

PROFILE OF *LACTOBACILLUS* SPECIES IN CAMEL'S AND COW'S MILK

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ABSTRACT

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A total number of 60 samples of camel's and cow's milk (30 samples of each) were investigated for the presence of *Lactobacillus* species in the present study. Enumerations of *Lactobacilli* in the examined camel's and cow's milk were carried out using usual media by the classic method (MRS media and broth). Typing of *Lactobacilli* strains isolated from the examined milk samples was determined by phenotypic characterization, growth at different temperatures and at different levels of salt and by the carbohydrates fermentation profile. The percentages of positive samples that contain *Lactobacilli* were 46.7 and 60 % in camel's and cow's milk samples respectively, and its total counts ranged from 37×10^3 to 43×10^5 cfu/ml with an average of 12×10^5 cfu/ml in camel's milk and ranged from 5×10^3 to 28×10^5 with an average of 5×10^5 cfu/ml in cow's milk. Three types of *Lactobacillus* species were recovered from camel's milk samples (*L. bulgaricus* detected in 23.3 %, *L. planetarium* detected in 13.3 % and *L. casei* detected in 10 % of the examined camel's milk samples). On the other hand only two species were detected in cow's milk (*L. bulgaricus* detected in 20 % and *L. acidophilus* detected in 40 % of the examined cow's samples). It could be concluded that *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, and *Lactobacillus casei* which are detected in camel's milk and *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* which are isolated from cow's milk could be used in the development of starter cultures for the production of fermented camel and cow's milk. However, further research works are needed to evaluate the performance of these isolates especially if it is used as mixed starter cultures.

Key words: *Lactobacillus*, camel's milk, cow's milk.

INTRODUCTION

Lactic acid bacteria (LAB) have a long history of application in fermented foods because of their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. They cause rapid acidification of the raw material through the production of organic acids, ethanol, aroma compounds, bacteriocins, exopolysaccharides and several enzymes of importance (Luc and Fré'deric, 2007). They have long been consumed by people in several fermented foods such as dairy products. Today, LAB are a focus of article in press intensive international research for their essential role in most fermented food, for their ability to produce various antimicrobial compounds promoting probiotic properties (Temmerman *et al.*, 2002) including antitumor activity (DeVuyst and Dege-est, 1999 and Hilde *et al.*, 2003), reduction of serum cholesterol (Desmazeaud, 1996 and Jackson *et al.*, 2002),

alleviation of lactose intolerance (De`Vrese *et al.*, 2001), stimulation of the immune system (Isolauri *et al.*, 2001) and stabilization of gut micro-flora (Gibson *et al.*, 1997).

LAB strains that produce exopolysaccharide are employed in the manufacture of fermented milk to improve its texture and viscosity (Curk *et al.*, 1996 and Ruas-Madiedo *et al.*, 2002). Some LAB strains are known to produce mannitol which is claimed to have several health promoting effects (Wood and Holz-apfel, 1995 and Wisselink *et al.*, 2002). New sources of nutrients should be more exploited for varying the human diet and also to benefit from new functional ingredients and natural food components.

Camel milk is nutritionally and medicinally superior to cow milk and milk from other species. It is used therapeutically against jaundice, problems of spleen, T.B., anemia and piles (Rao *et al.*, 1970 and Khalid *et al.*, 2012). When camel milk is left to stand, its

acidity rapidly increases due to presence of LAB (Ohris and Joshi, 1961, Ahmed and Kanwal, 2004). *Lactobacillus salivarius*, *Lactobacillus casei* and *Lactobacillus plantarum* were the common LAB recovered from camel's milk (Khedid *et al.*, 2009 and Seifu *et al.*, 2012). Growth of *L. acidophilus* is supported by camel milk so it grows more rapidly in camel milk than others. *L. acidophilus* converted 56 and 74 % of lactose into lactic acid. Strains isolated from camel milk were best for acid production and coagulated the milk in less time, so better results can be obtained by coagulating milk with starter culture prepared from strains isolated from camel milk (Ahmed and Kanwal, 2004).

