

MICROBIOLOGICAL STUDIES ON BACTERIAL COMMUNITIES IN SOME LOCAL FERMENTED MILK PRODUCTS (LABAN) IN SAUDI ARABIA

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ABSTRACT:

The present investigation was conducted to identify and characterize bacterial communities including starter bacteria as well as pathogenic ones, on some local fermented milk products (Laban) produced in Saudi Arabia in order to ensure human health. The isolated strains were characterized by phenotypic, physiological and biochemical methods, including API 50 CHL kit. The results showed that lactic acid bacteria (LAB) were present in high counts. The dominated species found were *Streptococcus salivarius* ssp. *thermophilus* (47.7%), *Lactobacillus delbrueckii* ssp. *bulgaricus* (22.2%), *Lactobacillus acidophilus* (22.2%), *Leuconostoc lactis* (5.5%), *Lactococcus lactis* ssp. *lactis* (2.2%). The results obtained demonstrated that the pathogenic bacteria in five examined products are *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus cloacae*, *Enterococcus durans*, *Enterococcus casseliflavus*, *Pseudomonas capacia*, *Streptococcus mitis*, *Bacillus cereus*, and *Corynebacterium* ssp. .

Keywords: Lactic acid bacteria (LAB), pathogenic bacteria, fermented milk products (Laban), Saudi Arabia.

INTRODUCTION

Laban is a fermented milk product produced in Saudi Arabia and some Arab countries using traditional and industrial manufacturing practices (Chammas *et al.* 2006). Lactic acid bacteria (LAB) have long been consumed by people in several fermented foods such as dairy products (Khedid *et al.*, 2006) Nowadays, LAB are a focus of 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.*, 2003) reduction of serum cholesterol (Desmazeaud, 1996; Jackson *et al.*, 2002) including antitumor activity (De Vuyst and Degeest, 1999; Hilde *et al.*, 2003) stimulation of the immune system (Isolauri *et al.*, 2001); stabilization of gut microflora (Gibson *et al.*, 1997). According to Tamime and Robinson (1999) laban is obtained through the lactic fermentation of heat-treated milk by using thermophilic starters, composed of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* strains. The thermophilic lactic acid bacteria are best known as starters for fermented milks. Several varieties of fermented milks originate from countries in Asia Minor and the Balkans, like Armenia, Turkey and Bulgaria. These products have emerged from spontaneous acidification of raw milk by indigenous organisms. Although these organisms have by no means been exhaustively characterised, they consist largely of thermophilic lactic acid bacteria, probably due to the relatively high incubation temperature determined by the prevailing climate. The first description of milk fermentations by these

bacteria can be found in the literature of some hundred years ago (Weigmann, 1905). Several attempts were made at that time to identify the bacteria dominating the flora in yoghurt-like products and they were given the names *Bacillus bulgaricus* and *Diplostreptococcus*. These spontaneous fermentations of milk into yoghurt have now been developed into microbiologically well-controlled industrial processes. The two most frequently used starter bacteria are now classified as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*, generally shortened as *Lb. bulgaricus* and *S. thermophilus*, respectively. Furthermore, many researches have studied the behavior of pathogenic bacteria in fermented milks (Al Shaikli, 1980; Salji et al, 1983; Nasser *et al.*, 2004; Ombui and Nduhiu, 2005; Aygun and Pehlivanlar, 2006; Zelalem *et al.*, 2007).

The main goals of this study were to isolate and identify LAB and pathogenic bacteria from 60 fermented milk samples belonging to 5 commercial companies were gathered at 5 different sites of Riyadh city in Saudi Arabia.

MATERIALS AND METHODS

Samples collection:

A total of 60 samples of fermented milk products (Laban) belonging to 5 commercial companies were collected from 5 different sites in the north, east, south, west, and central regions of Riyadh city. The samples were obtained from both large trade centers and small markets at each site. All samples have the same production dates. The samples were immediately cooled and transported to the laboratory in icebox 4° C and analyzed for the content of LAB and pathogenic bacteria on the arrival.

