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Identification of lactic acid bacteria in traditional curd in the Sudanian zone of Chad

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Abstract

In Chad, milk and its products are consumed by many people including the curd in its various forms. This product still called "Laban Rayib" has also an important role in the labor market. In fact, these different types of curds are made without real respect basic standards of technology and hygiene. This situation will be improved if lactic ferments are well identified and taking into account their synergy. The Scientists can propose to the producers the formulas to improve the curds. However the studies in this field are rare to have reliable scientific information. It is in this context that this study was conducted to identify lactic acid bacteria in milk curd to provide scientific data needed for improve the fermented product. Thus, one hundred samples of 100 ml were collected from producers due to 10 samples / producer. The analyses were performed in the laboratory of Hygiene and Industry of Food of Origin Animal (HIDAOA) of the Inter-States School of Sciences and Veterinary Diseases (EISMV) of Dakar in Senegal and at the Veterinary and Zoo Technical Research Laboratory of Farcha (LRVZ) in Chad. The culture media used are M17 broth and agar, broth and de Man, Rogosa and Sharpe (MRS) for the search of these lactic acid bacteria. The identification of bacteria isolated and purified was made using the API 50 CH galleries biochemical, 20 STREP and software WEB API galleries. The lactic species identified are Lactoccocus diacetylactis. Streptococcus thermophilus, and Lactococcus lactis subsp lactis. Isolated Lactobacilli are Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus fermentum, Lactobacillus brevis and Lactobacillus acidophilus. The results of this work could be a good tool for information and awareness to improve the characteristics of the curd.

Keywords: Lactic acid bacteria, Sudan zone, Moundou, Chad.

INTRODUCTION

Milk is a staple food for humans. It is highly nutritious for its richness in carbohydrates, lipids, vitamins and minerals (Ahmed et al., 2010). In addition to their nutritional values the consumption of dairy products is also associated with beneficial effects on health (Takahiro et al., 2007). Rastall et al. (2005) stresses that fermented dairy products are used as lactic ferments to remedy the gastrointestinal disorders. Fermented dairy products in particular are very popular with consumers because their tastes and flavors. The preparation of such milk curd is to let the milk at room temperature for one to two days until its coagulation. Then the milk is churning followed by addition of water at the end. In the process of fermentation, lactic acid bacteria play an important role. Own different fermented milks characteristics are due to the specific changes in certain factors such as the composition of the milk, the incubation temperature or the seeds used (Luquet and Corrieu, 2005). The characteristics of various fermented milks are due to the particular variation of certain factors, such as the composition of the milk, the incubation temperature or

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used enzymes (Luquet and Corrieu, 2005). The fermentation of milk involves primarily bacteria lactic acid (Zamfir et al., 2006). Lactic acid bacteria are a very interesting group of microorganisms, which are characterized by the ability to ferment carbohydrates into lactic acid, a weak acid conducive to the conservation and improvement of the organoleptic quality of food (Zadi-Karam and Karam, 2007). The interest of lactic acid bacteria in the food industry lies mainly in their ability to convert some sugars into lactate and so to acidify the surrounding environment (Raynaud, 2006). Lactic acid can be depending on the group of only product of bacterial fermentation in homofermentative bacteria or with other excreted metabolites such as acetic acid, ethanol, CO₂, among heterofermentative bacteria. The first group follows the Embden Meyerhoff and the second the way of Dickens Horecker and Entner Doudoroff (Kandler and Weiss, 1986). Leveau and Bouix (1993) indicate that the lactic acid bacteria produced as commercial starter cultures are pure cultures or a mix belonging to the genera Lactococcus, Streptococcus, Lactobacillus. Leuconostoc. Pediococcus and Bifidobacterium. Also, throughout the world, there are a variety of milk products (FAO, 1995). Moreover, many studies (Savadogo et al., 2004; Chammas et al., 2006; Zamfir et al., 2006; El-Baradei et al., 2008; Ouadghiri et al., 2009) were conducted on dairy products fermented. In Chad, little information exists on the strains that may have desirable characteristics in fermented dairy products. The present work which is to identify lactic acid bacteria in traditional curd "Laban Rayib" consumed in Chad is part of a quality approach to improve the technology scale processing of milk. In addition this research work complements the studies that we have made in other areas of Chad (Doutoum et al., 2013).

