



# Identification of Lactic Bacteria of Milk Quail in Chad

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## ARTICLE INFO

### Article history:

Received on: 22/11/2013

Revised on: 6/12/2013

Accepted on: 14/12/2013

Available online: 31/12/2013

### Key words:

Chad, curdled milk, lactic acid bacteria

## ABSTRACT

In Chad dairy products, especially milk curds are traditionally very popular with the public. Unfortunately, piecemeal investigations already conducted have shown that the ideal conditions are not met to increase the economic life of products. Given this situation, we decided to work on lactic acid bacteria in five agro-ecological zones of the country. The microbiological tests have identified the bacteria on culture media M17 and MRS. These bacteria belong to thirteen species of lactic streptococci and lactobacilli. Knowing the properties of these bacteria and the synergies they come, recommendations have been made to ensure access to quality dairy products.

## 1. INTRODUCTION

Milk is a highly nutritious food for its richness in carbohydrates, fats, vitamins and minerals [1; 2]. In addition to their nutritional values the consumption of dairy products is also associated with beneficial effects on health [3]. The system of production and sale of milk and dairy products in Africa in general and in particular in Chad are traditional [4]. In Chad, N'Djamena is actually by far the largest center of consumption of milk and dairy products [5]. The supply chain of the city of N'Djamena in milk local has grown considerably due to the emergence and multiplication small dairy from 1990 companies in development outside the home sweet whole milk consumption, and the success of "rayeb", a local fermented whole milk [6]. Indeed, the dairy product comes in many forms depending on the region and varied tastes. The supply of milk in the city basin based on farms within a radius of 65 km [7]. The curd is especially appreciated by the Chadian consumers. Fermented milk has always been traditionally consumed in many countries [8]. In the process of fermentation, lactic acid bacteria play an important role. Studies qualities carried out in this sector show microbial contaminations largely above international standards [9,10,11]. Among the lactic acid bacteria with habitat as milk, we have *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Aerococcus* [12]. But lactic acid bacteria also have other interests in their industry are acquired long ago. They provide

special aroma and texture features and good food security through organic acid products (lactic and acetic acids) that lower the pH in the middle [13]. Also, many studies [14; 15; 16] were conducted on dairy products fermented. In Chad, there is not enough information on strains that can be desirable to improve the quality characteristics of fermented dairy products. That is why this study was conducted in five locations for two seasons in order to identify lactic acid bacteria curd depending on the climate.

The general objective of this work is to know the lactic flora of different curds in Chad. Specifically this study helps to know the lactic flora specific to each area, about the proliferation of lactic flora in relation to season and location. This will capitalize on scientific information in order to improve the quality advantage curds marketed and consumed in Chad.

## 2. MATERIALS AND METHODS

This work was performed at the Laboratory of Veterinary and Zootechnical Research of Farcha in Chad in the Hygiene Services Industry and Food Commodities of Animal Origin.

### 2.1 Sampling areas

Five locations were selected for this study. This is N'Djamena, Sarh, Moundou Abeche and Bongor (Figure 1). These areas were chosen based on animal concentration in the dry season but also depending on the development of a trade for the dairy industry.

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Fig. 1: Location of study areas indicated by blue circles (Source: Jeune Afrique, 2006, amended).

Table 1: Lactic acid bacteria isolated in cold dry season (Abeche).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	41%	10.73	17.31
<i>Lactococcus lactis diacetylactis</i>	19%	8.36	23.62
<i>Lactococcus lactis lactis</i>	6%	12.5	23.4
<i>Lactococcus lactis cremoris</i>	4%	14.87	17.75
<i>Lactobacillus acidophilus</i>	5%	7.6	22.6
<i>Lactobacillus fermentum</i>	7%	20.14	14.42
<i>Lactobacillus plantarum</i>	7%	11	12.5
<i>Lactobacillus lactis</i>	3%	12.33	9.83
<i>Lactobacillus bulgaricus</i>	8%	11.18	12.62

## 2.2 Sampling

As Chad has several agro-ecological regions, it was necessary to have a representative in the quantity and quality of production areas curd samples. And for a period of two years, one thousand (1000) samples are taken at random, five hundred during the cold dry season (October to December) and five hundred during the hot dry season (March-May) in the same sites. By producer we take 10 samples of 250 ml who all come from different cans of 40 liters.