Isolation of the strains:

- LAB:

Aliquots of 10 ml of fermented milk sample was homogenized with 90 ml of peptone water to make an initial dilution (10^{-1}). The suspension was used for making suitable serial dilution up to 10^{-9} by incorporating 1 ml into 9 ml of sterile peptone water in sterile tubes. Enumeration of LAB was determined using various selective media, MRS agar (pH 5.3) (Biokar, France) and M17 agar (pH 6.2) (Biokar, France). After incubation at 30° C (24 - 48 h) colonies were enumerated, recorded as colony forming units (CFU/g cheese). The colonies were randomly picked from plates with 30- 300 colonies, and transferred in 10 ml of appropriate broth. The selected colonies were purified by repeated streaking on the appropriate ager media. LAB strains were kept on media slant at 4°C and streaked every 4 weeks. Prior to use, LAB strains were activated in broth media at 30°C for 24 h, and subcultured in MRS agar at 30°C for 24 h.

- Pathogenic bacteria:

Ten ml of fermented milk sample was homogenized with 90 ml of saline water to make an initial dilution (10^{-1}). The suspension was used for making suitable serial dilution up to 10^{-8} by incorporating 1 ml into 9 ml of

sterile saline water in sterile tubes. Enumeration of pathogenic bacteria was determined using various selective media, Nutrient agar (The total bacterial count), MacConkey agar (Coliform), Bile Aesculin agar (Enterococci), Mannitol salt agar (Staphylococcus), and Bacillus cereus selective agar Base (Bacillus). After incubation at 37°C (24 - 72 h) colonies were enumerated, recorded as colony forming units (CFU /ml) of cheese. The colonies were picked from plates, and transferred in 10 ml of appropriate broth. The selected colonies were purified by repeated streaking on the appropriate agar media. Pathogenic bacteria strains were kept on slants of the suitable media at 4°C.

Identification of the studied bacterial isolates:

LAB strains were identified by morphological, physiological and biochemical techniques according to methods recommended by several authors (Facklam and Collins, 1989; Charteris *et al.*, 2001; and Klein, 2001). All strains were initially subjected to Gram staining, catalase test, growth at 10 - 45 °C in MRS and M17 broth, and gas production from glucose. All strains were tested for their sugar fermentation patterns using API 50 CHL, and duplicated for each isolate. Pathogenic bacteria strains were identified by morphological, physiological and biochemical techniques according to methods recommended by several authors (Van Netten, 1989; Martin *et al.*, 1967; and Holbrook and Andersson, 1980). All strains were initially subjected to Gram staining and catalase test. Identification of bacteria was carried out using the methods recommended in Bergey's Manual of Systematic Bacteriology Vol. 1 (Holt, 1984).

RESULTS AND DISCUSSION

LAB counts in fermented milks:

The M17 agar medium was used to estimate the numbers of *Streptococcus salivarius* ssp. *thermophilus* while MRS agar medium was used to elucidate the numbers of *Lactobacillus*. The results obtained show that the estimated starter bacterial numbers on M17 agar media were ranged from 10⁸ to 10⁹ while those estimated on MRS agar media were ranged from 10⁶ to 10⁷ (CFU/ml) (Table 1). Statistical analysis shows that there were no significant differences between the means of the starter bacterial numbers on the M17 agar media for the samples in the large centers as compared with those in the small markets the beginning of the production. The results showed that there were decrease in the numbers of the starter bacteria on M17 agar media for the samples stored in the larger centers at the expire date as compared with those at the beginning. Further decrease was noticed in the small markets.

Also, there were no significant differences between the means of the starter bacterial numbers on the MRS agar media for the samples in the large centers as compared with those in the small markets at the beginning of the production. Also, the recorded data showed that there were decrease in these numbers on the same media for the samples stored in large centers at

the expired date as compared with those at the production date. Additional decrease was also recorded in the small markets.

Table 1: The mean numbers of starter bacteria from fermented milk grown on M17 agar and MRS agar media

Cheese products	M17 agar	MRS agar
Product I	1.67×10 ⁹	1.09×10 ⁷
Product II	1.64×10 ⁹	1.6×10 ⁶
Product III	2.30×10 ⁹	2.3×10 ⁶
Product IV	9.40×10 ⁸	9.11×10 ⁷
Product V	2.19×10 ⁸	0

Identification of isolates:

A total of 250 isolates were isolated from fermented milk samples gathered from 5 trade companies. A preliminary identification for these isolates was carried out on selective media. The identification for all isolates was continued by gram stain and catalase test.