Lactobacilli are known to produce many types of bacteriocins like acidophilin, acidolin, lactocidin, bulgarican, lactolin, lactobacillin and lactobrevin (Alvarez-Olmos and Oberhelman, 2001). The functional properties and safety of probiotics of particular strains of *L. casei*, *L. lactis* and *L. acidophilus* from various sources have been extensively studied and proved to be efficient for prevention of infectious diseases (Halami *et al.*, 1999). *L. rhamnosus* and *L. plantarum* had strongest antagonistic potential against *Salmonella typhi* followed by *Proteus vulgaris* and *Klebsiella pneumonia* (Tambekar and Bhutada, 2010).

Lactobacillus spp. is used to treat antibiotic diarrhea, yeast infection and urinary tract infection. It was studied for its possible benefit in protection against colon cancer and the adverse effects of chemotherapy and radiotherapy. Also some strains of *L. casei* are known to have anti-inflammatory effects and offer immune support enhancing the body defense against diseases (Sara, 2010).

African and Arab countries, where the breeding conditions for cows are severe and fastidious, can get over this situation by developing a breeding system for local animals such as camel. The beneficial microbiota of camel milk represented by LAB is a potential source of biological materials to be used in dairy technology. The transformation of camel milk by fermentation is not easy and more research for elucidating the process is needed. Cow's milk was widely investigated, up until now, little studies were undertaken on the camel's milk to characterize its microflora especially LAB. Thus, this study aims: 1- Isolation and characterization of *lactobacillus* bacteria from the raw camel's and cow's milk produced in Assiut and NewVally Governorates, Egypt. 2- Comparing the total count, percentage and different types of *lactobacilli* recovered from examined milk samples of both species.

MATERIALS and METHODS

Sampling: A total number of 60 samples of camel's and cow's milk (30 samples of each) were collected from different regions at Assiut and Newvally Governorates. Samples were immediately cooled and transported to the laboratory in an icebox (4 °C) and investigated for the content of *Lactobacilli* on the arrival.

Isolation and enumeration of *Lactobacilli*: Ten (10) ml of milk sample were homogenized with 90 ml of sterile saline solution to make an initial dilution (10^{-1}). The suspension was used for making suitable serial dilutions up to 10^{-5} by incorporating 1 ml into 9 ml of sterile saline solution in sterile tubes.

Enumeration of *Lactobacilli* was determined using the elective media, DeMan, Rogosa and Sharpe (MRS) agar (CMO 361 Oxoid England) according to DeMan *et al.* (1960). 15–20 ml sterile MRS agar (45–50 °C) was poured into sterile petri dishes containing 1 ml of diluted test sample. The inoculums distributed throughout the medium by gentle rotation of plates in one direction and then in the reverse direction. The inoculated plates were left for 5 -10 minutes to solidify, and then incubated at 37 °C for 48 -72 hours in CO₂ Incubator. *Lactobacilli* colonies are smooth, white and convex with regular edges. After incubation, colonies were enumerated and recorded as colony forming units (cfu) per milliliter of milk.

The colonies were randomly picked from plates and transferred in 10 ml of appropriate MRS broth. The selected colonies were purified by repeated streaking on the appropriate agar media. *Lactobacilli* strains were kept on MRS agar slant at 4 °C and streaked every 4 weeks. Prior to use, *Lactobacilli* strains were activated in MRS broth at 30 °C for 24 hrs, and subculture in MRS agar at 30 °C for 24 hrs. Gram staining, catalase reaction, gas production, growth at different temperatures and growth at different sodium chloride concentrations were tested for a preliminary separation of different isolates of lactobacilli. Biochemical identification (Lactose, sucrose, mannitol, xylose, maltose and trehaloze fermentation as well as production of NH₃ from arginine) was then conducted.