Identifications are made in indelible ink. This with a view to avoid sampling curd having the same origin, or at least whose manufacturing practices are similar. Quickly, we introduce samples into the cooler to prevent microbial growth. Thus the chain is maintained to the storage location. This continues until one hundred samples suburban area. The Thousand (1000) samples were collected as aseptic rules respecting rigorous hygiene standards.

It consists of plastic pots of 250 ml volume. After sampling, the samples were quickly introduced into a cooler with bottles of dry ice and crushed ice to maintain samples at very low temperatures. We used as thermo-hygrometers and pH meter probe to allow us to measure the temperature, moisture and pH levels. All samples are kept in cold rooms of the Research Laboratory Veterinary and Zootechnical (LRVZ).

## 2.3 Sample Preparation

All samples studied have undergone preliminary treatment to obtain dilutions according to standard NF V08 -010 (March 1996). In STOMACHER<sup>ND</sup> bag are introduced 10 ml of the sample to which are added 90 ml MRS broth or broth or M17.

## 2.4 Search mesophilic lactic acid bacteria

Lactic flora was enumerated according to the NF ISO 15214 (September 1998). The culture media used are: broth and agar de MAN ROGOSA and SHARPE (MRS) for the enumeration of lactobacilli and M17 medium for the enumeration of streptococci.

For each sample, the morphological characteristics of the colonies (size, shape, color) are observed. Lactobacillus colonies are round, lenticular, vary in size (1-4 mm), those of Streptococcus are round or lenticular, with regular contours, a white opaque.

## 2.5 Expression of results

For the expression of results is the following formula is used.

$$N = \frac{\Sigma c}{V (n_1 + 0.1 n_2) d}$$

N = N: number of cells /

$g \Sigma C$  = sum of counted on all boxes used two successive dilutions colonies, V = volume of the inoculums applied to each box (in ml)

$n_1$ : number of boxes used for the first dilution  $n_2$ : number of boxes used the second dilution of: dilution in the first box selected.

## 2.6 Identification and confirmation method (NF ISO 15214, September 1998)

We must clarify that the wells after receiving tabs reagents are covered by a plastic cover to prevent contamination. For bacteria grown in MRS agar, we use the API 50 CH galleries - L. On the cup with 50 stalls, we put a small amount of distilled water to be able to fix the plates that have compartments containing chemicals that are capable of reacting with the bacterial suspension with or without color change water. Indeed, it there's a note that explains the positivity or negativity of a reaction depending on the color and tell us the number to write. And so we get a code number and we will consult the grid ID for the name of the bacterium with a percentage of identification and testing against.

For bacteria grown in M17 agar, the cup is more simplified and has only ten cubicles allowing the strip containing the reagents to adhere by putting a small amount of distilled water. Each stall is filled with the suspension is at half or three-quarters full. Some stalls are filled entirely from the paraffin oil for achieving anaerobiosis.

For the M17, there's a reading that it takes 2 hours and 24 hours. Whereas MRS reading is at 24 h only. After reading the bacteria are identified. But to avoid any confusion the APIWEB TM software is used for the second time with the same code.

## 2.7 Statistical Analysis

The collected data are entered in the spreadsheet Microsoft Excel version 2003. Then to compare means, we used the SPSS 16 software. 0 from WINDOWS including Student's t test and analysis of variance (ANOVA) with a factor.

## 3. RESULTS

We have isolated and identified a total of 13 species of bacteria from the curds collected in Abeche, N'Djamena, Bongor, Sarh and Moundou in hot dry season of 2006 (March to May) and cold dry season of 2008 (October to December).