The results of the identification revealed that the isolates grown on M17 agar medium have spherical shape to oval or short rods, usually present in pairs or chains, immotile, catalase negative and gram positive. While results of identification for the isolates grown on MRS agar medium have uniform rod shape. However, some isolates take short rod shape. They were gram positive, catalase negative and obligate anaerobic. A total of 90 isolates from these lactic acid bacteria representing all the studied samples were randomly selected. Then they were identified by the identification ribbons API 50 CHL. The results recorded show that the lactic acid bacteria present in the samples belonging to the trade company-I were *Streptococcus salivarius* ssp. *thermophilus* (present in about 35%), *Leuconostoc lactis* (15%) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (50%) (Fig. 1). *Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus acidophilus* were recorded in the samples of the trade company-II with ratio of 50% for each type (Fig. 1). Considering bacterial types noticed in the samples of the trade company-III were *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus acidophilus* and *Leuconostoc lactis* with ratios of 40, 50 and 10%, respectively (Fig.1). Also, *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Lactococcus lactis* ssp. *lactis* were detected with ratios 45, 50 and 5%, respectively in the samples of the trade company IV (Fig. 1). The gained results revealed that *Streptococcus salivarius* ssp. *thermophilus* represents the most pronounced bacterial type followed by *Lactococcus lactis* ssp. *lactis* recognized in the samples of the trade company V. They were recorded with ratios 90 and 10%, respectively (Fig. 1).

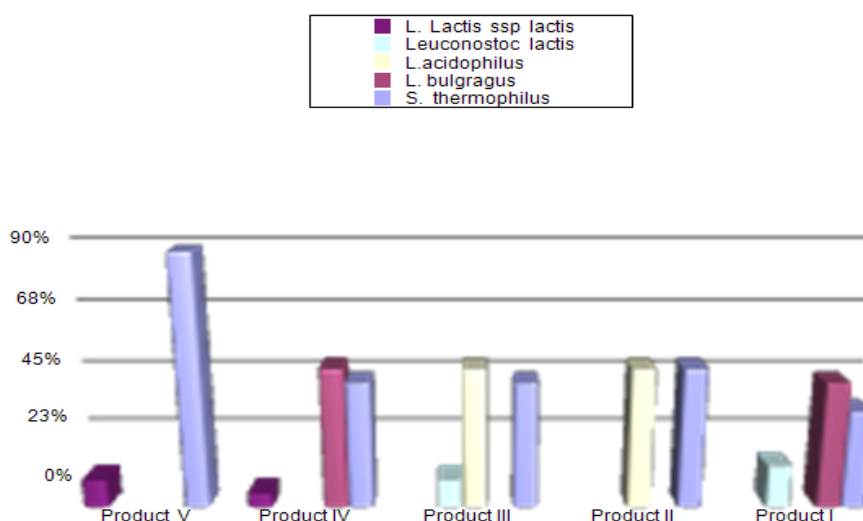


Fig. 1 Lactic acid bacteria species isolated from fermented milk products.

In general, mean ratios of *Streptococcus salivarius* ssp. thermophilus, *Lactobacillus dellberckii* ssp. bulgricus, *Lactobacillus acidophilus*, *Leuconostoc lactis* and *Lactococcus lactis* ssp. lactis in all samples gathered from the five trade companies were 47.77%, 22.22%, 22.22%, 5.5% and 2.2%, respectively (Table 2) (Fig. 2).

Table 2: Identification of the Lactic acid bacteria species isolated from fermented milk products

Isolates	Total of isolates	Present
<i>Streptococcus salivarius</i> ssp. thermophilus	43	47.77%
<i>Lactobacillus dellberckii</i> bulgricus	20	22.2%
<i>Lactobacillus acidophilus</i>	20	22.2%
<i>Leuconostoc lactis</i>	5	5.5%
<i>Lactococcus Lactic</i> ssp. lactis	2	2.2%

By considering their phenotypical characteristics, all strains isolated from fermented milk samples belonged to *S. thermophilus* and *L. bulgaricus*. This confirmed that laban is fermented milk product similar to yogurt, as already reported by Tamime and Robinson, 1999.