Lactobacilli strains were characterized according to methods recommended by several authors (Harrigan and McCance, 1976; Sharpe, 1979; Schleifer *et al.*, 1985; Kandler and Weiss, 1986; Schleifer and Kilpper-Ba'lz, 1987; Facklam and Collins, 1989; Curk *et al.*, 1996; Charteris *et al.*, 2001; Klein, 2001, Ahmed and Kanwal, 2004 and Khedid *et al.*, 2009).

Table 5: Types and incidence of the isolated *Lactobacilli* spp. that recovered from positive cow's milk samples.

<i>Lactobacillus</i> spp.	No.	%	Min.	Max.	Average
<i>L. bulgaricus</i>	6	20	27×10 ³	28×10 ⁵	8×10 ⁵
<i>L. acidophilus</i>	12	40	5×10 ³	13×10 ⁵	3.5×10 ⁵

No.: Number of positive milk samples. Percentages calculated according to the No. of total milk samples of each animal species.

DISCUSSION

Lactobacillus spp. were isolated from camel's and cow's milk and identified. Up to now, camel's milk produced in Egypt was not deeply investigated for the characterization of the microflora. The isolated strains were investigated to retrieve the number of species and varieties of the different genera. Tests were repeated two times to avoid confusing results in the identification.

In the present study the percentages of positive samples that contain *Lactobacilli* were 46.7 and 60 % in camel's and cow's milk samples respectively (Table, 2). The obtained results of *Lactobacilli* counts revealed that it ranged from 37×10³ to 43×10⁵ cfu/ml with an average of 12×10⁵ cfu/ml in camel's milk samples and ranged from 5×10³ to 28×10⁵ with an average of 5×10⁵ cfu/ml in cow's milk samples (Table, 3). These results indicated that the percentage of positive samples that contain *Lactobacilli* were higher in cow's milk than that of camel's milk, in contrary to the total count, which was higher in camel's milk than that of cow's milk. Khedid *et al.* (2009) isolated *lactobacilli* in camel's milk samples in Morocco with levels varied from 2.5×10² to 6×10⁷ cfu/ml with an average of 7.5×10⁶ cfu/ml. Aziz *et al.* (2009) identified, *Lactobacillus bulgaricus* in 28 % of examined cow milk samples. Fatehenti *et al.* (1979), Tornadijo *et al.* (1995) and Badis *et al.* (2004) reported that *Lactobacilli* counts found in camel milk were higher compared to that reported in other types of milk such as goat's milk. However, similar counts recorded in this study were reported in ewe's milk (Devoyod *et al.*, 1968; Fernandez del Pozo *et al.*, 1988; Pouillet *et al.*, 1991).

Typing of *Lactobacilli* strains isolated from camel's and cow's milk samples was determined by phenotypic characterization, growth at different temperatures and at different levels of salt and by the carbohydrates fermentation profile (Table, 1). Three types of *Lactobacillus* species were recovered from camel's milk samples. 1. *L. bulgaricus* detected in 23.3 % of the examined camel's samples with total counts ranged from 37×10³ to 12×10⁵ and an average of 11.4×10⁵ cfu/ml. 2. *L. planetarium* detected in 13.3 % of the examined camel's samples with total counts ranged from 11×10⁵ to 43×10⁵ and an average of

19×10⁵ cfu/ml and 3. *L. casei* detected in 10 % of the examined camel's samples with total counts ranged from 93×10³ to 6×10⁵ and an average of 3.8×10⁵ cfu/ml (table, 4).

On the other hand only two species were detected in cow's milk, *L. bulgaricus* detected in 20 % of the examined cow's samples with total counts ranged from 27×10³ to 28×10⁵ and an average of 8×10⁵ cfu/ml and *L. acidophilus* detected in 40 % of the examined cow's samples with total counts ranged from 5×10³ to 13×10⁵ and an average of 3.5×10⁵ cfu/ml (table, 5). Khedid *et al.* (2009) reported that lactic acid bacteria species, frequency *Lactobacillus* are present in camel's milk, the authors' isolated *Lactobacillus casei* and *Lactobacillus plantarum* from camel's milk in similar proportion.