Microbiological analyzes were performed on curds collected. The species identified are *Streptococcus thermophilus*; *Lactococcus lactis diacetylactis*; *Lactococcus lactis cremoris*; *Lactococcus lactis lactis*; *Leuconostoc ssp*; *Loctobacillus lactis*; *Lactobacillus casei*; *Lactobacillus helveticus*; *Lactobacillus plantarum*; *Lactobacillus bulgaricus*; *Lactobacillus fermentum*; *Lactobacillus acidophilus*; *Lactobacillus brevis*. We present below the details of results in tables 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

## 4. DISCUSSION

Results of our work have identified various species of bacteria in cold dry season and hot dry season.

**Table. 2:** Lactic acid bacteria isolated in hot dry season (Abeche).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	51%	7.65	16.67
<i>Lactococcus lactis diacetylactis</i>	28 %	11.01	13.77
<i>Lactococcus lactis lactis</i>	12%	5.87	13.81
<i>Lactococcus lactis cremoris</i>	2%	11	2.75
<i>Leuconostoc ssp</i>	2%	2.25	10.5
<i>Lactobacillus acidophilus</i>	2%	10.3	10.66
<i>Lactobacillus fermentum</i>	1 %	3.5	9.5
<i>Lactobacillus plantarum</i>	2 %	5.5	2.5
<i>Lactobacillus bulgaricus</i>	4 %	4.33	12.75

**Table. 3:** Lactic acid bacteria isolated in cold dry season (N'Djamena).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	25%	14.18	15.46
<i>Lactococcus lactis diacetylactis</i>	15 %	13.23	15.1
<i>Lactococcus lactis cremoris</i>	4 %	18.12	21.37
<i>Leuconostoc ssp</i>	5 %	18.4	22.2
<i>Lactobacillus acidophilus</i>	6 %	14.5	12.33
<i>Lactobacillus fermentum</i>	11 %	13.22	13.04
<i>Lactobacillus plantarum</i>	11 %	13.36	16.54
<i>Lactobacillus lactis</i>	6 %	15.5	8.66
<i>Lactobacillus bulgaricus</i>	10 %	15.3	17.7
<i>Lactobacillus helveticus</i>	4%	25.37	15
<i>Lactobacillus brevis</i>	3%	7	8.83

**Table. 4 :** Lactic acid bacteria isolated in hot dry season (N'Djamena).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	52%	10.06	13.65
<i>Lactococcus lactis diacetylactis</i>	31%	9.02	10.50
<i>Lactococcus lactis cremoris</i>	3%	13.66	8.66
<i>Leuconostoc ssp</i>	3 %	10.83	17.83
<i>Lactobacillus lactis lactis</i>	3 %	0.55	6.8
<i>Lactobacillus fermentum</i>	1 %	0.1	13
<i>Lactobacillus plantarum</i>	2 %	0.85	10.25
<i>Lactobacillus lactis</i>	2 %	17.25	17.25
<i>Lactobacillus bulgaricus</i>	1 %	5.7	8.5
<i>Lactobacillus acidophilus</i>	2%	27	18.50

**Table. 5 :** Lactic acid bacteria isolated in cold dry season (Bongor).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	38%	52.84	10.56
<i>Lactococcus lactis diacetylactis</i>	31 %	14.09	11.56
<i>Lactococcus lactis lactis</i>	6%	21.75	18.66
<i>Lactococcus lactis cremoris</i>	7%	19.5	11.14
<i>Leuconostoc ssp</i>	2 %	16.25	15
<i>Lactobacillus fermentum</i>	3 %	8.66	9.66
<i>Lactobacillus bulgaricus</i>	6 %	18.66	19.66
<i>Lactobacillus acidophilus</i>	2%	11	9
<i>Lactobacillus lactis</i>	5%	21.5	14

