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MICROBIOLOGICAL STUDIES OF BACTERIAL COMMUNITIES IN SOME LOCAL CHEESE PRODUCTS 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 cheese products produced in Saudi Arabia in order to ensure human health. The isolated strains were characterized by phenotypic, physiological and biochemical methods, including API 20 Strep. kit. The results showed that high counts of lactic acid bacteria (LAB) were found. The dominated species found were *Streptococcus salivaius ssp. thermophilic* (50%), *Leuconostc spp.* (24%), *Lactococcus lactic ssp. lactic* (4%), *Streptococcus mitis* (2%), *Enterococcus faecium* (12%), *Streptococcus acidominimus* (4%), *and Enterococcus durans* (4%). The results obtained demonstrated that the pathogenic bacteria in five examined products are *Enterobacter Cloacae, Enterococcus faealis, Pseudomonas spp., staphylococcus aureus, Klebsilla sp., Bacillus spp., and Corynebacterium* (*Diptheroid*) *spp.*

Keywords: Lactic acid bacteria (LAB), pathogenic bacteria, cheese products, Saudi Arabia.

INTRODUCTION

Fermented foods are consumed widely worldwide (IDF, 1988). 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.*, 2002) reduction of serum cholesterol (Desmazeaud,1996; Jackson *et al.*, 2002) including antitumoral 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). The thermophilic lactic acid bacteria play an essential role in the manufacture of some cheese types. New strains of LAB have been isolated from cheeses (Callon *et al.*, 2004; El-Baradei *et al.*, 2007; Gonzalez *et al.,* 2007; Nikolic *et al.,* 2008). Furthermore, many researches have studied the behavior of pathogenic bacteria in cheeses (Ramon- Blarco *et al.*, 1999; Al-Jedah & Robinson 2001; Nasser *et al.*, 2004; Aygun, 2005; Jerome Mounier *et al.*, 2005, 2007).

This study was undertaken in order to isolate and identify LAB and pathogenic bacteria from 45 cheese samples collected from 5 different sites at Riyadh city in Saudi Arabia.

MATERIALS AND METHODS

Cheese Samples:

A total of 45 samples of white cheese 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:

Aliquot of 10g of cheese sample (surface and core) 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 elective media, MRS agar (pH 5.3) (Biokar, France) and M17 agar (pH 6.2) (Biokar, France). After incubation(24 - 48 h) colonies were enumerated, recorded as colony forming units (CFU/g) cheese. The colonies were randomly picked from plates containing 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 maintained on slants of suitable agar media at 4°C and subcultured every 4 weeks. Prior to use, LAB strains were activated in broth media 30°C for 24 h, and sub-cultured in MRS agar at 30°C for 24 h.

- Pathogenic bacteria:

Ten g of cheese 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. Enumeration of pathogenic bacteria was determined using various elective media, Nutrient agar (The total bacterial count), MacConkey agar (Coliform), Bile Aesculin agar (Enterococci), Mannitol salt agar (*Staphylococcus*), Salmonella Shigella agar (Salmonella and Shigella), Bacillus cereus selective agar Base (*Bacillus*), Listeria selective agar (Listeria), and Chocolate agar (Brucella). After incubation (24 - 72 h) colonies were enumerated, recorded as colony forming units (CFU /g) 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 media slant 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, acid and gas production from glucose. All strains were tested for their sugar fermentation patterns using API 20 Strep. and duplicated for each isolate. Pathogenic bacteria strains were identified by morphological, physiological and biochemical techniques according to methods recommended by 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 (1984).

RESULTS AND DISCUSSION

LAB counts in cheese:

The results obtained revealed that samples collected from both cheese surfaces and cores in summer and grown on M17 agar medium had more or less the same mean numbers of starter bacteria. The number reached a value of 10^9 CFU/g in all samples studied (Table 1) . In winter, the mean numbers of starter bacteria were lower, they reached a value of 10^8 CFU/g for both samples taken from surfaces and cores for all samples studied. Also, there were significant differences in the mean numbers of starter bacteria between surface and cores for the same cheese product. They showed that the mean numbers in cores were larger than those on surfaces in all examined products.

Table 1: The mean numbers of starter bacteria from both cheese surfaces and cores in summer and winter grown on M17 agar medium.