This group is best known as starter for fermented dairy products because of the following criteria: production of large amounts of acid in milk, synthesis of vitamin (Forsse'n *et al.*, 2000), flavor compounds, production of folic acid and production of EPS that has an effect on the rheological properties of yoghurt. The mesophilic homofermentative *lactobacilli* represented by *Lactobacillus casei*, and *Lactobacillus plantarum* has been reported that they are usually found in cheese (Devoyod and Mu'ller, 1969). Strains of this group can be used to adjunct starter culture to accelerate ripening and to produce desirable flavors and to eliminate defects by adventitious nonstarter LAB (NSLAB) since they inhibit their outgrowth. Herreros *et al.* (2003) showed that some strains of *Lactobacillus casei subsp. casei* were developing acid activity as rapidly as some *lactococci*, the authors explained that *lactobacilli* metabolize lactose more slowly than *lactococci* but the final acid production can be similar to or even higher than *lactococci*.

It could be concluded that in the present study, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, and *Lactobacillus casei* were the detected *Lactobacillus* bacteria in camel's milk while *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were the *Lactobacillus* bacteria in cow's milk. More studies are needed to complete the isolation and the characterization of *Lactobacillus* strains that could be present in camel milk. These isolates from the present study could be used in the

development of starter cultures for the production of fermented camel milk under controlled environment in the future. However, further research work is needed to evaluate the performance of these isolates when used especially if it is used as mixed cultures.

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صورة بكتريا اللبن العصوية (اللاكتوباسيلس) في اللبن الجمال والابقار

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٦٠ عينة من ألبان الجمال والابقار (٣٠ عينة من كل نوع) تم فحصها في هذه الدراسة لمعرفة ما بها من بكتريا اللبن العصوية. أعتمد تصنيف بكتريا اللبن العصوية المعزوله على شكلها وصفاتها الظاهرية وعلى قدرتها على النمو عند درجات حرارة مختلفة وعلى مستنبتات تحتوي على تركيزات مختلفة من محلول كلوريد الصويوم، وكذلك على قدرتها على تخمر الكربوهيدرات. أظهرت نتائج البحث أن ٤٦.٧ % و ٦٠ % من عينات ألبان الجمال والابقار على التوالي تحتوي على بكتريا اللبن العصوية وبتراكيزات تراوحت من ٣٧×10^٢ : ٤٣×10^٤ (متوسط ١٢×10^١ /cfu مل) في اللبن الجمال وتراوحت من ٥×10^٢ : ٢٨×10^٤ (متوسط ٥×10^١ /cfu مل) في ألبان الأبقار. تم عزل ثلاث عترات من بكتريا اللبن العصوية في ألبان الجمال وهي: *L. bulgaricus* والتي تواجدت في ٢٣.٣ % من العينات و *L. planetarium* والتي تواجدت في ١٣.٣ % و *L. casei* والتي تواجدت في ١٠ % من العينات المختبرة. بينما تم عزل عترتين فقط من بكتريا اللبن العصوية في ألبان الأبقار وهما *L. bulgaricus* والتي تواجدت في ٢٠ % من العينات و *L. acidophilus* والتي تواجدت في ٤٠ % من العينات المختبرة. من هذه الدراسة يمكن ان نستنتج ان معظم ألبان الجمال تحتوي على عترات مختلفة من بكتريا اللبن العصوية (*Lactobacillus bulgaricus* *Lactobacillus plantarum* ، *Lactobacillus casei* ، وكذلك ألبان الأبقار تحتوي ايضا على سلالات اخرى (*Lactobacillus bulgaricus* and *Lactobacillus acidophilus*) ، هذه السلالات من بكتريا اللبن العصوية يمكن الاستفادة منها واستخدامها في تصنيع بادئات تخثر تفيد في تصنيع منتجات البان الجمال والابقار.