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RESEARCH ARTICLE

THE EFFECT OF CAMEL MILK WHEY PROTEINS ON LACTIC ACID BACTERIA ISOLATED FROM CAMEL AND COW MILKS IN EGYPT: A COMPARATIVE STUDY.

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Abstract

Camel milk also has valuable nutritional properties as it contains a high proportion of antibacterial substances that found naturally in camel milk or derived by lactic acid bacteria. This study was carried out to evaluate the effect of camel's milk whey on lactic acid bacteria (LAB) isolated from cows' and camels' milk. Fifty-eight lactic acid bacteria species (41, 17 isolates were obtained from camel milk and cow milk respectively) were isolated from 14 camel's milk samples and 5 samples of raw cow's milk and 2 samples of zabady were collected from different regions in Egypt and Sudan, followed by pre-identification using phenotypic and genotypic methods using rep-PCR techniques. Then All LAB strains isolated from both camel's and cow's milk were examined to grow at constant concentration of whey camel's milk and its ability to grow was determined as optical density during 24 hours. Based on phenotypic and genotypic identification, Lactobacilli isolates were identified as: *Lb. rhamnosus* (8.6%), *Lb. fermentum* (10.3%), *Lb. acidophilus* (8.6%), *Lb. delbrueckii* subsp. *lactis* (7%), *Lb. plantarum* (13.8%). Among cocci isolates, *Lc. lactis* subsp. *lactis* were 13.8%, *Lc. garvieae* were 1.7%, *Lc. lactis* subsp. *cremoris* were 1.7%, *Enterococcus faecium* were 27.6% and *Streptococcus thermophilus* were 5.2%. Whereas, 1.7% of isolates were identified as *Bidobacterium animalis*. All LAB that isolated from camel's milk showed a good ability to grow at the concentration of whey 20% compared with those isolated from cow's milk.

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Introduction:-

In the last decades, many studies have indicated that camel milk is an important food source because it has functional and healthy benefits due to the presence of biological substances active in it (Asres and Yusuf, 2014).

In Egypt, the majority of people consume cow's milk regularly than camel milk, due to the fact that cows and buffaloes give much more milk and require less maintenance and labor. Unfortunately, people are not aware about the nutritional facts and healthy benefits of camel's milk. Camel's milk is regarded to be abundant source of proteins for people living in arid lands of the world. These proteins are found with high concentration in the whey of camel

milk and are rich in protective components include lysozyme, lactoferrin, lactoperoxidase (LP) and peptidoglycan recognition protein (PGRP) which only detected in camel's milk (Singh *et al.*, 2006; Kappelr *et al.*, 2004), IGA and IgG immunoglobulin's that are compatible with human ones and provide effective defense against several viral and bacterial pathogens (Khitam, 2003). Camel's milk consumption may also be helpful in reducing the nutritional deficiencies and morbidities in adult community (Agrawal, *et al.*, 2005; Singh *et al.*, 2009).

Lactic acid bacteria (LAB) are considered as a Gram positive, non-spore forming, catalase- negative and acid tolerant microorganisms that produce lactic acid as the primary end-produced during carbohydrate fermentation (Carr *et al.*, 2002). (LAB) are one of the most important beneficial microbiota in camel milk, which is vitality of an important source for various dairy technology (Khedid, *et al.*, 2009). LAB are among the important groups of bacteria providing health benefits to human, animal and plant (Song, *et al.*, 2012). Using LAB in food is one of the ancient known food preserving techniques. LABs are widely spread. It was found in many different food products (dairy, meat, beverages and vegetables), but they are also present in the mouth, digestive system and vagina of mammals (Hayek and Ibrahim, 2013). Also LAB plays an important role in the food industry, as they are used for health improvement, production of macromolecules, enzymes and metabolites (Pfeiler and Kleinhammer, 2007).

Bacteria in general, require an appropriated biochemical and environment to grow and express normal metabolic activities. There are many factors that affect the growth of LAB strains, including those related to intrinsic the food itself (water activity; pH value; nutrient content; antimicrobial substances and mechanical barriers to microbial invasion; redox potential.), including extrinsic related to the environment (the temperature of storage; the atmosphere surrounding the food). Also, implicit factors are the factors related to the microorganisms themselves and processing factors. (Hamad, 2012, Hayek and Ibrahim, 2013).

Biochemical conditions including stimulatory, preferred, and essential nutrients have significant impact on the growth and studied throughout the world and it approved that camel's milk contains several antimicrobial agents including lactoferrin, lactoperoxidase, lysozyme and PGRP (Reiter, 1985; Renner, *et al.*, 1989; Kappler, *et al.*, 2004;) that found with high concentration in whey of camel's milk. The effect of this antimicrobial agent against pathogenic bacteria such as *Escherichia coli* and Rotavirus has been studied in several investigations but its effect on LAB is not clear till now. Furthermore, isolating and classifying this bacterial group from camel's milk and selecting them as starter cultures are important steps for the better use of camel's milk.

Thus, the aim of this investigation is to evaluate the effect of whey camel's milk (that is rich in antimicrobial proteins) on the behavior of lactic acid bacteria isolated from camel and cow's milk in parallel.

Materials and Methods:-

Sampling:-

Total 14 samples of camel's milk and 5 samples of raw cow's milk and 2 samples of zabady (a traditional fermented product from cow milk) were collected from different regions in Egypt and Sudan. Camel's milk samples were collected from several regions in Egypt including; King Maryout (5 samples) and El Amrya (2 samples) at the Alexandria governorate; Matrouh (2 samples) and El Omyed (2 samples) at the Marsa Matrouh governorate; Halayeb and Shalteen (1 samples) at the New Valley governorate and one sample from north of Sudan. Samples of camel milk were collected at the same season (winter-spring for year 2015-2016) and milking was performed randomly during different periods ranging from one day to 3 months after parturition whereas, cow milk samples were collected from Alexandria governorate at the winter season. All samples were immediately cooled and transported to the laboratory in an icebox (4 °C) and analyzed for the content of LAB on the arrival.