**Table. 6 :** Lactic acid bacteria isolated in hot dry season (Bongor).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	30%	12.25	8.16
<i>Lactococcus lactis diacetylactis</i>	49 %	10.56	9.75
<i>Lactococcus lactis cremoris</i>	5 %	15.2	19.2
<i>Lactobacillus plantarum</i>	1 %	13.00	13.00
<i>Lactobacillus fermentum</i>	1 %	2.50	7.50
<i>Lactobacillus bulgaricus</i>	9 %	13.33	11.55
<i>Lactobacillus acidophilus</i>	3 %	13.33	11.33
<i>Lactobacillus lactis</i>	2 %	10.5	21.5

**Table. 7:** Lactic acid bacteria isolated in cold dry season (Sarh).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	31%	20.40	17.06
<i>Lactococcus lactis diacetylactis</i>	13 %	27.23	21.80
<i>Lactococcus lactis lactis</i>	10%	14.45	11.1
<i>Lactococcus lactis cremoris</i>	6 %	15.16	13.58
<i>Lactobacillus acidophilus</i>	5 %	21.2	20.8
<i>Lactobacillus fermentum</i>	6 %	12.33	14.91
<i>Lactobacillus plantarum</i>	11 %	23.31	23.22
<i>Lactobacillus lactis</i>	4%	18.37	15.25
<i>Lactobacillus bulgaricus</i>	11 %	18.27	16.63
<i>Lactobacillus casei</i>	3%	8.83	11.5

**Table. 8 :** Lactic acid bacteria isolated in hot dry season (Sarh).

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	45%	18.93	16.71
<i>Lactococcus lactis diacetylactis</i>	14 %	8.42	9.10
<i>Lactococcus lactis cremoris</i>	10%	26.35	28.4
<i>Lactococcus lactis lactis</i>	3%	0.5	7.83
<i>Lactobacillus fermentum</i>	7 %	20.14	14.42
<i>Lactobacillus plantarum</i>	11 %	21.72	23.54
<i>Lactobacillus bulgaricus</i>	10%	26	23.6

**Table. 9:** Lactic acid bacteria isolated in cold dry season (Moundou)

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	26%	14.88	20.57
<i>Lactococcus lactis diacetylactis</i>	28 %	18.31	21.22
<i>Lactococcus lactis cremoris</i>	10%	21.5	22.65
<i>Lactobacillus lactis lactis</i>	10 %	9.5	12.2
<i>Leuconostoc ssp</i>	6%	14.41	15.5
<i>Lactobacillus acidophilus</i>	6 %	14	17
<i>Lactobacillus plantarum</i>	9 %	23.11	23.88
<i>Lactobacillus fermentum</i>	6 %	13.33	13.33

**Table. 10 :** Lactic acid bacteria isolated in hot dry season (Moundou)

Lactic acid bacteria	Percentage	Number in MRS X x 10 <sup>9</sup>	Number in M17 X x 10 <sup>9</sup>
<i>Streptococcus thermophilus</i>	36%	11.81	17.93
<i>Lactococcus lactis diacetylactis</i>	28 %	18.95	21.38
<i>Lactococcus lactis cremoris</i>	8%	9.22	13.56
<i>Lactococcus lactis lactis</i>	6%	7.23	9.75
<i>Lactobacillus lactis</i>	2%	11.35	16.25
<i>Lactobacillus casei</i>	2 %	7.45	10.75
<i>Lactobacillus helveticus</i>	3 %	16	19.5
<i>Lactobacillus plantarum</i>	7%	21.71	22.35
<i>Lactobacillus bulgaricus</i>	9%	9.77	12.55

