

Short Communication

Identification of the lactic microorganism responsible for milk coagulation in Abeche (Chad)

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Milk and dairy products are consumed by many people in its various forms in Chad. This product has a major share in the labor market. Milk is turned into curds of various types and other dairy products empirically, but there is not much information reported about the lactobacilli species responsible for curd production in Chad. Therefore we have chosen to locate and identify them. We started with the suburban area of Abeche. This research conducted aimed at identifying the lactic acid bacterial strains in 100 samples of milk curd (laban) obtained from the suburban area of Abeche, Chad. Identification of lactic acid bacteria was made using the API 20 STREP and API 50 CHL galleries. The bacteria were cultured in de Man, Rogosa and Sharpe (MRS) and M17 medium. In addition to the identification, confirmation was done at the Pasteur Institute of Dakar with the API MT web software. Results have identified *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis* as well, other contaminants were identified, including *Enterococcus durans* and *Enterococcus faecium*. *Lactobacillus plantarum* identified in all samples analyzed.

Key words: Laban, *Lactococcus*, *Lactobacillus*, Identification, Abeche.

INTRODUCTION

Milk is the staple food in a domestic economy. In Chad, the local artisanal dairy industry has developed considerably based on micro-enterprise collection and dairy processing (Koussou, 2008). Milk is a highly nutritious food for its richness in carbohydrates, fats, vitamins and minerals (Aggad et al., 2009; Ahmed et al., 2010). Indeed, the dairy product comes in many forms depending on the region and varied tastes. The curd is especially appreciated by the Chadian consumers. Fermented milk has always been traditionally consumed in many countries (Nakasaka et al., 2008). Most studies on traditional fermented products showed that mesophilic lactic acid bacteria are the main cause of these fermentations group

(Ben Amor et al., 1998). Lactic acid bacteria also through their lipase can decompose fat and free fatty acids in milk, resulting in the appearance of rancid odor in the dairy product. When lipolysis is not controlled or exaggerated, there is the appearance of strong and rancid odors (Vignola, 2002). Among the lactic acid bacteria with habitat as milk, we have *Streptococcus*, *Lactobacillus*, *Enterococcus*, *Leuconostoc* and *Aerococcus* (Luquet and Corrieu, 2005). In Chad, especially in Abeche, no study has been done about this subject. This lack of scientific information led us to seek and identify these bacteria in different varieties of "Laban", which is very well consumed by the population. The objective of this study was therefore

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Table 1. Biochemical analysis of isolated strains with API 20 STREP.

Biochemical parameter	SLA1	SLA2	SLA3	SLA4	SLA5	SLA6	SLA7	SLA8	SLA9	SLA10
Hydrolysis hypurate	+	+	+	+	+	+	+	+	+	+
Hydrolysis of esculin	+	+	+	+	+	+	+	+	+	+
Galactosidase	-	-	-	-	-	-	-	-	-	-
Glucuronidase	-	-	-	-	-	-	-	-	-	-
Phenylalanine deaminase	-	-	-	-	-	-	-	-	-	-
Leucinearyl aminase	+	+	+	+	+	+	+	+	+	+
Hydrolysis of arginine	+	+	+	+	+	+	+	+	+	+
Hemolysis	-	-	-	-	-	-	-	-	-	-
Profile fermentation										
Ribose	+	+	+	-	-	+	+	+	-	+
Arabinose	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	-	+	+	+	+	-	-	+
Sorbitol	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	-	+	+	+	+
Raffinose	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	+	+	-	+	-	-
AGlycogene	-	-	-	-	-	-	-	-	+	-

SLA, Strain lactic Abeche

to identify some species of lactic acid bacteria present in "Laban".

MATERIALS AND METHODS

Sampling

In the suburban area of Abeche, we collected aseptically and randomly 100 samples of 250 ml of milk curd in sterile plastic jars. Before introducing the curd in the jars, the technician washes hands first with soapy water and take precautions to avoid contact with the curds. After collection, the samples were quickly introduced into a cooler box containing bottles of dry ice and transported at a low temperature to the laboratory for analyses.

Isolation and Identification of lactic acid bacteria

de Man, Rogosa and Sharpe (MRS) and M17 as agar media and broth were used to isolate lactic acid bacteria after incubating at 30°C for 48 h. One milliliter 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. Gallery API 20 STREP and API 50 CH gallery-L with their reagents (Biomerieux, France) were used in the identification using software API Web TM (Biomerieux, France).

RESULTS

Isolation and identification of lactic flora

The Table 1 shows the results of biochemical analyses of isolated strains with API 20 STREP. The test showed that the positive and negative reactions tend to equilibrate.

The Table 2 shows the digital profile and identification of strains. It was observed that there was a predominance of *Lactococcus lactis* subsp. *lactis*. The results of analyzes based on the biochemical and physiological criteria of isolated bacteria are shown in Table 3. Captured in Table 4 are the biochemical analyses of isolated strains with API 50 STREP.