MATERIALS AND METHODS

The samples were collected in the periurban area of "Moundou". The town of "Moundou" is the second city of Chad after N'Diamena. This economic capital has grown from the right bank of the river "logone". We collected from each producer, 10 samples of 250 ml, which all come from different containers of 40 liters. . The indelible ink used to mark bottles to avoid the mixing of samples. Then the samples are quickly placed in an ice containing cooling elements to prevent microbial growth. Thus the chain is maintained until the place of storage. In total, we collected at random from the sampling requirements 100 samples of curd. Each sample is a plastic jar containing 250 ml of curd. The samples were then quickly transferred to LRVZ and HIDAOA which were conducted analyzes. All samples studied were treated to obtain preliminary dilutions according to standard NF V08-010

(March 1996). To form the stock solution, 10 ml of the sample are introduced in a bag STOMACHERND which are added 90 ml of MRS broth or M17 broth. And then one ml of each milk sample was homogenized in 9 ml of sterile saline. Lactic acid bacteria were enumerated by serial dilutions in sterile saline that were placed on the appropriate agar medium. The incubation was carried out at 37 °C for 48 hours.

Regarding the different identifications and confirmations of lactic acid bacteria, we first used two types of galleries: API 20 STREP (Biomerieux, France) gallery and Gallery 50 API CH-L with their boxes of reagents. Then, we used the identification catalogs for confirmation and then the software API Web TM (Biomerieux, France).

RESULTS

Table 1 shows the digital profile and identification of lactococcal strains by API 20 STREP Galleries. The digital profile and identification of *Lactobacillus* strains by API 50 CH galleries are presented in the table 2.

DISCUSSION

In this study, we worked on ten (10) isolates of Lactococcus and ten (10) isolates of Lactobacilli. In the light of our research, we have identified from the broth and agar M17 and then using the API 20 STREP gallery, strains of lactic acid bacteria following: three (3) strains of Lactococcus diacetylactis, four (4) Lactococcus lactis (2) strains of Streptococcus subsp lactis, two thermophilus and finally a strain of Streptococcus bovis. These bacteria were isolated from the curds collected in cold dry season. This could explain the rarity of isolated thermophilic strains (one strain of ten isolates). Bacteria of the genus Lactococcus are dominant in the traditional curds of Chad. This confirms the results we have obtained in four (4) other areas during the same period (Doutoum et al., 2013). Indeed, in the suburban area of Abeche (Chad), we identified six (6) strains of Lactococcus lactis subsp lactis, two (2) strains of Lactococcus lactis subsp cremoris and a strains of contamination: Enterococcus faecium and Enterococcus durans. Jiwoua and Milliere (1990) in Cameroon have reported similar results in the curds dominated by Streptococcus thermophilus and Leuconostoc, and other Enterococcus. Our results differ from those of Badis et al. (2005) who obtained a significant percentage of Lactobacilli. It is clear from the results that the thermophilic flora is rare only met eugenic conditions to be found in large numbers in the curds. In their studies, the authors did not isolate flora contamination. Indeed, according to their investigations, the habits of making curds differ. We also