#### 4.1 *Streptococcus thermophilus*

Curd of five areas analyzed showed that this bacterial species is predominant in the traditional curds of Chad. The percentage is higher in the hot dry season than in cold dry season except in the town of Bongor where it was found an inverse evolution. The percentage increase of the bacterial species in the four sites can be explained by the fact that this bacterium multiplies when the temperature is high hence ; it's *Streptococcus thermophilus*. According to other authors, the multiplication of the bacteria depends on the ambient temperature and pH. According to Walter *et al.*, [17], *Streptococcus thermophilus* is the only species of lactic streptococci which grow at 45 ° C but does not believe at 10 ° C. Other authors also reported that *Streptococcus thermophilus* develops when the ambient temperature is very high and when the pH is very low around 3; 5 [18; 19]. Our results abound in the same direction as those of Baroudi *et al.*, [20] and Ngassam [21] whose reported the the predominance of *Streptococcus thermophilus* in fermented milks studied.

#### 4.2 *Lactococcus lactis diacetylactis*

In the peri-urban area of Abeche , curd analyzed in cold dry season gave a percentage of 19 % of the bacterial species against a percentage of 28 % obtained during the hot dry season. In the area of N'Djamena, the percentage of the bacteria increased from 15 % to 31% over the next two seasons, the double. In the town of Bongor the percentage of the bacterial species evolved from 31% to 49% against the town of Sarh, changes in the rate of this bacterial species is not significant in the two seasons since the

percentage is increased from 13 % to 14% only. Finally, in the town of Moundou, the percentage is certainly important, but it has not changed over the past two seasons (28%). If the localities of Sarh and Moundou, this species has not been a change, it may be due to the temperature is low has held the climate is sub -Bissau in these communities. However, in areas where the temperature is high, this bacterial species has experienced a sharp increase in the cold dry season to the hot dry season. As in the previous species, *Lactococcus lactis diacetylactis* is a thermophilic bacterium. Many authors whose Ben Amor *et al.*, [22]; Baroudi *et al.*, [20]; Tantoui *et al.*, [23] and Jraidi Guizani [24] identified this species.

#### 4.3 *Lactococcus lactis lactis*

In curd peri- urban area of Abeche, this species was identified at a rate of 6% during the cold dry season. This percentage has increased from one to two during the hot dry season. In the area of N'Djamena this species has not been identified during the cold dry season. But during the hot dry season it has been identified as a percentage of 3%. Area Bongor rate of this species during the cold dry season is 6%, however this bacterium was not found during the hot dry season. In the town of Sarh, curd analyzed gave a percentage of about 10% in cold dry season but during the second season rate is only 3%. Finally, in the town of Moundou in cold dry season *Lactococcus lactis lactis* represented a percentage of 10 % against 6% in the hot dry season. This bacterial species even if she does not know the evolution of the previous species was still identified in each locality. Like other lactic streptococci bacteria, this bacteria often occurs in synergy

with other species in obtaining traditional curd. In effect, El-Baradei *et al.*, [25] have identified in the Egyptian traditional curd milk (Domiat) the species *Lactococcus lactis* subsp *lactis*. Also, further more studies [26; 27; 28] reported the prevalence of *Lactococcus*. Baroudi *et al.*, [20], Jraidi *et al.*, [24] and Ben Amor *et al.*, [22] also have identified this species in the traditional curd in combination with other species. But Lafarge *et al.*, [29]; Ngassam [21] noted for their majority presence of *Lactococcus lactis lactis* same as *Streptococcus thermophilus*.

#### 4.4 *Lactococcus lactis cremoris*

In the town of Abeche, this bacterial species in cold dry season represents a percentage of 4 %, while in hot dry season rate is only 2 %. In area of N'Djamena, the rates was 4% in cold dry season and 3% during the hot dry season. In the area of Bongor this bacterial species have a percentage of 7% during the cold dry season, it is 5 % during the hot dry season. In the area of Sarh, the evolution of this bacterial species has the same profile as other lactic streptococci, there is an increased rate during the hot season. Indeed, *Lactococcus lactis cremoris* which a percentage of 6% cold dry season sees its increased to 10% in the warm season rates. Finally, in the region of Moundou this species was identified during the two seasons of study, 10% in cold dry season and 8% during the hot dry season. Even though this bacterial species has not changed according to the temperature, it was found in all five locations with a neighbor of 10%. This means that this bacterium plays a fundamental role in obtaining traditional curds. Indeed it is often identified with the previous two *Lactococcus* side. Our results are similar to those of many authors [20; 30; 22]. Also Doutoum *et al.*, [31] identified *Lactococcus lactis cremoris*.