Isolation of Lactic Acid Bacteria:-

Thirty ml of each milk sample were incubated at 30°C (for mesophilic LAB), 42°C (for thermophilic LAB) and 37°C (Both LAB) until coagulation for 24 hours to facilitate their isolation. The cultures were streaked on different media included M17 agar (Biolife Italy) for lactococci, MRS agar (de Man *et al.*, 1960) (for lactobacilli) at 30°C, 37°C and 42°C respectively, ST agar for *Str. thermophilus* and SF agar for enterococci at 42°C for two days. Representative colonies were collected according to their shape and colour. Isolates were purified by streaking two times on the respective isolation medium and temperature. The isolation of Bifidobacteria spp. were performed by enumerating coagulated samples on modified MRS broth (MRS supplemented with sodium acetate (10gm/L), 0.5g /L L.cystein and pH was adjusted to 5.4 by acetic acid) followed by streaking at the same medium and isolated as described before.

Identification of isolates:-**Phenotypic identification:-**

All isolates were microscopically examined for Gram stain reaction, cell morphology and cellular arrangement. Catalase activity was examined by adding drop of 3 percent hydrogen peroxide on a clean microscopic slide. A visible amount of bacterial growth was added with the inoculating loop. Both were mixed and observed for gas bubble production. Only Gram-positive and catalase-negative isolates were identified at species level. The production of carbon dioxide from glucose was carried out as follows, 0.5 ml of the purified isolate was inoculated in 2 ml medium (at 40 - 45°C) after that 2 ml water agar (1.5%) was added as an agar plug, incubated at 37°C and observed for gas production after 24 - 48 h. A control was carried out using positive culture for carbon dioxide production "*Saccharomyces lactis* N.C.Y.C. 571".

Growth at 45°C and at 10°C:-

The ability of isolates to grow at 45°C or at 10°C were carried out as follows, each isolate was inoculated in broth medium; MRS of lactobacilli and M17 for lactococci (1%) and then incubated at 45°C or at 10°C for 48 h. The growth was observed in the medium; the control was an un-inoculated tube and incubated under same condition.

Growth in the presence of 6.5% salt:-

This test was limited to the lactococci only; salt tolerance was assessed after 3 days of incubation at 6.5% NaCl in M17 broth.

Growth in SF medium:-

This test was limited to the lactococci, the growth of strains was assessed after 48 h of incubation in SF medium at 42°C.

Molecular Identification of Lactic Acid Bacteria Species:-

The isolates that isolated from fresh samples only were subjected to their genotypic characterization using BOX and ERIC PCRs as described below:

Reference strains and cultivation:-

The reference strains were obtained from Institute National de Recherche Agronomique (INRA), Center National de Recherches Zootechnique Jouy- en Josas, France (CNRZ), American Type Culture Collection (ATCC) and (DSM) Deutsche Sammlung von Mikroorganismen. These strains had been previously characterized on the basis of microscopic examination, Gram staining and catalase reaction. Stock cultures were maintained at -40°C in reconstituted skimmed milk plus 15 % glycerol and working cultures will be prepared from frozen stocks through MRS or M17 broth.

DNA Extraction:-

For DNA extraction, 1ml of an overnight culture was added to a 1.5ml microcentrifuge tube and Centrifuged at 13,000–16,000 × g for 2 minutes to pellet the cells. The supernatant was removed. DNA was extracted from cells pellet using Wizard Genomic DNA Purification Kit (Promega, Madison, USA) as described by its manufacture.

Repetitive -Polymerase chain reaction:-

The DNA concentration of each sample was adjusted to 25 ng/μl in a 25 μl PCR mixture. Amplification was performed in a 25 μl reaction as described by Mohammed et al. (2009) and Křížová et al. (2008). The molecular sizes of the amplified DNA fragments were estimated by comparison to a 100 bp DNA ladder (Promega, Madison, USA) and were photographed using polaroid film. The Rep profiles were processed using the Gel ComparII version 5.00 software (Applied Maths, Kortrijk, Belgium). Similarity coefficients for pairs of tracks were calculated by using Pearson product moment correlation coefficient and strains were grouped by using the unweighted pair group method with arithmetic average (UPGMA).

Preparation of camel milk whey:-

Ten ml of camel milk was centrifuged at 5000xg for 30 min at 10°C to remove fat components. Whey was obtained after acid precipitation of casein at pH 4.6 by adding 1ml of 10% (v/v) acetic acid and incubated at 37°C for 10 min. Following it was neutralized 1/10 vol of sodium acetate and centrifuged at 10.000 xg for 5 min at 4°C. The supernatant was then dialyzed for 48hrs and stored at 4°C until used.

Determination of total whey proteins:-

Twenty-five ml of whey (that was naturalized previously by 1/10 vol. of 1M sodium acetate) was added in a breaker. Then, 1ml potassium oxalate solution (28%) and 0.25ml phenolphthalein (2%) was added into the sample. After mixing, the solution was titrated against sodium hydroxide (N/7) until the pink color appeared and then 5ml. of neutralized formalin solution (40%) was added to disappear the pink color followed by second titration against NaOH (N/7) until a faint pink color appears again and the second reading was represented as protein%.

Resistance to whey of camel milk:-

Two hundred millimeter of camel milk whey (that included 12.2gm of soluble whey protein) was added to 800ml MRS media. Then, 1% of lactic acid bacteria strains were inoculated in media supplemented with whey. The optical density was determined at 650nm during 3, 6 and 24 hours compared with control (supplemented media without bacterial inoculation).

