Identification of the microbiota involved in the production of Lait caillé, a spontaneously fermented milk product from Burkina Faso

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Conclusion

This study revealed that *Lactococci lactis* was the major lactic acid bacteria involved in the fermentation process of Lait caillé and that *Saccharomyces cereviscae* was the major yeast in the final Lait caillé product. These microorganisms can, together with *Leuconostoc mesenteroides*, be considered for starter culture development.

The fermentation process helped to reduce the level of the presumptive pathogenic *Candida parapsilosis, Candida orthopsilosis.*

Microbial quality defects were revealed in Lait caillé with high levels of enterobacteriaceae and enterococci species.

This study constitutes a first step towards implementation of starter cultures in order to up-grade the food sector in West Africa.

Introduction

Lait caillé is a traditional spontaneously fermented yoghurt-like product from Burkina Faso. Its production brings nutritional benefits and value added market in particular to vulnerable groups like rural people, and women who are the main processors and traders. However, the microbial quality defects limits the attractiveness of this fermented milk (Abel et al., 2016). This is partly due to insufficient manufacturing practices in conjunction with the spontaneous nature of the fermentation. Furthermore, no previous studies on monitoring the microbiota changes during the fermentation process is yet available.

Therefore, the aim of this investigation was to identify the predominant microbial groups involved in the fermentation of Lait caillé from the raw milk to the end product, in the south-west area of Burkina Faso, by using both phenotypic and genotypic methods.

Materials and methods

Lait caillé processing and sampling

Lait caillé was processed the traditional way (Fig.1). Sampling was performed at specific time points during the fermentation process. Furthermore, samples of end-products from 3 markets were included, for hygienic quality determination.

Fig.1. Flow diagram of Lait caillé processing

Enumeration and isolation of microorganisms

Microbial count was performed on agar media. Total mesophilic flora and Enterobacteriaceae were determined on plate count agar and violet red bile glucose agar, respectively. Yeasts, lactic acid bacteria (LAB) and lactococci colonies were counted and isolated from Sabouraud CAF, Man, Rogosa and Sharpe and M17 agar media, respectively. Yeast and LAB colonies were isolated and purified by successive streaking.

Phenotipic characterization

Micro- and macro morphological description of the isolates were performed as initial phenotypic characterization. Carbohydrates profiles and growth tests (15° C, 45° C, NaCl 6.5%) were later performed to differentiate within the species.

Genotypic characterization of LAB and yeasts

Following the initial phenotypic characterization, a total of 261 presumptive LAB and 171 presumptive yeasts isolates were grouped by $(GTG)_5$ based rep-PCR fingerprints (Fig 3 & 4). Sequencing of the 16S/26S rRNA genes were done on representative LAB/yeast isolates (Nielsen et al., 2007). Sequencing of internal transcribed spacer region was performed to differentiate within the group of *Candida parapsilosis* species (Esteve-Zarzozo et al., 1999). *Lactobacillus plantarum/ Lactobacillus pentosus* were differentiated by multiplex PCR.

Results

Microbial count and hygienic quality

During the 59h of Lait caillé fermentation, the pH dropped from 6.7 in the fresh milk (0h) to 4.4 in the end-product (59h) (Fig. 2). Meanwhile aerobic mesophilic bacteria and LAB counts increased from 5.6 to 8.8 log CFU/g and from 5.4 to 8.7 log CFU/g, respectively. Similarly, lactococci counts increased from 5.4 to 8.5 log CFU/g. Yeast level varied from 3.6 (0h) to 6.5 log CFU/g (59h), whereas the level of Enterobacteriaceae raised from 2.7 to 6.7 log CFU/g during the same period. The average level of Enterobacteriaceae in market samples was 7.6 log CFU/g.