Counting and identification of pathogenic bacteria in the tested fermented milks samples:

In this study contamination of the fermented milk samples collected from the five trade companies were determined. The numbers of pollutants were counted using selective media at the beginning of production date as well as at the expired date. The results obtained revealed that only two out of the five sample collections have high contamination growth while the other

three collections did not show any contamination growth (Table 3). The results also show that contaminant types were *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter cloacae*, *Enterococcus durans*, *Enterococcus casseliflavus*, *Pseudomonas capacia*, *Corynebacterium ssp.*, *Streptococcus mitis*, and *Bacillus cereus*.

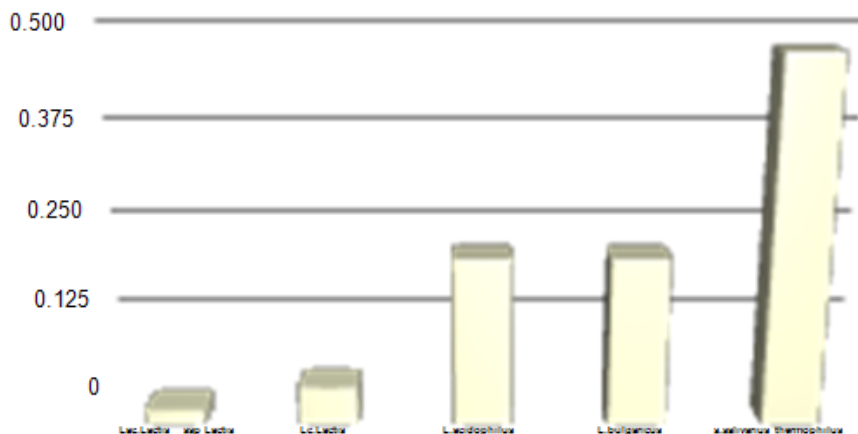


Fig. 2 Identification of the Lactic acid bacteria species isolated from fermented milk products

Table 3: Pathogenic bacteria in the tested fermented milk samples

Cheese products	Nutrient Agar	Nutrient Agar 10 °C	MacConakey agar	Mannitol Salt agar	Bacillus Cereus Agar	Bile Aesculin Agar	Ss agar
I	1.3×10 ⁸	0	3.28×10 ⁴	6.9×10 ²	0	4.24 ×10 ⁷	0
II	1.8×10 ⁷	0	1.27×10 ⁵	0	1.9×10 ⁴	1.8×10 ²	0
III	3.3×10 ⁵	0	0	0	0	0	0
IV	1.9×10 ⁵	0	0	0	0	0	0
V	9.3×10 ⁴	0	0	0	0	0	0

In fact fermented food products are usually considered safe because of the low pH and production of antimicrobial substances by fermenting organisms.

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دراسات ميكروبية للمجتمعات البكتيرية في بعض منتجات الالبان المتخمرة المحلية (اللبن) في المملكة العربية السعودية

ندى الحربي ، حسام الدين زكي حسن و كوثر عابد
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أجريت هذه الدراسة لتحديد وتوصيف مجتمعات البكتيريا بما في ذلك بكتيريا البادئ فضلا عن الأنواع الممرضة منها في بعض منتجات الالبان المتخمرة المحلية (اللبن) التي تنتج في المملكة العربية السعودية من أجل ضمان صحة الإنسان. تم تعريف السلالات المعزولة باستخدام الشكل الظاهري و الاختبارات الفسيولوجية والبيوكيميائية بالإضافة في (LAB) وأظهرت النتائج ارتفاع أعداد بكتيريا حمض اللاكتيك API 50 CHL. إلى استخدام نظام *Streptococcus salivaius* ssp. thermophilic (47.7%) العينات المختبره . و قد هيمنت الأنواع

Lactobacillus dellberckii ssp. bulgricus (22.2%), *Lactobacillus acidophilus* (22.2%), *Leuconostoc lactis* (5.5%), *Lactococcus lactis* ssp. lactis (2.2%)

كما أظهرت النتائج المتحصل عليها أيضا أن البكتيريا الممرضة في الخمسة المنتجات محل الدراسة هي *Escherickia coli*, *Enterococcus faecalis*, *Enterococcus cloacae*, *Enterococcus durens*, *Enterococcus casselifavus*, *Pasudomonus capacia*, *Streptococcus mitis*, *Bacillus ceres*, and *Corynebacterium* ssp

قام بتحكيم البحث

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