#### 4.5 *Leuconostoc ssp*

This bacterial species is pseudolactique because it produces lactic acid as other lactic acid bacteria as well as other substances such as acids and alcohols. She was not identified in the curd of Abeche suburban area during the cold dry season. However, it was found during the hot dry season with a percentage of about 2 %. In the area of N'Djamena this species represented a percentage of 5 % during the cold dry season and 3% during the hot dry season. By cons of the area Bongor, it was found that during the cold dry season at a rate of 2%. In the town of Sarh this bacterial species is absent in the two seasons. In the area of Moundou if this species represented a large percentage of the order of 6% during cold dry season, it was absent in the curd of this area during the hot dry season. This bacterial species does not seem to grow in areas where the ambient temperature is very high. This is why it is highly recognized in the curd of Guinean zones or Sahel during the cold season. Our results are similar to those of Baroudi *et al.*, [20]; Tantawi *et al.*, [23]; Jraidi *et al.*, [24] who reported that *Leuconostoc lactis* and *Leuconostoc cremoris* are part of lactic acid bacteria that are isolated in the eastern leben. Saidi and Kihal [32] identified in the Algerian leben 10% *Leuconostoc sp*. These authors argue that the acidification of milk leben in North African countries and the Mediterranean basin is mainly due to

*Lactococcus*, mainly *Leuconostoc ssp*. Isono et Shimuzu [33] have also isolated this species lactic in traditional curds of Masai in Tanzania. Moreover, Ouaghiri [34] points out that the most common in Moroccan raw milk lactic acid bacteria belonging to the species *Lactococcus lactis*, *Leuconostoc pseudomesenteroides*, *Leuconostoc mesenteroides* and *Lactobacillus plantarum*.

#### 4.6 *Loctobacillus lactis*

In the town of Abeche this bacterial species was identified during the cold dry season with a percentage of about 6 % while it is absent in the curd of the same area taken during the hot dry season. As against the curd of the suburban area of N'Djamena, the lactic species was identified in both seasons, 6% in cold dry season and 2% during the hot dry season. In the area of Bongor *Loctobacillus lactis* was identified in the two study seasons with 5% in cold dry season and 2% during the hot dry season. In the town of Sarh, this lactic species has been isolated during the cold dry season with a percentage of around 4 %. Finally, in the town of Moundou *Loctobacillus lactis* was isolated with a percentage of 2% during the hot dry season. The evolution of this species does not appear to lactic increasing temperature and decreasing pH because it is often isolated regardless of the place or season. But its action on getting traditional curd is not removed because it is involved in the production of lactic acid. Our results are similar to those of Khosrof [35] whose study of the Tunisian leben has shown that a very strong association of different species of lactobacilli was present among other *Lactobacillus lactis*. The results reported by Pissang [9] in the area of N'Djamena mention the massive presence of *Loctobacillus lactis* (21 %) in the curd of this region. Note further that in her study of the bacteria of raw milk in Algeria, Bekhouche [13] indicates that about the genus *Lactobacillus*, the species *Lactobacillus cellobiosus* is largely dominant over other species isolated.