Results and Discussion:-**Identification of Lactic acid bacteria:-**

The isolation of LAB from natural source have always been the most powerful means for obtaining useful and genetically-stable strains for industry important products

Pre-identification using morphological tests and biochemical reactions:-

In the present study, fifty-eight isolates that including 41 isolates obtained from camel milk and 17 isolates were obtained from cow milk. Pre-identification of isolates of different camel's milk and cow's milk samples is illustrated in Tables (1-2).

Table1: Pre-Identification Results Of Strains Isolated From Raw Camel And Cow Milk Samples That Collected From Different Origins In Egypt

Strain No.	Origin	Gram stain	Catalase	Growth on 6.5%Salt	Growth at 10°C	Growth at 45°C	CO ₂ production	Growth on SF media	Pre Identification
1CM	AM	+	-	-	+	-	-	-	<i>Lactococcus</i>
2CM	AM	+	-	-	+	-	-	-	<i>Lactococcus</i>
3CM	AM	+	-	-	+	-	-	-	<i>Lactococcus</i>
4CM	AM	+	-		+	-	-		<i>Lactobacillus</i> G.B
5CM	AM	+	-		+	-	-		<i>Lactobacillus</i> G.B
6CM	AM	+	-	-	-	+	-	+	<i>Enterococcus</i>
7CM	AM	+	-	-	+	+	-	+	<i>Enterococcus</i>
8CM	AM	+	-	-	-	+	-	+	<i>Enterococcus</i>
9CM	HSH	+	-		-	+	+		<i>Lactobacillus</i> G.C
10CM	HSH	+	-		-	+	-		<i>Lactobacillus</i> G.A
11CM	HSH	+	-		+	-	-		<i>Lactobacillus</i> G.B
12CM	HSH	+	-		-	+	-		<i>Lactobacillus</i> G.A
13CM	HSH	+	-	-	+	+	-	+	<i>E. faecium</i>
14CM	HSH	+	-			+			<i>Bifidobacterium</i>
15CM	HSH	+	-	-	+	-	-	-	<i>Lactococcus</i>
16CM	HSH	+	-	-	+	-	-	-	<i>Lactococcus</i>
17CM	KM	+	-		-	+	+		<i>Lactobacillus</i> G.C
18CM	KM	+	-		-	+	+		<i>Lactobacillus</i> G.C
19CM	Hb	+	-		-	+	-		<i>Lactobacillus</i> G.A

CM= camel milk, AM=EL Amrya ,HSH=Halayeb&Shalteen , KM= King mayot,Hb=Halayeb, EO= G.A= , G.B= , G.C=

Table1:-(cont.): Pre-identification results of strains isolated from raw camel samples.

Strain No.	Origin	Gram stain	Catalase	Growth on 6.5% Salt	Growth at 10°C	Growth at 45°C	CO ₂ production	Growth on SF media	Pre Identification
20CM	KM	+	-		-	+	+		<i>Lactobacillus</i> G.C
21CM	KM	+	-		+	-	-		<i>Lactobacillus</i> G.B
22CM	KM	+	-		+	-	-		<i>Lactobacillus</i> G.B
23CM	KM	+	-		+	-	-		<i>Lactobacillus</i> G.B
24CM	KM	+	-	-	+	+	-	+	<i>Enterococcus</i>
25CM	KM	+	-		+	-	-		<i>Lactobacillus</i> G.B
26CM	EO	+	-	+	-	+	-	+	<i>Enterococcus</i>
27CM	EO	+	-	+	+	+	-	+	<i>Enterococcus</i>
28CM	EO	+	-	+	+	+	-	+	<i>Enterococcus</i>
29CM	EO	+	-	-	-	+	-	-	<i>Streptococcus</i>
30CM	EO	+	-	-	+	-	-	-	<i>Lactococcus</i>
31CM	NS	+	-		-	+	-		<i>Lactobacillus</i> G.A
32CM	NS	+	-		-	+	+		<i>Lactobacillus</i> G.C
33CM	NS	+	-	-	-	+	-	-	<i>Streptococcus</i>
34CM	NS	+	-	-	+	+	-	+	<i>Enterococcus</i>
35CM	NS	+	-	-	+	+	-	+	<i>Enterococcus</i>
36CM	NS	+	-	-	+	+	-	+	<i>Enterococcus</i>
37CM	MM	+	-	+	+	-	-	-	<i>Lactococcus</i>
38CM	MM	+	-	-	+	+	-	+	<i>Enterococcus</i>
39CM	MM	+	-	+	+	-	-	-	<i>Lactococcus</i>
40CM	MM	+	-	-	+	-	-	-	<i>Lactobacillus</i> G.B
41CM	MM	+	-	-	+	-	-	-	<i>Lactobacillus</i> G.B

CM= camel milk, KM= King mayot, EO= El Omyed, NS= North Sudan, MM= MarsaMatrouh G.A= , G.B= , G.C=

Forty- one isolates of Gram positive and catalase negative of none starter lactic bacteria (wild strains) (NSLAB) were isolated from 14 samples of camel's milk and 17 isolates were isolated from 5 samples of raw cow's milk and 2 samples of Zabady. The isolates were differentiated according to their morphological and physiological characteristics into five genus of LAB as follow: *Enterococcus*, *Lactococcus*, *Streptococcus*, *Lactobacillus* and *Bifidobacterium*. Isolated lactobacilli were classified into 3 groups according to Kandler and Weiss, (1986); group A, (obligate-homo-fermentative), B (facultative-hetero-fermentative) and C (obligate-hetero-fermentative).

Pre-identification using morphological tests and biochemical reactions of different camel's milk and cow's milk samples is showed in Tables (3).