Cheese products	Summer		Winter	
	Core	Surface	Core	Surface
Product I	2.38×10^9	1.82×10^9	2.2×10^8	1.58×10^8
Product II	2.27×10^9	1.58×10^9	2.2×10^8	1.60×10^8
Product III	2.34×10^9	2.13×10^9	2.28×10^8	1.81×10^8
Product IV	2.30×10^9	2.01×10^9	2.33×10^8	1.81×10^8
Product V	2.24×10^9	1.61×10^9	2.17×10^8	1.97×10^8

Also, the gained data demonstrated that samples collected from both cheese surfaces and cores in summer and grown on MRS agar medium had approximately the same mean numbers of starter bacteria. They reached a value of 10^8 CFU/g in all studied samples (Table 2) . In winter, the mean numbers of starter bacteria were lower. They reached a value of 10^7 CFU/g for both samples taken from surfaces and cores of all samples studied. In the contrary, there were significant differences in the mean numbers of starter bacteria between surfaces and cores of the same cheese product .

Table 2: The mean numbers of starter bacteria from both cheese surfaces and cores in summer and winter grown on MRS agar medium.

Cheese products	summer		winter	
	Core	Surface	Core	Surface
Product I	2.41×10^8	2.16×10^8	2.4×10^7	1.93×10^7
Product II	2.34×10^8	2.05×10^8	2.14×10^7	1.71×10^7
Product III	2.29×10^8	2.0×10^8	2.29×10^7	2.0×10^7
Product IV	2.16×10^8	2.0×10^8	2.28×10^7	1.9×10^7
Product V	2.30×10^8	2.01×10^8	2.23×10^7	1.92×10^7

Identification of isolates:

A total of 250 isolates were randomly isolated and purified using a single colony isolation technique to obtain pure colonies. The isolates were identified using culture-dependant and morphological methods. The results of such identification for all isolates were confirmed by the catalase test followed by the gram stain test. These tests have been demonstrated that the bacteria grown on both MRS agar and M17 agar media are of spherical shape, some of them take the form of oval or short rods, and are present in pairs or chains. The cells are non-motile, non-spore former, catalase positive and facultatively anaerobic. Further identification using API 20 Strep. was carried out for 10 randomly chosen isolates from each of the products studied; this method is a standard technique which includes 20 biochemical tests. The results showed that the most pronounced bacterial type in product I is the genus *Leuconostoc spp.* Which is present in 50% of isolates (Fig. 1). Also, *Enterococcus durans* was identified in 20%. Other types of bacteria were recognized at equal rates (10%). These types are *Streptococcus salivarius ssp. thermophilus, Lactococcus lactis ssp. lactis,* and *Enterococcus faecium*. Similarly, the most frequent bacterial type in the product II is *Streptococcus salivarius ssp. thermophilus* which was recorded in 50% of the isolates whereas each of *Leuconostoc* spp. and *Streptococcus acidominimus* were detected at a rate of 20%. The least common one is *Streptococcus mitis* which was recorded with a ratio of 10% (Fig. 2). *Leuconostoc spp*. was found to be the most frequent one in the product III where it was detected at a rate of 40%. *Streptococcus salivarius ssp. thermophilus, Enterococcus faecium* were detected in the product III with a rate of 30% for each of them (Fig 3). Considering product IV, *Streptococcus salivarius ssp. thermophilus* represent the most recognized type (70%) followed by *Enterococcus fecium* (20%) then *Leuconostoc spp*. (10%) (Fig.4). Product V includes *Streptococcus salivarius ssp. thermophilus* (90%) and *Lactococcus lactis ssp. lactis* (10%) (Fig. 5).

In general, the results obtained show that a total of 25 isolates of *Streptococcus salivarius ssp. thermophilic* were isolated. It represents the most frequent genus being present in 50% of samples. Also, a total of 12 isolates of *Leuconostoc spp.* were isolated with a value of 24%. Two isolates of *Lactococcus lactic ssp. lactic* were isolated with a rate of 4%.

Fig. 1: Lactic acid bacteria species isolated from product I.

Leuconostoc spp ***S. salivarius thermophilus*** ***S. mitis*** ***S. acidominimus***

isolates

Fig. 2: Lactic acid bacteria species isolated from product II.

isolates

Fig. 3: Lactic acid bacteria species isolated from product III.

isolates

Fig. 4: Lactic acid bacteria species isolated from product IV.

Fig. 5: Lactic acid bacteria species isolated from product V.

Only one isolate of *Sterptococcus mitis* was isolated giving it a rate of 2%. *Enterococcus faecium* was isolated from 6 isolates with a rate of 12%. *Streptococcus acidominimus* was isolated from two isolates with a rate of 4%. Finally, *Enterococcus durans* has been isolated from two isolates with a rate of 4% (Table 3).