DISCUSSION

The results of this study have identified six strains of *Lactococcus lactis* subsp. *lactis*, two strains of *Lactococcus lactis* subsp. *cremoris* and one flora contamination represented by a strain of *Enterococcus faecium* and *Enterococcus durans* strain. Ben Amor et al. (1998) found similar results of 12 isolates. They showed that the mesophilic lactic flora is represented by three species of *Lactococcus* flora associated with contamination of the *Enterococcus* genus. Moreover, Ngassam's studies (2007) on 10 samples of curd have identified a single species in all samples (100%): *Lactococcus lactis* subsp. *lactis* with a percentage of identification of 98%; while El-Baradei et al. (2007) have identified in the Egyptian traditional curd milk (Domati) the species *Lactococcus lactis* subsp. *lactis*, *Lactococcus garvieae* and *Lactococcus raffinolactis*. Previously Lafarge et al. (2004) have also determined the curd pre-dominant presence of *Lactococcus lactis* subsp. *lactis* species by molecular methods. Also, further more studies (Katinan et al., 2012; Conte, 2008; Badis et al., 2005) reported the prevalence of *Lactococcus*

Table 2. Digital profile and identification of strains of *Lactococci* by the API 20 STREP galleries.

Souche	Identification			% Identification	Tests against
	Genus	Species	Subsp.		
SLA1	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	43.8	2
SLA2	<i>Lactococcus</i>	<i>lactis</i>	<i>cremoris</i>	82.2	1
SLA3	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	95.3	1
SLA4	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	97.5	2
SLA5	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	99.8	0
SLA6	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	59.9	2
SLA7	<i>Lactococcus</i>	<i>lactis</i>	<i>cremoris</i>	85.7	1
SLA8	<i>Lactococcus</i>	<i>lactis</i>	<i>lactis</i>	98.9	0
SLA9	<i>Enterococcus</i>	<i>faecium</i>		93.6	3
SLA10	<i>Enterococcus</i>	<i>durans</i>		52.8	3

SLA, Strain lactic Abeche.

Table 3. Biochemical analysis of isolated strains with API 50 STR.

Growth condition	LBA1	LBA2	LBA3	LBA4	LBA5	LBA6	LBA7	LBA8	LBA9	LBA10
15°C	+	+	+	+	+	+	+	+	+	+
45°C	-	-	-	-	-	-	-	-	-	-
6.5% NaCl	+	+	+	+	+	+	+	+	+	+
pH 9.6	-	-	-	-	-	-	-	-	-	-
Milk sunflower	ACr	ACr	ACr	ACr	ACr	ACr	ACr	ACr	ACr	ACr
ADH	-	-	-	-	-	-	-	-	-	-
Type of fermentation	Homo	Homo	Homo	Homo	Homo	Homo	Homo	Homo	Homo	Homo

DHA, Arginine dihydrolase

Table 4. Biochemical analysis of isolated strains with API 50 STREP.

Profile fermentation	LBA1	LBA2	LBA3	LBA4	LBA5	LBA6	LBA7	LBA8	LBA9	LBA10
D-Arabinose	-	-	-	-	-	-	-	-	-	-
L-Arabinose	+	-	+	+	+	+	+	-	-	+
Ribose	+	+	+	+	+	+	+	+	+	+
D-Xylose	-	-	-	-	-	-	-	-	-	-
L-Xylose	-	-	-	-	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-	-	-
Rhamnose	+	-	+	+	+	+	+	-	-	+
Inositol	-	-	-	-	-	-	-	-	-	-
Mannitol	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	-	+	+	+	+	+	-	-	+
N-Acetyl Glucosamine	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+
Gluconate	±	+	+	+	+	+	±	+	+	+

in milk. Ouadghiri et al. (2009) studies indicate that Morocco "Lben" is typically dominated by *Lactococcus lactis* species (41%), *Leuconostoc pseudomes enteroides* (36%) and *Lactobacillus plantarum* (15%). Our results are also consistent with those found by Biatcho (2006) with lactobacilli in large quantities in the craft milk. However, dairy technology, lactococci play a role of "bioconserverator." It is recognized *Lactococcus lactis* having a role of initiator agent for curdling milk.

Compared to biochemical tests and API 50 CHL tests, our results show that the germs identified belong to the *Lactobacillus* genus, including *Lactobacillus plantarum*. The percentage of identification obtained by the API software web MT are the order of 99.9% for most strains except LBA2, LBA8 and LBA9 that are a little discriminating profile compared to *Cornobacterium piscicola* Identification with a percentage of 52.6% and 3 test against (ribose, melibiose and sorbitol). Our results are similar to those of Ben Amor et al. (1998). These authors on eight strains were able to identify seven strains of *Lactobacillus plantarum* and strain *Cornobacterium piscicola* with a percentage of 50.7% and identification of three tests against. *Lactobacillus plantarum* has also been isolated (16 isolates) in raw goat milk from two Algerian goat populations (Badis et al., 2005). Ngassam (2007) meanwhile isolated a variety of six species of the *Lactobacillus* genus. The differences in species and their diversity may be explained by the origins of milk.

Conclusion

This work has identified certain lactic acid bacteria in curd available in Abeche. The study identified some lactic acid bacteria in the "Laban" consumed in of Abeche town. These include *Lactococcus lactis* subsp. *lactis* *Lactococcus lactis* subsp. *cremoris* and *Lactobacillus plantarum*. But these results are far to be completed because the country is too large as well as the agro-ecological zones are varied. Knowledge of most lactic acid bacteria in Chad will help in the production of dairy products with longer shelf-life. Other studies, including the characterization of molecular biology of lactic acid bacteria is necessary to provide benefit information to improve the technological and sanitary quality of milk.

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