Table 3:-Pre-identification using morphological tests and biochemical reactions

Genus	Raw Camel milk	Cow milk (raw and fermented)
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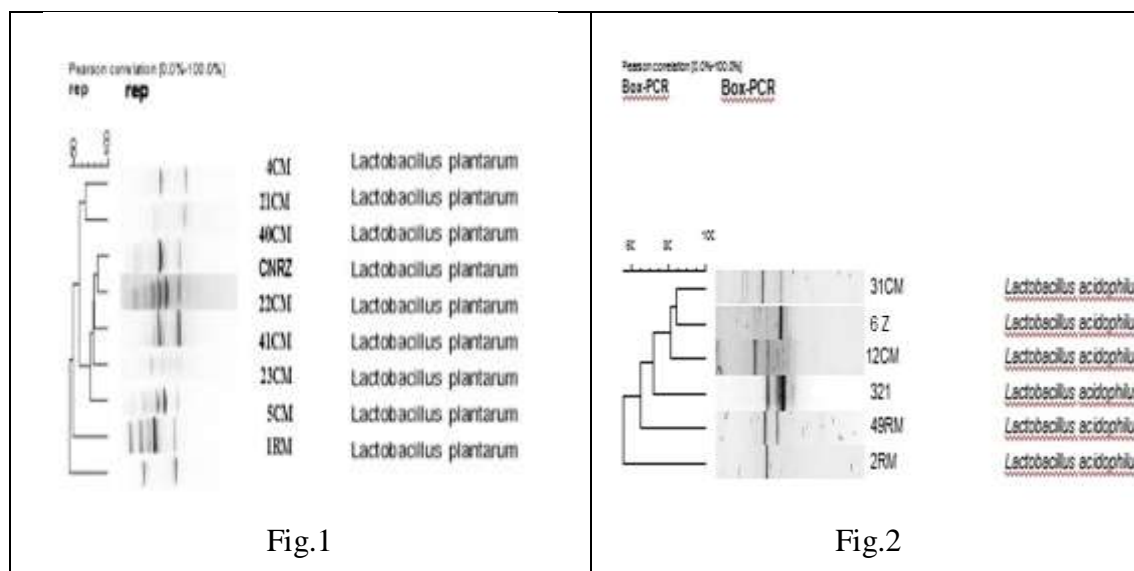
Lactobacillus , Group A	4	5
Lactobacillus , Group B	9	4
Lactobacillus , Group C	5	1
<i>Enterococcus</i> spp.	12	4
<i>Lactococcus</i> spp.	8	2
<i>Streptococcus</i> spp.	2	1
Bifidobacteria	1	0

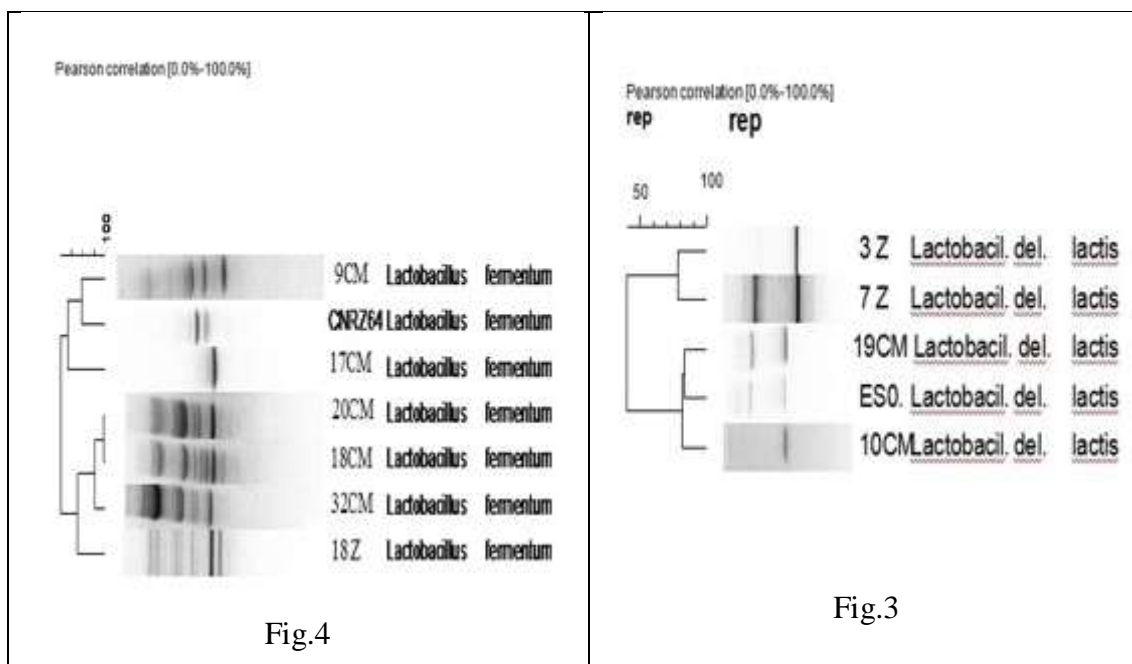
The results obtained from camel's milk showed that Lactobacilli species (43.90%) were divided to group A (22.22%), group B (50%), and group C (27.78%). *Enterococcus* spp. isolates represented 29.27%, whereas *Lactococcus* spp. represented 19.51%. The remaining isolates were *Streptococcus* and Bifidobacteria that represented as 4.88% and 2.44% respectively. While the isolates of cow's milk were Lactobacilli species (58.82%) that divided to group A (50%), group B (40%), and group C (10%), *Enterococcus* spp. isolates (23.52%), *Lactococcus* spp. (11.76%) and *Streptococcus* spp. (5.88%).

According to research results, *Enterococcus* and *Lactococcus* genus seem to be dominating in camel's milk. Similar results on predominant cocci microorganisms of camel milk compared with other species' milk have been already reported by Ashmaing *et al.*, (2009); Khedid *et al.*, (2009); Rahman *et al.*, (2009); Irad *et al.*, (2013); and Akhmetsadykava *et al.*, (2015).