By considering their phenotypical characteristics, all strains isolated from both cheese surfaces and cores samples belonged to *Leuconostoc spp., S. thermophilus Enterococcus durans, Lactococcus lactis ssp. lactis,* and *Enterococcus faecium,* as already reported by Ercolini *et al.* 2003; Psoni and Litopoulou-Tzanetaki, 2003).

Table 3: Identification of the Lactic acid bacteria species isolated from products

Isolates	Total of isolates	Present
Streptococcus salivarius ssp. thermophilus	25	50%
Leuconostoc spp	12	24%
Enterococcus faecium	6	12%
Enterococcus durans	2	4%
Lactococcus Lactis ssp. lactic	2	4%
Streptococcus acidominimus	2	4%
Streptococcus mitis	1	2%

Counting and identification of pathogenic bacteria in the tested cheese samples:

The bacterial numbers recorded in 5 local cheese products grown on

nutrient agar medium and on the studied selective media were counted. All examined samples showed bacterial growth on all media except lesteria medium (Table 4). Also, it was recorded that bacterial numbers grown on selective media were lower compared with the numbers grown on nutrient agar and MaConky media. The comparison of means of numbers and percentages of bacterial cells isolated from cheeses belonging to other trade companies and grown on the feeding agar medium were in the range of 10^6 CFU/g.

Table 4: Pathogenic bacteria in the tested cheese samples

Cheese products	Nutrient Agar	Salmonella Shigella Agar	Listeria Selective Agar	MacConkey Agar	Bile Aesculin Agar	Mannitol Salt Agar	Bacillus Cereus Selective Agar	Chocolate Agar
I	9×10^6	10.8×10^4	0	14.8×10^6	5.3×10^5	13.5×10^5	3.2×10^6	8.7×10^5
II	11.3×10^6	1.4×10^4	0	2.7×10^6	10.1×10^4	2.5×10^6	13.4×10^5	3.2×10^5
III	6.1×10^6	2.9×10^4	0	3.3×10^6	5.2×10^5	2.2×10^6	1.8×10^6	5.7×10^3
IV	2.9×10^6	2×10^4	0	5.4×10^6	2.4×10^5	3×10^5	6.4×10^5	14.7×10^5
V	4.7×10^3	0	0	0	3.4×10^2	0	0	1.9×10^2

The results obtained showed an increase in the means of bacterial numbers isolated from cheeses from the trade companies I and III (Fig. 6). They increased their presence to 47.3% and 22.1 %, respectively. The means of the bacterial numbers from product V were decreased to 10^3 CFU/ml and their ratio reached to 0.01%. This is a good indication for such cheese products. The obtained results demonstrated that the pathogenic bacteria in the five examined products are *Enterobacter cloacae, Enterococcus faecalis, Pseudomonas spp., Staphylococcus epidermidis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Klebsilla spp., Bacillus ssp., Corynebacterium* (Diptheroid).

Fig. 6: The means of pathogenic bacteria numbers isolated from cheese products

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دراسات ميكروبية للمجتمعات البكتيرية في بعض منتجات الاجبان المحلية في المملكة العربية السعودية

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أجريت هذه الدراسة لتحديد وتوصيف مجتمعات البكتيريا بما في ذلك بكتيريا البادئ فضلا عن الآنواع الممرضة منها في بعض منتجات الآجبان المحلية التي تنتج في المملكة العربية السعودية من أجل ضمان صحة الإنسان. تم تعريف السلالات المعزولة باستخدام الشكل المظهري و الاختبارات الفسيولوجية والبيوكيميائية بالإضافة الى استخدام نظام .API 20 Strep التعريف في العينات المختبره . و قد (LAB)وأظهرت النتائج ارتفاع آعداد بكتيريا حمض اللاكتيك
Streptococcus salivaius ssp. thermophilic (50%), *Leuconostc* spp %ى
Lactococcus lactic ssp. lactic (4%), *Streptococcus mitis* (2%), *Enterococcus faecium* (12%), *Streptococcus acidominimus* (4%), and *Enterococcus durans* (4%)سادت الأنواع .(٢٤

كما أظهرت النتائج المتحصل عليها ايضا أن البكتيريا الممرضة في الخمسة المنتجات تحت الدراسة هي
Enterobacter cloacae, *Enterococcus faealis*, *Pseudomonas* spp., *Staphylococcus aureus*, *Klebsilla* spp., *Bacillus* spp. and *Corynebacterium* (Diptheroid

قام بتحكيم البحث

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