#### 4.7 *Lactobacillus casei*

This lactic acid bacteria has not been identified in the curd of Abeche suburban area, or during the cold season or during the hot season. On the outskirts of N'Djamena also the lactic acid bacterium was not isolated during the two seasons. Similarly, in the area of Bongor during the two seasons of study, this bacterium has not been isolated. These three areas are not located in Guinean zones. In the region of Sarh, *Lactobacillus casei* was isolated in the curd during the cold season with a percentage of 3%. Finally, in the town of Moundou this species has been identified in the curd in hot dry season with a percentage of 2%. Our results show that this species seems confined to lactic Guinean zone where the relative humidity is very high. Our results are similar to those of Ngassam [21] who also identified this species lactic in curd in the suburban area of Dakar with a percentage of about 3 %. Saied and Boudabous [30] isolated from dairy products local *Lactobacillus casei* with a small percentage of 4%. Saidi and Kihal [32] identified in the Algerian leben *Lactobacillus casei* with a percentage of 5%.

#### 4.8 *Lactobacillus helveticus*

In the curd of Abeche suburban area this species has not been isolated or during the cold dry season or during the hot dry season. However in the area of N'Djamena, this species was identified during the cold dry season with a percentage of 4%. In area Bongor analysis curd did not isolate this species in both seasons. In the area of Sarh, microbiological studies curds were not able to isolate lactic this species. In the suburban area of Moundou, *Lactobacillus helveticus* was isolated in a percentage of about 3%. It is a kind lactic plays a synergistic role with other lactobacilli in obtaining dairy products but the rate seems pretty low. Pissang [9] did not identify the lactic curd in the suburban area of N'Djamena species. Similarly Ngassam [21] did not isolate the bacteria in the curd the suburban area of Dakar. Our results are similar to those Khosrof [35] who identified *Lactobacillus helveticus* in the Tunisian leben. The same results were obtained by Saidi and Kihal [32] who identified *Lactobacillus helveticus* in leben.

#### 4.9 *Lactobacillus plantarum*

Curd of suburban areas of Abeche analyzed identified *Lactobacillus plantarum* in cold dry season (7%) against (2%) during the hot dry season. In the area of N'Djamena, curd analyzed during the two seasons has given the following percentages: 11% in cold dry season against 2% during the hot dry season. Curd locality of Bongor analyzed, was used to isolate *Lactobacillus plantarum* only during hot dry season with a small percentage of the order of 1 %. In Sarh region, this bacterial species was identified in the two seasons with a percentage of 11%. Finally, in the suburban area of Moundou, curds have sampled and analyzed to isolate *Lactobacillus plantarum* during the two seasons, 9% during the cold dry season and 7% during the hot dry season. *Lactobacillus plantarum* was isolated from all five locations with a higher percentage in curd in southern areas. Doutoum *et al.*, [31] was also isolated *lactobacillus plantarum* in traditional curd in the Sudanian zone of Chad. Our results are similar to those of Pissang [9] who isolated the bacteria in lactic curd around N'Djamena with a high percentage (43%). Our results are also similar to those of Ngassam [21] who isolated the percentage of lactic acid bacteria in the curds of the Dakar region (36%). Saied and Boudabous [30] isolated *Lactobacillus plantarum* in the Tunisian leben with a percentage of about 13 %. Bouzaine *et al.*, [36] have isolated in the Tunisian leben among lactobacilli as *Lactobacillus plantarum* in addition to lactococci. These results are very different from ours. As against the results of Saidi and Kihal [32] are close to ours because they have identified in the Algerian leben many lactococci but also a high percentage of *Lactobacillus plantarum* in the order of 10.4%.

#### 4.10 *Lactobacillus fermentum*

Microbiological analyzes of curds of Abeche suburban area during the two seasons have isolated this lactic acid bacterium which is the percentage of 7% in cold dry season and 1% during

the hot dry season. The study of curd in N'Djamena also identified *Lactobacillus fermentum* with a rate of 11% in cold dry season but only 1% during the hot dry season. In the town of Bongor, this lactic acid bacterium was isolated during two seasons with 3% for the cold dry season and 1% for the hot dry season. In the town of Sarh also this bacterium was identified with a rate of 6 % in cold dry season and 7% in hot dry season. Finally, in the town of Moundou, curd analyzed allowed isolation of this bacterium only during the cold dry season with a rate of 6 %. This lactic acid bacterium which it is identified at very high rates does not seem to be influenced by changes in temperature. Our results have shown that the same bacteria grows much in areas where the temperature is not very high.