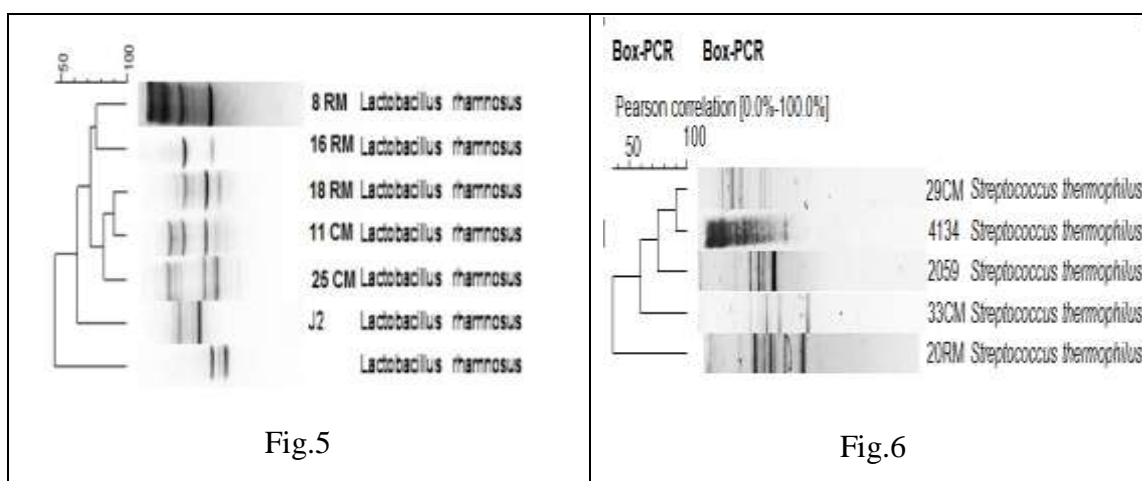
Identification by rep-PCR fingerprints:-

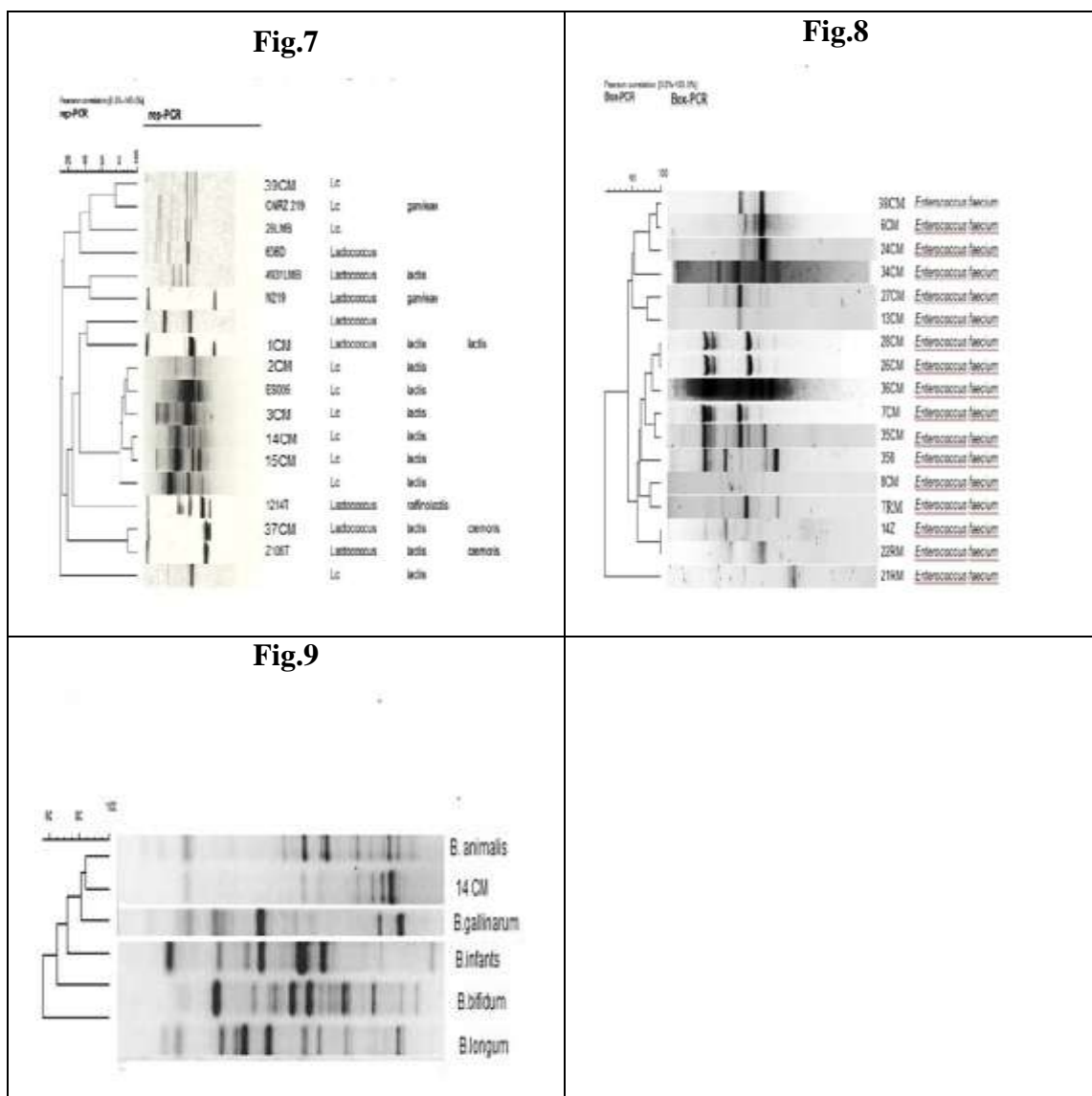
Fifty-eight isolates were further characterized to species and subspecies level using Rep-PCR technique using BOXA1R primer for LAB. Identification was based on similarity coefficients for pairs of tracks calculated by using Pearson correlation coefficient and strains were grouped by using the unweighted pair group method with arithmetic average. Based on phenotypic and biochemical characteristics, the pre-identified isolates that belonging to group A, group B and group C for Lactobacilli, *Enterococcus*, *Lactococcus* and *Streptococcus* for lactococci and *Bifidobacteria* were subjected to genotypic identification using Rep-PCR as shown in Figures (1– 9).





