in eradication of pathogenic bacteria[34].

Fig (3) showed that (94.44 %,87.0%, and 92.59 %) of *L. acidophilus* strains had antibacterial effect on *Staph . aureus, E .coli* and *B. cereus*. This was in aggreament with Karthikeyan and Santhosh[35] who found that *L. acidophilus* was inhibitory to all the pathogens they tested except for the *Vibrio cholera* . Fig (4) showed that (93.20%) of *B.breve* had antibacterial effect against *Staph. aureus*, (91.52%) against *E .coli* and (88.13 %) against *B. cereus*. While Ghita *et al*[36] reported that *B. breve* did not exhibit any antibacterial activities against the used pathogenic indicators .

The inhibition activity of these strains could be attributed to their production of bio-substances with bactericidal or bacteriostatic activities such as bacteriocin , organic acid, and low molecular weight peptides that were inhibitory to the pathogens[18].

Traditional culture methods were labor-intensive and time-consuming. Identification based on phenotypic traits do not always provide clear-cut results, On the other hand, PCR with specific 16S rRNA-based oligonucleotide primers is a powerful method for the detection of target bacteria [37].

Lactobacilli isolates were examined by PCR using LA primer specific for *L. acidophilus*, LF primer specific for *L. fermentum* and LR primer specific for *L. rhamnosi*. The positive PCR samples were 130 samples. Out of these sample 51 *L. acidophilus* (39.2%), 49 *L. fermentum* (37.7%) and 30 *L. rhamnosi* (23.1%) were detected.The sensitivity of the conventional methods was 94.7% and the specificity was 93.86 %.

A total of 154 positive *Bifidobacteria* isolates were examined by using *BiDEN* primer specific for of *B. dentium*, *BiBIF* primer specific for *B. bifidum* and *BiBRE* primer specific for *B. breve*. The positive PCR samples were 149 samples, 59 were *B. berve* isolates (39.6%), 40 *B. dentium* isolates (26.8%) and 50 *B. bifidum* isolates (33.6%). The sensitivity of the conventional methods was 95.97% and specificity was 94.79 %.

From the obtained data, PCR assay provides a more rapid, specific and more sensitive technique alternative to biochemical reaction.

All of the *Lactobacillus* and *Bifidobacteri*a strains exhibited strong activities of leucine arylamidase, valine arylamidase and amino peptidase, which accelerate fermentation of cheese[38]. Also esterase, acid phosphatase and β-galactosidase. This result was similar

to Arias *et al*, (2013)[39] who reported that some strains of *Lactobacilli* showed high *β*-galactosidase activity which are related to reduce lactose intolerance.

None of the tested probiotics showed undesired β-glucuronidase activity, which is associated with the conversion of precarcinogens to carcinogens this result was similar to Bassyouni *et al*[40].

CONCLUSION

Rayeb, yogurt milk powder and milk based cereals can be used as potential source of probiotics. Isolated *Lactobacillus and Bifidobacterium* species exhibited good probiotic characteristics and can therefore be used for dairy fermentations and contribute health benefits to consumers. They tolerated acidic media, bile salts. They were non heamolytic with good antibacterial activity against other foodborne pathogenic. They had beneficial enzymatic activities. PCR was a rapid method for identification of different probiotic strains.