Lactococcal	Code	Identification		Identification	Appreciation	
strains		Genus	Species	Subspecies	%	
SM1	1050400	Streptococcus	thermophilus	-	99.7	good identification
SM2	7167711	Streptococcus	thermophilus	-	66.4	low identification
SM3	1054411	Streptococcus	thermophilus	-	98.9	proper identification
SM4	1053411	Streptococcus	thermophilus	-	98.9	proper identification
SM5	1050400	Streptococcus	thermophilus	-	99.7	good identification
SM6	1052453	Streptococcus	thermophilus	-	99.6	proper identification
SM7	1053411	Streptococcus	thermophilus	-	98.6	proper identification
SM8	3050400	Streptococcus	thermophilus	-	99.9	excellent identification
SM9	7167711	Streptococcus	, thermophilus	-	66.4	proper identification
SM10	2050400	Streptococcus	, thermophilus	-	94.0	proper identification
SM11	1252400	Streptococcus	thermophilus	-	99.9	excellent identification
SM12	1250410	Streptococcus	thermophilus	-	87.4	identification acceptable
SM13	1250400	Streptococcus	thermophilus	-	99.4	good identification
SM14	1242400	Streptococcus	thermophilus	-	99.9	excellent identification
SM15	1240410	Streptococcus	thermophilus	-	81.9	identification acceptable
SM16	1240400	Streptococcus	thermophilus	-	99.2	good identification
SM17	1072400	Streptococcus	thermophilus	-	95.5	proper identification
SM18	1070400	Streptococcus	thermonhilus	_	89.1	identification acceptable
SM19	1062400	Streptococcus	thermonhilus	_	93.0	proper identification
SM20	1060400	Streptococcus	thermonhilus	_	85.1	identification acceptable
SM21	1050440	Streptococcus	thermonhilus	_	96.8	proper identification
SM22	1050410	Streptococcus	thermonhilus	_	81.0	identification acceptable
SM23	1050410	Streptococcus	thermonhilus	_	98.9	proper identification
SM24	1050410	Streptococcus	thermonhilus	_	90.5 91 7	low identification
SM25	1050000	Streptococcus	thermonhilus	_	45 7	proper identification
SM26	2050400	Streptococcus	thermonhilus	_	43.7	excellent identification
SM27	1252400	Streptococcus	thermophilus	_	94.0	identification acceptable
SM29	1252400	Streptococcus	thermophilus	_	99.9 97 /	good identification
SM20	1250410	Streptococcus	thermophilus		07.4	excellent identification
SM29	1230400	Streptococcus	thermophilus		99.4	identification acceptable
SMOT	1242400	Streptococcus	thormophilus	-	99.9	good identification
SIVIST	1240410	Streptococcus	thormophilus	-	00.2	proper identification
SIVI32 SM22	1240400	Streptococcus	thermophilus	-	99.2 05.5	identification acceptable
SIVI33	1072400	Streptococcus	thermophilus	-	90.0	proper identification
SIVI34 SM25	1070400	Streptococcus	thermophilus	-	09.1	identification acceptable
SMOC	1062400	Streptococcus	thermophilus	-	93.0	proper identification
SIVI30	70400	Sirepiococcus	linermoprinus	- dia anti-da atia	85.1 00.7	identification acceptable
SM37	7043010	Laciococcus	laclis	diacelylaciis	98.7	proper identification
SM38	7043410	Lactococcus	lactis	diacetylactis	88.9	proper identification
SM39	7043511	Lactococcus	lactis	diacetylactis	98.9	excellent identification
SM40	7043510	Lactococcus	lactis	diacetylactis	97.6	excellent identification
SM41	7043401	Lactococcus	lactis	diacetylactis	99.9	excellent identification
SM42	7043151	Lactococcus	lactis	diacetylactis	99.9	proper identification
SM43	5053011	Lactococcus	lactis	diacetylactis	99.9	excellent identification
SM44	5053401	Lactococcus	lactis	diacetylactis	93.6	nroner identification
SM45	5053011	Lactococcus	lactis	diacetylactis	99.9	proper identification
SM46	5053411	Lactococcus	lactis	diacetylactis	98.9	and identification
SM47	5057511	Lactococcus	lactis	diacetylactis	91.1	good identification
SM48	5057411	Lactococcus	lactis	diacetylactis	99.6	good identification