Figures 1 to 4:- Dendrograms generated from rep-PCR fingerprints of LAB isolates. The dendrogram was constructed using the unweighted pair group method using arithmetic averages with correlation levels expressed as percentage compared with reference strains of each species.





Figures 5 to 9:- Dendrograms generated from rep-PCR fingerprints of LAB isolates. The dendrogram was constructed using the unweighted pair group method using arithmetic averages with correlation levels expressed as percentage compared with reference strains of each species.

Figure (1) shows that seven isolates obtained from camel milk and one isolate obtained from cow milk were identified as *Lactobacillus plantarum* (*Lb. plantarum*) with similarity level 75% and 50% respectively comparing with CNRZ 5050 as a reference stain. On the other hand, two isolates obtained from camel milk and three isolates obtained from cow milk that were pre-identified as group A previously, were identified as *Lactobacillus acidophilus* (*Lb. acidophilus*) by Rep-PCR fingerprints at 75% and 60% similarity level respectively as shown in Figure (2), as well as, two isolates obtained from both camel and cow milks were identified as *Lactobacillus delbrueckii* subsp *lactis* (*Lb. del. lactis*) at similarity level 90% comparing with CNRZ 250 as a reference strain (Figure 3). Belonging to group C lactobacilli, five isolates from camel milk and one isolate from cow milk were identified as *Lactobacillus fermentum* (*Lb. fermentum*) with similarity level 85% and 80% respectively comparing with CNRZ64 as a reference strain (Figure4).

As illustrated in Figure (5) two isolates from camel milk and three isolates from cow milk were identified as *Lactobacillus rhamnosus* (*Lb. rhamnosus*) with similarity level 80% comparing with the reference strain.

Twenty-nine from lactic acid cocci that were pre-identified previously as *Lactococcus*, *Enterococcus* and *Streptococcus* were distinguished by genotypic identification. All *Streptococcus* isolates (2 obtained from camel milk and one from

cow milk) were clearly identified as *Streptococcus thermophiles* (*Str. thermophiles*) with a similarity level 60% comparing with DMS 20259 as a reference strain (Figure 6). Whereas six isolates from camel milk and two isolates obtained from cow milk were identified as *Lactococcus lactis* subsp. *lactis* (*Lc. lactis.lactis*) with similarity level 90% compared with ES005 as a reference strain and two isolates obtained from camel milk only were identified as *Lactococcus lactis* subsp. *cremoris* (*Lc. lactis cremoris*) and *Lactococcus garvieae* (*Lc. garvieae*) that were detected for the first time in camel milk comparing with Z 105T and CNRZ 219 as a reference strains respectively as described in Figure (7).

Among Enterococcus twelve isolates obtained from camel milk and four isolates obtained from cow milk were identified as *Enterococcus faecium* (*En. faecium*) using Rep-PCR amplification with similarity level 65% comparing with CNRZ 131 as a reference stain (Figure 8). On the other hand, one isolate was identified as *Bifidobacterium animalis* (*B. animalis*) and represented as a minor species of LAB in camel milk (Figure 9). This result was in agreement with Savadogo et al.,(2004) who found detected *Bifidobacterium* species in traditional fermented camel milk and it represented 10% of LAB isolated from samples studied.

The percentage of LAB strains from camel and cow milks were illustrated at Table (4).

Table 4:- Distribution of lactic acid bacteria species isolated from camel's and cow's milk

Genus	Species	Number of isolates	% of total isolates
Lactobacillus	Lb. plantarum	8	13.8
	Lb. acidophilus	5	8.6
	Lb. fermentum	6	10.3
	Lb. delbrueckii subsp. lactis	4	7
	Lb. rhamnosus	5	8.6
Lactococcus	Lc. lactis subsp. lactis	8	13.8
	Lc. lactis subsp. cremoris	1	1.7
	Lc. garvieae	1	1.7
Enterococcus	Enterococcus faecium	16	27.6
Streptococcus	Streptococcus thermophilus	3	5.2
Bifidobacteria	Bifidobacterium animalis	1	1.7
Total		58	100

These results revealed that *En. faecium* was the dominant species in samples of camel and cow milks and represented 27.6% of total isolates. These results were in agreement with Khadid *et al.*, (2009); Hamed and El-Attar, (2013); Jrad *et al.*, (2013) who found that *Enterococcus* was predominant specie in camel milk samples collected from Morocco, Egypt and Tunisia respectively. *Lactobacillus plantarum* as well as *Lc. lactis lactis* were predominant species with percentage 13.8 followed by *Lb. fermentum* (10.3%) of total isolates from both camel and cow milks. The remaining species including *Lb. rhamnosus*, *Str. thermophilus*, *Lc. lactis cremoris*, *Lc. garvieae* and *B. animalis* were detected as a sub dominant and minor species in both camel and cow milk as shown at Table (4). Thermophilic lactic acid bacteria such as *Str. thermophilus* and *Lb. acidophilus* were found in camel milk samples collected Halayeb and Shalateen and north Sudan and could not be detected in the remaining samples. This result was in agreement with Jans, Christoph, (2011) who found *Str. thermophiles* in camel milk collected from Kenya and Somalia, and Estiphano *et al.*, (2016) who found the same results in raw camel's milk obtained from Ethiopia.