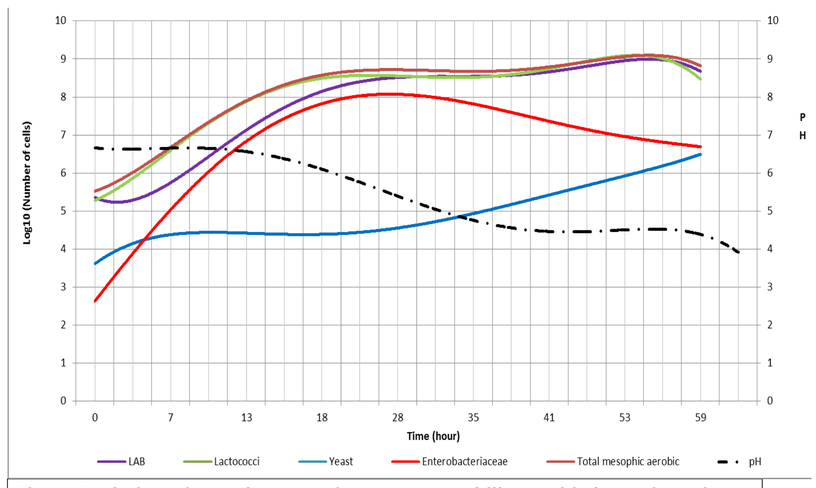

Fig. 2. Evolution of LAB, lactococci, yeast, mesophilic aerobic bacteria and Enterobacteriaceae counts and pH during fermentation of Lait caillé.

Microbial succession of LAB and yeasts

At the onset of the fermentation (Table 1), *Pediococcus pentosaceus* and *Weissela paramesenteroides* were the dominant LAB, each representing 40% of the LAB at 0h. They were followed by *Leuconostoc mesenteroides* (representing 20% of the LAB at 0h). From this point their level decreased and, throughout the rest of the fermentation the predominating LAB were *Lactococcus lactis* on average comprising 40.9%±19.3% of the LAB, together with *Enterococcus lactis* (21.6%±15.6% of the LAB), followed by E*nterococcus hirae* (16.8%±11.2% of the LAB). *Leuconostoc mesenteroides* was present throughout all the fermentation on average comprising 15.0%±6.0% of the LAB. Less frequently isolated LAB were *Macrococcus caseolyticus*, *Lactobacillus plantarum* and *Enterococcus faecium* together comprising 4.2% of the total LAB.

For yeast (Table 2), *Saccharomyces cerevisiae* (51.7% of the total yeast) was predominant. During the first 18h, however, *Candida parapsilosis* were predominating, on average comprising 69.9%±14.4% of the yeast. Where after it decreased until it was not detected after 53h. From 18h and throughout the rest of the fermentation, *Saccharomyces cerevisiae* was the dominant yeast on average comprising 80.9%±22.3% of the yeasts. Less frequently yeast isolates were *Lecythophora* sp., *Candida orthopsilosis* and *Ascomycota* sp. together comprising 4.7% of the total yeasts.

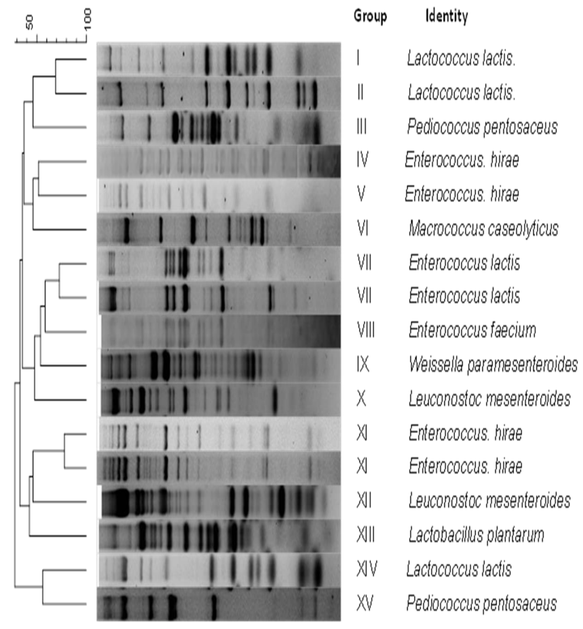

Fig. 3. Dendrogram of rep-PCR cluster analysis of LAB isolates from Lait caillé fermentation. The cluster is based on Dice's coefficient of similarity using the unweighted pair group method with arithmetic average clustering algorithm (UPGMA). Only a representative sub-sample of sequenced isolates is shown.