Our results are similar to those of Pissang [9] who identified *Lactobacillus fermentum* in curd of N'Djamena region with a percentage of 10%. As against our results are different from those of Saied and Boudabous [30] and Xanthopoulos *et al.*, [37] who noted a high percentage of this bacterial species ( 16%).

#### 4.11 *Lactobacillus acidophilus*

In the town of Abeche, curd analysis was used to isolate the bacterium in both seasons. The rate is 5% in cold dry season and 2% during the hot dry season. In the area of N'Djamena, this bacterium has been identified with a rate of 6 % in cold dry season and 2% during the hot dry season. Curd of the suburban area of Bongor analysis was used to isolate the lactic acid bacterium with a percentage of 2% in cold dry season and 3% in the second season. In the town of Sarh, the lactic acid bacteria have been identified in cold dry season with a rate of 5%. Finally, the curd of the suburban area of Moundou analyzed was used to isolate the bacterium only during the cold dry season with a rate of 6 %. If the evolution of the lactic acid bacterium according to the temperature is not well known, it is certain that it multiplies in the very acid curd. Our results are similar to those of Johnson *et al.*, [38] with the isolation of this bacterium in curds very acidic and Ahn *et al* [18], Kim *et al.*, [39], by which this species grows in areas where the pH is very low in the order of (3.5). Bae *et al.*, [40]; Yoon and Woon [19] reported high acid values neighbors 2.

#### 4.12 *Lactobacillus bulgaricus* (thermophilic)

Microbiological analyzes of the curd in Abeche suburban areas have isolated *Lactobacillus bulgaricus* during the two seasons with a rate of 8 % in cold dry season and 4% during the hot dry season. In the area of N'Djamena analyzes have isolated the bacterial species with a rate of 10% in cold dry season and 1% during the hot dry season. In the town of Bongor during the cold dry season, *Lactobacillus bulgaricus* has been identified with a rate of 6 % against 9% for the hot dry season. In the town of Sarh, in cold dry season rate of this lactic acid bacterium is 11% against 10% during hot dry season. For the locality of Moundou against microbiological analyzes have led to the isolation of *Lactobacillus bulgaricus* during the hot dry season with a rate of 9 %. And with few exceptions this bacterium was identified in all five locations

with a larger increase during the hot dry season because it is a *Thermophilic bacterium*. It occurs most often in synergy with *Streptococcus thermophilus* in the manufacture of sophisticated such as yogurt dairy products. Our results are similar to those of Baroudi *et al.*, [20]; and Loons (41) who reported that in the Lebanese and Iraqi laban, there is always presence of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*.

#### 4.13 *Lactobacillus casei*

It is a bacterial species that have been identified in the southern part of Chad. Other authors [21] isolated this species in the traditional lactic curd in Dakar region, but still a small percentage.

### CONCLUSION

This study allowed us to identify several species of bacteria including species of lactic streptococci and lactobacilli. Bacteria were isolated in the hot dry season and cold dry season in five localities in Chad belonging to different regions. The study confirms that there are differences in the identification of bacteria according to the seasons. We note for example that some bacteria such as *Lactobacillus casei* does not exist or the cold dry season or hot dry season in the three regions namely N'Djamena Abeche and Bongor. Against by the same bacterium was identified in regions of Moundou and Sarh where the relative humidity is high. Other studies are needed to characterize lactic acid bacteria according to the seasons.

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**How to cite this article:**

Doutoum A A., Tidjani A., Balde M., Tidjani.S.M.T, Abdelaziz Arada I, Sylla K. S. B., Seydi MG, Toguebaye B. S. Identification of Lactic Bacteria of Milk Quail in Chad. *J App Biol Biotech.* 2013; 1 (04):007-015