The obtained results revealed that *Lc. lactis* subsp. *cremoris* and *Lc. garvieae* that detected at the first time in camel milk were found in samples collected from Marsa matrrouh governorate that located near the Mediterranean sea. The presence of this species in this sample of camel milk may be due to its high resistance to salt concentration as *Lc. garvieae* always found in marine environment.

In this study the biodiversity of LAB obtained from camel milk from different regions in Egypt showed that noticed belonging to *Lactobacillus acidophilus* and *Lb. del. lactis* that could not be distinguished in camel milk samples obtained from north Egypt and found in samples collected from Halayeb and Shalateen and north Sudan, and this also was in agreement with Estiphano *et al.*, (2016) who found the same species in raw camel milk from Ethiopia. These results may be due to the climate conditions in Sudan and east of Africa countries where high temperature encourages thermophilic bacteria to grow and become dominant LAB species in these regions.

Our results regarding to the biodiversity of LAB isolated from raw camel milk were in agreement with many researchers who considered these species of LAB isolated from raw camel's and cow milks and its traditional fermented dairy products. (Hamma et al., 1991; Isoro et al., 1994; Watabe et al., 1998; Yoneya et al., 1999; Nakamora et al., 1999; Mathara et al., 2004; Abdalla, 2007; Daimasso et al., 2008; Ashmaing et al., 2009; Khedid et al., 2009; Rahman et al., 2010; Hamed and El-Attar, 2011; Jrad et al., 2013; Manu and Adel, 2014; Akhmetsadykova et al., 2015 and Estiphano et al., 2016).

The ability to resist whey of camel milk:-

All LAB strains isolated from both camel and cow milks were tested to grow at constant concentration of whey camel's milk Figure (10).

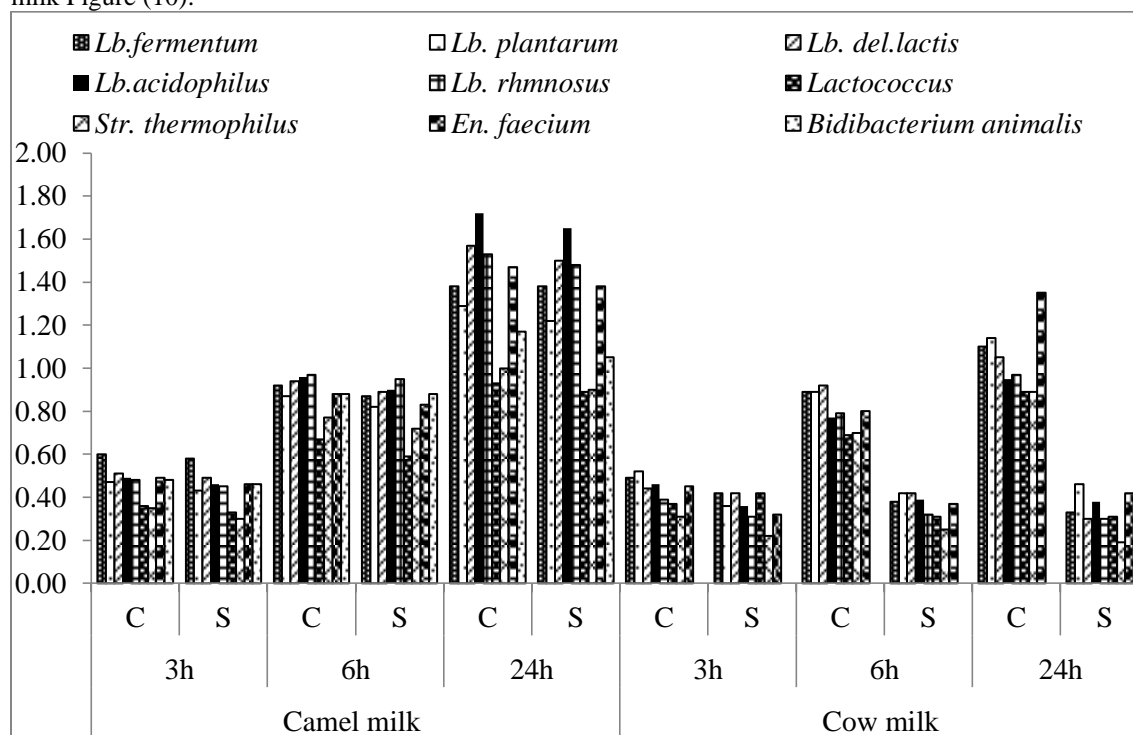


Fig.10:- Growth of LAB isolated from cow and camel milks expressed by optical density at 650nm during 24 hours of incubation comparing with control of each species.

interprets the averages of optical density values of bacterial growth at 650nm. The obtained results revealed that all LABS that isolated from camel's milk showed a good ability to grow at the 20% of camel milk whey during 24hours compared with those isolated from cow milk. A gradual increase of bacterial growth was observed through 3,6 and 24 hours of incubation regarding LAB strains isolated from camel milk, whereas, a sharp decline was noticed of the growth of strains isolated from cow milk when examined to grow in cultures supplemented with whey. Camel milk strains belonging *Lb.acidophilus*, *Lb. del. lactis* and *Lb. rhamnosus* show the highest ability to resist the inhibition effect of Camel whey and there optical densities (O.D) were 1.65, 1.53 and 1.5 respectively after 24hrs of incubation comparing with the growth of the control ones. In contrast the same species that isolated from cow milks could not grow at this concentration of whey camel milk and their O.D was 0.38 for *Lb acidophilus* and 0.3 for *Lb.rhamnosus* comparing with control ones that recorded 0.95 and 0.97 respectively at the same time. The same behaviors were noticed on *Lb. fermentum* and *Lb. plantarum* isolated from camel milk that show a good ability to grow in the presence of camel whey and their O.D after 24hrs were 1.38 and 1.22 respectively comparing with control that examined to grow in media without camel whey. By comparison the same species that isolated from cow milks to grow in medium supplemented with whey, it is clear that cow milk *Lb. fermentum* and *Lb. plantarum* could not resist the inhibition effect of whey proteins and its O.D was 0.33 for *Lb.fermentum* and 0.46 for *Lb.plantarum*, even so its controls recorded 1.1 and 1.14 for same strains respectively after 24hrs incubation.