 Table 1. Digital profile and identification of lactococcal strains

Table 1	I. (Cont	tinue
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Lactococcal	Code	Identification			Identification	Appreciation	
strains	-	Genus	Genus Species Subspecies		%		
SM49	5507111	Lactococcus	lactis	diacetylactis	99.7	excellent identification	
SM50	5057011	Lactococcus	lactis	diacetylactis	99.9	proper identification	
SM51	5053551	Lactococcus	lactis	diacetylactis	98.0	proper identification	
SM52	7043010	Lactococcus	lactis	diacetylactis	98.7	identification acceptable	
SM53	7043410	Lactococcus	lactis	diacetylactis	88.9	proper identification	
SM54	7043511	Lactococcus	lactis	diacetylactis	98.9	proper identification	
SM55	7043510	Lactococcus	lactis	diacetylactis	97.6	excellent identification	
SM56	7043401	Lactococcus	lactis	diacetylactis	99.9	excellent identification	
SM57	7043151	Lactococcus	lactis	diacetylactis	99.9	excellent identification	
SM58	5053011	Lactococcus	lactis	diacetylactis	99.9	proper identification	
SM59	5053401	Lactococcus	lactis	diacetylactis	93.6	excellent identification	
SM60	5053011	Lactococcus	lactis	diacetylactis	99.9	proper identification	
SM61	5053411	Lactococcus	lactis	diacetylactis	98.9	proper identification	
SM62	5057511	Lactococcus	lactis	diacetylactis	91.1	good identification	
SM63	5057411	Lactococcus	lactis	diacetylactis	99.6	excellent identification	
SM64	7007411	Lactococcus	lactis	lactis	99.9	excellent identification	
SM65	7007411	Lactococcus	lactis	lactis	99.9	good identification	
SM66	7003511	Lactococcus	lactis	lactis	99.7	excellent identification	
SM67	7007411	Lactococcus	lactis	lactis	99.9	excellent identification	
SM68	7007410	Lactococcus	lactis	lactis	99.9	good identification	
SM69	7003510	Lactococcus	lactis	lactis	99.5	proper identification	
SM70	5353411	Lactococcus	lactis	cremoris	91.1	proper identification	
SM71	5210440	Lactococcus	lactis	cremoris	99.6	proper identification	
SM72	1040473	Lactococcus	lactis	cremoris	94.0	proper identification	
SM73	5353411	Lactococcus	lactis	cremoris	91.1	proper identification	
SM74	5353411	Lactococcus	lactis	cremoris	91.1	proper identification	
SM75	5210440	Lactococcus	lactis	cremoris	99.6	proper identification	
SM76	1040473	Lactococcus	lactis	cremoris	94.0	proper identificat	
SM77	5353411	Lactococcus	lactis	cremoris	91.1		

Lactic streptococci identified in curd show that there is a predominance of *Lactococcus lactis* subsp *lactis* and *Lactococcus lactis* subsp *cremoris*.

Table 2. Digital profile and identification of Lactobacillus strains

lactococcal strains	Identification		Identification %	Tests against
	Genus	Species		
SLM1	Lactobacillus	Plantarum	99.9	0
SLM2	Lactobacillus	plantarum	99.6	1
SLM3	Lactobacillus	plantarum	99.9	0
SLM4	Lactobacillus	helveticus	99.9	2
SLM5	Lactobacillus	fermentum	99.6	2
SLM6	Lactobacillus	plantarum	98.4	2
SLM7	Lactobacillus	brevis	99.9	0
SLM8	Lactobacillus	fermentum	99.9	1
SLM9	Lactobacillus	brevis	99.9	0
SLM10	Lactobacillus	acidophilus	88.4	3

Lactobacilli identified the following species: Lactobacillus plantarum, Lactobacillus helveticus, Lactobacillus fermentum, Lactobacillus brevis and Lactobacillus acidophilus.