REFERENCES

1. Rijkers GT, De Vos WM, Brummer RJ, Morelli L, Corthier G and Marteau P: Health benefits and health claims of probiotics: Bridging science and marketing . British Journal of Nutrition, 2011; 106 (9): 1291–1296.
2. Rakib MRH, Kabir MA and Amanullah SM: Starter Cultures Used in the Production of Probiotic Dairy Products and Their Potential Applications: A Review. Chemical and Biomolecular Engineering, 2017; 2(2): 83-89.
3. El Helim MA, Hashem S, Essam T, Omar M: Cholesterol Lowering Capability of some Lactobacillus Strains and its Effect on Mice Fed a High Cholesterol Diet. Clin Microbiol, 2016; 5:253,2327-5073.
4. APHA American public Health Association: Standard Methods for the Examination of Dairy Products. 16th Ed., American Public Health Association, New York, 1992.
5. Lamiaa M, Ali Elshreef: Profile of Lactobacillus species in camels and cow's milk , Assiut Vet. Med. J., 2014; 60 (143): 89-94.
6. Kim ER, Cho YH, Kim YH, Park SO, Woo and Chun HN: Comparison of bifidobacteria selective media for the detection of bifidobacteria in Korean commercial fermented milk products. Korean Journal for Food Science, 2010; 30 (1): 154-162.
7. Baron EJ, Peterson LR and Finegold SM: Bailey and Scott's, Diagnostic Microbiology,9th ed.,st. Louis, Baltimore, USA, 1994; 34:pp.457-473.
8. Koneman EW, Allen SD, Janda WM, Shcreckenberger and Wihn WC: Diagnostic microbiology 4th ed. Philadelphia, 1992.
9. Cheesbrough M.: Collection, transport and examination of specimens. Cited by: Cheesbrough, M. (ed)., Medical Labolatory Manual for Tropical Countries, 4th ed., pp. 100-195, Worth-Heinemann, Oxford, 2004.
10. Diba FS, Hossain KM, Azim and Hoque MM: Isolation, Characterization and Determination of Antimicrobial properties of Lactic Acid Bacteria from Human Milk .Jordan Journal of Biological Sciences , 2013; 6 (2): 111-116.
11. Samelis J, Maurogenakis F and Metaxopoulos J: characterization of lactic acid bacteria isolated from naturally fermented Greek dry salami. International Journal of Food Microbiology, 1994; 23(2) :179-196.
12. Mahrous H, Badran EM and El-Bagoury ARM: Identification Of Potentially Probiotic Bacteria From Traditional Dairy Product (Laban Rayeb) Of Egypt . International Journal of World Research, 2014; 1(5): 2347-2359.
13. Singh AK, Pandey A, Sharma M and Singh A.: probiotic activities of lactic acid bacteria isolated from human breast milk .Journal of Biological Engineering Research and Review, 2014; 1(2):7-12.
14. Soliman AHS, Sharoba AM, Bahlol HEM, Soliman Radi OMM: Evaluation of Lactobacillus acidophilus, Lactobacillus casei and Lactobacillus plantarum for probiotic characterstics .Middl East Journal of Applied Sciences, 2015; 5 (1) :94-101.
15. Tambekar DH and Bhutada SA: An evaluation of probiotic potential of Lactobacillus sp. from milk of domestic animals and commercial available probiotic preparations in prevention of enteric bacterial infections. Recent Research in Science and Technology, 2010; 2(10) :1-6.
16. Jamaly N, Benjouad A and Mohammed M.: Probiotic Potential of Lactobacillus strains Isolated from Known Popular Traditiona l Moroccan DairyProducts. British Microbiology Research Journal , 2011; 1(4): 79-94.
17. Zinedine A and Faid M: Isolation and characterization of strains of Bifidobacteria with probiotic proprieties in vitro. Worled Journal of Dairy & food Science, 2007; 2 (1): 28-34 .
18. Njoki WJ, Boga HI, Kutim PM, Maina MJ and Kadere: Probiotic potential of lactic acid bacteria isolated from coconut (Cocos Nucifera) wine (Mnazi) in Kenya .International Journal of Life Sciences Research, 2015; 3(1): 113-120.
19. Shah D, Shringi S, Besser T and Call D.: Molecular detection of foodborne pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, 2009; Pp. 369-389.