On the other hand, *Lactococcus* and *Str.thermophilus* strains show a less ability to grow through medium supplemented with camel whey as their O.Ds after 24 hrs were 0.9 and 0.89 comparing with controls one that recorded 0.93 for *Lactococcus* and 1 for *Str.thermophilus*.

Strains belonging to *Lb. fermentum*, *Lb. plantarum* and *Lb. acidophilus* were the highest resistant to whey followed by *Lb.delbrueckii lactis*, *Str.thermophilus*, *Enterococcus* and *Lc.lactis* respectively. Strains 14CM that identified as *B.animalis* and 39 CM that identified as *Lc.garvieae* demonstrated a good ability two grow at this level of whey and the same species could not be isolated from cow milk in our study.

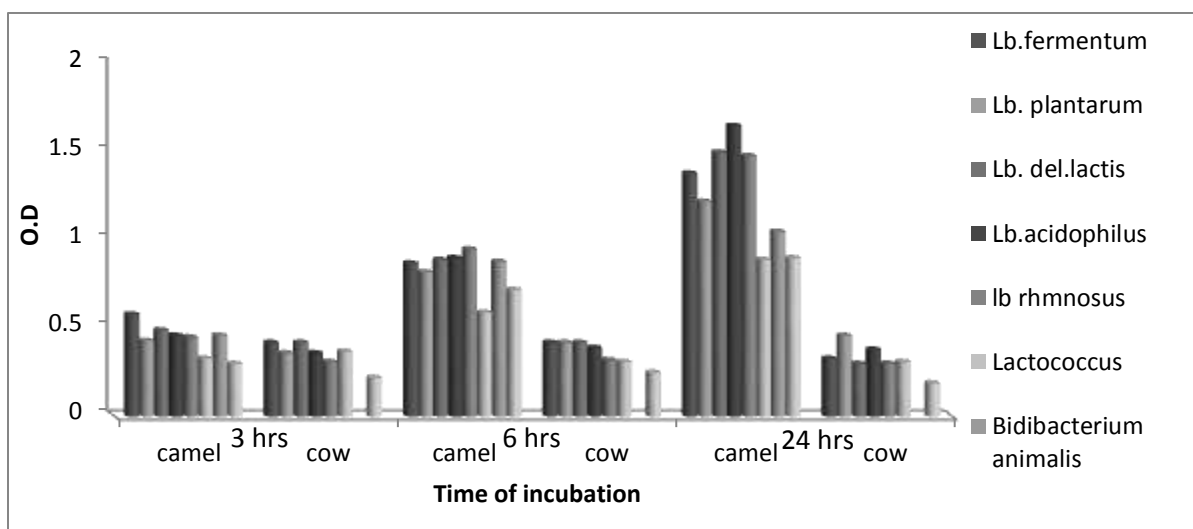


Fig.11:- Comparison the behaviors of LAB strains obtained from camel and cow milk during 24 hours of incubation.

Figure (11) illustrated the comparison of the bacterial growth between those isolated from camel and cow milks when it subjected to grow at the constant concentration of camel whey. Through the first three hours of incubation there were no significant differences between the growth of LAB strains obtained from cow and camel milks however, an evident differences were observed during the remaining 24 hours of incubation. After 6 hours of incubation LAB strains that obtained from camel milk grew increasingly whereas, the same species that obtained from cow milk could not able to progress and its optical density was almost constant. As represented in Figure (11) there is an apparent difference at the growth of LAB that obtained from the two sources. The optical density values of the bacterial growth after 24 hours of incubation established that LAB strains isolated from camel milk can grow optimally in the presence of camel whey at the same activity wherever it absence. In comparison with the behavior of same species of LAB that obtained from cow milk, it was noticed that cow milk LAB lost their activity and their growth declined sharply after 24 hours of incubation at the medium supplemented with camel whey.

As camel milk is rich in antibacterial proteins that control the growth of gram negative bacteria and peptidoglycan recognition protein which control the growth of gram positive bacteria and it could not detected in other Ruminants milk (Kappler *et al.*, 2004), there is a need to examine the ability of LAB to resist the antibacterial effect of camel milk whey proteins.

Camel milk whey contains protective proteins such as serum albumin, lactoferrin, immune globulins and peptidoglycan recognition protein (PGRP) (Farah, 1993; Kappler *et al.*, 2004; Marin *et al.*, 2001).

According to Kappler *et al.* (2004) who detected that PGRP is found in camel milk only and the main role of this peptide is to control positive Gram bacteria and bound with peptidoglycan of the bacterial cell wall and thus inhibit LAB growth. Moreover, he reported that presence of exopolysaccharide (EPS) surroundings bacterial cell wall can avoid it from binding with PGRP. Based on these facts most of LAB isolated from camel milk should be EPS producers to save itself from the inhibition action of PGRP. This explanation is in agreement with Estiphano *et al.* (2016) who found that 85% of LAB isolated from camel milk had EPS production, and thus explain clearly the ability of LAB isolated from camel milk to resist its whey compared with those isolated from other sources such as cow milk.

Conclusion:-

This study showed new aspects about the behavior of LAB isolated from camel milk that can grow in complicated ecosystem like camel milk and it resists the camel whey that rich in antibacterial proteins. In addition it is recommended that these species should be further studied according to selection criteria for dairy industries like EPS production, acidifying activities and stimulation of immunological system and adhesion the epithelium tissue to can benefit from their unique characters to produce functional dairy products.

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