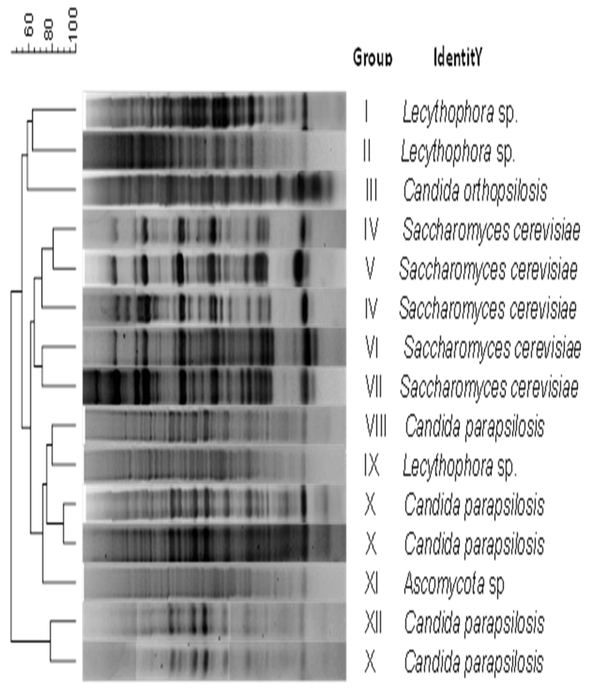

Fig. 4. Dendrogram of rep-PCR cluster analysis of yeast isolates from Lait caillé fermentation. The cluster is based on Dice's coefficient of similarity using the unweighted pair group method with arithmetic average clustering algorithm (UPGMA). Only a representative sub-sample of sequenced isolates is shown.

Table 1. Microbial succession, percentage occurrence, identification and count of LAB during Lait caillé fermentation.

Fermentation Time (Hours)	0	7	13	18	28	35	41	53	59
pH	6.7	6.6	6.6	6.2	5.3	4.7	4.6	4.4	4.4
Acidity (mg KOH/g)	1± 0.06	1.0 ±0.00	1.2±0. 06	1.1± 0.00	3.1± 0.01	3.3±0. 00	3.8± 0.01	4.2± 0.02	4.4±0. 10
Log(CFU/g)	5.36	5.72	7.23	8.00	8.61	8.57	8.58	9.00	8.66
%LAB									
Lactococcus lactis	-	13.3	33.3	56.0	30.6	69.6	61.8	29.0	33.3
Enterococcus hirae	-	6.7	15.2	12.0	8.3	17.4	8.8	39.5	26.7
Enterococcus faecium	-	-	6.1	-	-	-	-	-	-
Enterococcus lactis	-	23.3	27.3	28.0	52.8	4.4	8.8	7.9	20.0
Pediococcus pentosaceus	40.0	-	-	-	-	-	-	-	-
Leuconostoc mesenteroides	20.0	20.0	15.2	4.0	8.3	8.7	20.6	21.1	16.7
Weissella paramesenteroides	40.0	16.7	-	-	-	-	-	-	-
Lactobacillus plantarum	-	3.3	-	-	-	-	-	2.6	3.3
Macrococcus caseolyticus	-	16.7	3.0	-	-	-	-	-	-

Table 2. Microbial succession, percentage occurrence, identification and count of yeast during Lait caillé fermentation.

Fermentation Time (Hours)	0	7	13	18	28	35	41	53	59
pH	6.7	6.6	6.6	6.2	5.3	4.7	4.6	4.4	4.4
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Log(CFU/g)	3.6	4.6	4.2	4.4	4.8	4.9	5.2	6.1	6.5
%Yeast									
Saccharo-myces cerevissae	20	10	45.5	19.1	47.4	70.6	86.7	100	100
Candida parapsilosis	80	75	48.5	76.2	42.1	23.5	13.3	-	-
Candida orthopsilosis			3.0			5.9			
Lecytho-phora sp.	-	10	2.9	4.8	10.5	-	-	-	-
Ascomycota sp	-	5	-	-	-	-	-	-	-

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