noted that in the area of Moundou, sanitary measures are satisfactory. Ben Amor et al. (1998) have identified twelve (12) isolates, seven (7) Lactococcus lactis subsp lactis, Lactococcus lactis subsp cremoris and two (2) bacteria contamination. These same authors have conducted additional research identified Lactococcus diacetylactis. They have shown by their results that the mesophilic lactic flora is represented by three species of Lactococcus flora associated with contamination of Enterococcus genus. Many studies (Tantawi et al., 1983., Abo Elnaga et al., 1977; Jiraidi et al., 1996) in the Maghreb and the Middle East on the microbiological analysis of "Leben" or "Laban" showed that lactic flora dominant is that belonging to the lactic streptococcus represented by the following species: Lactococcus lactis subsp lactis, Lactococcus lactis subsp cremoris and Lactococcus subsp diacetylactis associated with flora contamination pseudolactique dominated by Enterococcus genus. These authors although worked in hot climates but they have not reported thermophilic lactic acid bacteria. Ouadghiri (2009) reported that "Lben" in Morocco is typically dominated by species Lactococcus lactis. Lactobacillus plantarum and Leuconostoc pseudomesenteroides. For Ngassam (2007), on ten (10) samples of curd during cold, he could identify with the M17 medium and API 20 STREP galleries ten (10) Lactococcus lactis subsp lactis exclusively. These results are similar to ours because we have not met flora contamination. El-Baradei et al. (2007) did not find in their research flora contamination. They were identified as lactic genres themselves even if the species different from ours. This is Lactococcus lactis subsp lactis, Lactococcus and Lactococcus garviae raffinolactis. Lafarge et al. (2004) have been confirmed by molecular methods the predominant presence of the species Lactococcus lactis subsp lactis. Pu et al. (2002) are enriched in the scientific field of lactic acid bacteria by the use of PCR. With few exceptions, these authors found similar to our own species: Lactococcus lactis subsp lactis, Lactococcus lactis subsp cremoris and Lactococcus lactis hordniae. As against the Lebanese and Iragi in the hot or cold season, they have always identified Streptococcus thermophilus and Lactobacillus bulgaricus associated with yeast as Kluyveromyces fragelis, Saccharomyces cerevisiae and Saccharomyces lactis which play an important role in the fermentation "Laban" (Abo Elnaga et al, 1977; Loons, 1994). The isolation of Streptococcus thermophilus and Lactobacillus bulgaricus is linked to the synergistic action of these two bacteria.

Regarding the ten (10) Lactobacilli isolates, we identified four (4) species of Lactobacillus plantarum, two (2) species Lactobacillus fermentum, two (2) species Lactobacillus brevis, one (1) species of Lactobacillus acidophilus and one (1) species of Lactobacillus helveticus. These results show that in Sudanian species

are different because in the Sahel of Chad we met only one species: Lactobacillus plantarum. Ben Amor et al. (1998) also identified seven (7) of Lactobacillus plantarum strains on seven (7) isolates. They argue that Lactobacilli are either absent (Leben Algeria and Tunisia), or in very low numbers for the Moroccan "Leben". But research on the Chadian "Laban" helped highlight several species of Lactobacilli. Similarly, Khosrof (1996) found on the Tunisian "Leben" an association of Lactobacilli: Lactobacillus helveticus, Lactobacillus acidophilus. Lactobacillus fructosus. Lactobacillus casei subsp pseuplantarum and Lactobacillus delbruekii subsp lactis. Weerkamp et al. (1996) in Europe have shown that lactic flora is a mesophilic : Lactococcus lactis subsp lactis, Lactococcus diacetylactis, Lactococcus lactis subsp cremoris, Leuconostoc lactis and Leuconostoc mesenteroides. This would mesophilic flora in relation to the climate of this continent which is unfavorable to the development of thermophilic bacteria. Saied and Boudabous (1994) isolated from local dairy products the following germs: Streptococcus, Lactobacillus, Lactococcus lactis subsp lactis. Lactococcus lactis subsp diacetylactis, Lactococcus lactis subsp cremoris. With regard to species and subspecies, the authors identified among other Lacbatocillus brevis, Lacbatocillus plantarum, Lacbatocillus casei. But these authors have not only worked on the curd. They also worked on the cheese and vogurt. They worked in the cold season, but they could find thermophilic species as in the present study.

CONCLUSION

These work in the Sudanian zone of Chad complemented those that have been made in the Chadian Sahel. Identified lactic species are: Lactococcus diacetylactis, Streptococcus thermophilus and Lactococcus lactis. Regarding Lactobacilli, we discovered the following species: Lactobacillus plantarum. Lactobacillus helveticus, Lactobacillus fermentum, Lactobacillus brevis and *Lactobacillus acidophilus*. It is clear from our study: Curd "Laban Rayib" contains a variety of species of lactic acid bacteria whose properties would be useful in the processing of milk. The study has enriched the knowledge of ecology of lactic acid bacteria. However, these preliminary results are far from exhaustive. A much more thorough study using molecular biology techniques could help to characterize these bacteria benefit. Such work would be more in improving the guality of fermented dairy products consumed and sold in Chad.

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