20. Furet J, Quénée P and Tailliez P: Molecular quantification of lactic acid bacteria in fermented milk using the RT quantitative PCR. International journal of food microbiology, 2004; 97 (2): 197-207.
21. Walter J, Tannock GW, Tilsala-Timisjarvi A, Rodtong S, Loach Munro K and Alatossava T: Detection and identification of gastrointestinal Lactobacillus species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Applied and environmental microbiology, 2000; 66(1): 297–303.
22. Byun R, Nadkarni MA, Chhour KL, Martin FE, Jacques NA and Hunter N: Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. Journal of clinical microbiology, 2004; 42 (7): 3128– 3136.
23. Matsuki T, Watanabe K, Tanaka R and Oyaizu H: Rapid identification of human intestinal Bifidobacteria by 16S rRNA-targeted species- and group-specific primers. FEMS Microbiol. Letters, 1988; 167 (2): 113-121.
24. Rasha HB, Walla SA, Mostafa GF, Saed A and Zeinat K: Characterization of Lactic Acid Bacteria Isolated from Dairy Products in Egypt as a Probiotic. Life Sci J 2012;9(4):2924-2933.
25. I.D.F. (International Dairy Federation): General standard of identity for fermented milks. 1992; 163.
26. Akin MB, Akin MS and Kirmac IZ: Effects of inulin and sugar levels on the viability of yogurt and probiotic bacteria and the physical and sensory characteristics in probiotic ice cream. Food Chemitry, 2007; 104:93–99.
27. Toscano M, Vecchi DE, Rodighiero V and Drago L: Microbiological and genetic identification of some probiotics proposed for medical use in 2011 . J. Chemother ,2013; 25 : 156-161.
28. Abbas MM and Mahasneh AM: Isolation of Lactobacillus strains with probiotic. potential from camels milk . African Journal of Microbiology Research , 2014; 8 (15) : 1645-1655 .
29. Pundir RK, Rena S, Kashyap N and Kaur A: Probiotic potential of lact ic acid bacteria isolated from food samples: an in vitro study. Journal of Applied Pharmaceutical Science, 2013; 3 (3): 85-93.
30. Sandra TS, Janine B, Adele C, Glenn RG and Ian R: In vitro elvaluation of the antimicrobial activity of a range of probiotics against pathogens: Evidence for the effects of organic acids . Journal Anaerobe , 2012; 18: 530-538.
31. Powthong P and Suntornthiticharoen P: Isolation, Identification and Analysis of Probiotic Properties of Lactic Acid Bacteria from Selective Various Traditional Thai Fermented Food and Kefir. Pakistan Journal of Nutrition , 2015; 14 (2): 67-74.
32. AL- Saadi, ZN: In vitro Antagonistic Activity of Bifidobacterium breve Isolated from Breast-fed Infants Human Gastrointestinal Microflora against Two Clinical Strains of Staphylococcus aureus (MRSA and MSSA). Journal of Advances in Biology & Biotechnology , 2016; 10 (2): 1-9.
33. Barakat OS, Ibrahim GA, Tawfik NF, El-kholy and El-Rab GD: Identification and probiotic characteristics of Lactobacillus strains isolated from Domitai cheese. International Journal of Microbiology Research, 2011; 3 (1): 59-66 .
34. Hemant C and Harshada J.: Antagonistic Effect of Lactobacillus Isolates from cow Milk on selected Pathogenic Bacteria. Research Journal of Recent Sciences, 2015; 4: 173-176 .
35. Karthikeyan V and Santhosh SW: Study of Bacteriocin as a Food Preservative and the L. acidophilus Strain as Probiotic .Pakistan Journal of Nutrition, 2009; 8 (4): 335-340.
36. Ghita I, Azzam MA, El Sayed NH, Sadek ZI, Abdel-Khalek AB and Emara AM: Probiotic Characteristics of Some Bifidobacteria and Leuconostoc Strains and Growth Behavior of the Selected Strains with Different Prebiotics. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2015; 6(6):789- 802.
37. Matsuki T, Watanabe K and Tanaka R: Genus- and Species Specific PCR for the Detection and Identification of Bifidobacteria. Curr.Issuses Intest. Microbiol. 2003; 4 : 61-69.
38. Azarnia S, Lee B, St-Gelais D, Kilcawley K and Noroozi E: Effect of free and encapsulated recombinant aminopeptidase on proteolytic indices and sensory characteristics of Cheddar cheese. LWT- Food Science and Technology, 2011; 4 (2): 570–575.
39. Arias LG, Fernández D, Sacristán N, Arenas R, Fresno JM and Tornadijo E: Enzymatic Activity, Surface Hydrophobicity and Biogenic Amines Production in Lactic Acid Bacteria Isolated from an Artisanal Spanish Cheese. African Journal of Microbiology Research, 2013; 7(19): 2114-2118.
40. Bassyouni RH, Abdel-all WS, Abdel-all MGFS & Kamel Z: Characterization of Lactic Acid Bacteria Isolated from Dairy Products in Egypt as a Probiotic. Life Sci. J. 2012; 9: 2924- 2933.