World Journal of Microbiology and Biotechnology (2019) 35:100
https://doi.org/10.1007/s11274-019-2672-3

ORIGINAL PAPER

Identification of the predominant microbiota during production of *lait caillé*, a spontaneously fermented milk product made in Burkina Faso

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Received: 13 February 2019 / Accepted: 7 June 2019 / Published online: 20 June 2019
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Abstract

The spontaneously fermented curdled milk product from Burkina Faso, *lait caillé* is prepared by traditional processing from raw unpasteurised milk. The fermentation lasts 1–3 days. This study aims to identify the predominant microbiota involved in *lait caillé* fermentation from cow milk. A survey on *lait caillé* end-products from local markets showed pH ranges of 3.5 to 4.2. Counts of total lactic acid bacteria (LAB) were 7.8 ± 0.06 to 10.0 ± 0.03 log CFU/g and yeast counts were 5.3 ± 0.06 to 8.7 ± 0.01 log CFU/g, together with considerate amounts of Enterobacteriaceae < 3.00 to 8.4 ± 0.14 log CFU/g. Sampling throughout the entire fermentation of *lait caillé* was performed at a traditional house-hold production site. A drop in pH from 6.7 ± 0.01 at 0 h to 4.3 ± 0.08 in the end-product (59 h) was found. Total LAB counts increased to 8.6 ± 0.02 log CFU/g in the end-product, while yeast and Enterobacteriaceae counts reached 6.4 ± 0.11 and 6.7 ± 0.00 log CFU/g, respectively. LAB and yeasts isolated during the fermentation were clustered by $(GTG)_5$ repetitive-PCR fingerprinting followed by 16S and 26S rRNA gene sequencing, respectively. Microbial successions were observed with *Leuconostoc mesenteroides* being the predominant LAB followed by *Pediococcus pentosaceus* and *Weissella paramesenteroides* at the onset, while *Lactococcus lactis* and *Enterococcus* spp. where the predominant LAB after 7 h of fermentation. During the first 18 h *Candida parapsilosis* was the dominant yeast species, while from 35 h to the end-product, *Saccharomyces cerevisiae* predominated. The microbial safety risk pointed out in this study, showed the need for implementation of good manufacturing practices including pasteurisation and use of well-defined starter cultures.

Keywords *Lait caillé* · Lactic acid bacteria · Yeasts · Fermented milk products · Food safety

Introduction

The spontaneously fermented curdled milk product from Burkina Faso, known as *lait caillé,* is consumed as a beverage or in combination with various cereal based products. Similar spontaneously fermented milk products exist in Africa with slight differences in the processing and consequently the final products. Specifically differences can be seen in microbial quality and sensorial characteristics. *Lait caillé* from Burkina Faso is prepared from raw cow milk or more seldom from raw goat milk by traditional processing using spontaneous fermentation. Typically the Fulani people of Burkina Faso prepare *lait caillé* by collecting the raw milk in calabashes, gourds, clay pots or plastic containers and leave it to ferment at ambient temperature until the preferred characteristics of *lait caillé* are obtained (Savadogo et al. 2004). *Lait caillé* processing is of social and socioeconomic value since it serves as income generation for both main

Geoffroy Romaric Bayili and Pernille Johansen contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11274-019-2672-3) contains supplementary material, which is available to authorized users.

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processors and traders, in most cases involving vulnerable women groups (Duteurtre 2007; Gonfa et al. 2001).

Lactic acid bacteria (LAB) play a key role in the production of fermented milk products. In African fermented milk LAB counts ranging between 7.0 and 10.0 log CFU/mL were found in the "Masai" from Tanzania, *kivuguto* from Rwanda, *leben* from Tunisia, *fènè* from Mali as well as *nyarmie* and *nunu* from Ghana (Akabanda et al. 2013; Karenzi et al. 2012; Obodai and Dodd 2006; Samet-Bali et al. 2012; Wullschleger et al. 2013). The most commonly identified LAB in African fermented milk products are *Lactococcus lactis*, *Lactobacillus* spp. and *Leuconostoc mesenteroides* or *Leuconostoc pseudomesenteroides*. Other LAB, such as *Enterococcus* spp. and *Streptococcus* spp., especially *Streptococcus infantarius*, have also been reported with high frequencies in some traditional fermented milk products (Jans et al. 2012; Mathara et al. 2004; Obodai and Dodd 2006; Walsh et al. 2017; Wullschleger et al. 2013). Yeasts in African fermented milk products are less reported in the literature. However, their importance was highlighted in *amasi*, a fermented milk from Zimbabwe with counts up to 8.1 log CFU/g (Gadaga et al. 2000). *Saccharomyces cerevisiae* is most often the dominant yeast species in African fermented milk products, usually in association with other species, such as *Candida* spp. and *Pichia kudriavzevii* (Akabanda et al. 2013; Jespersen 2003; Obodai and Dodd 2006).

Characterisation of LAB and yeasts in African spontaneous fermented milk products has mostly been done on end-products, identifying the microorganisms by phenotypic techniques with low discriminatory power (Akabanda et al. 2010; Beukes et al. 2001; Gadaga et al. 2000; Mathara et al. 2004; Savadogo et al. 2004), while later studies used genotypic techniques for microbial identifications (Akabanda et al. 2013; Jans et al. 2017; Karenzi et al. 2012; Parker et al. 2018; Walsh et al. 2017; Wullschleger et al. 2013). Further, microbial successions during processing of African fermented milk products have only been studied to a limited extent (Akabanda et al. 2013; Wullschleger et al. 2013), and LAB and yeasts involved in *lait caillé* production in Burkina Faso have, to the best of our knowledge, not been investigated before. Understanding the microbial successions is important, since the overall quality of the fermented products highly depends on the microorganisms present during the fermentation. Moreover, microbial successions likely arise from changes in nutrient availability, pH, temperature, concentrations of organic acids and oxygen availability (Jespersen et al. 2005). Previous reports have additionally revealed low quality linked to the hygienic conditions and processing of *lait caillé* and similar fermented milks, limiting shelf life and income from the production (Broutin et al. 2007; Koussou et al. 2007; Walsh et al. 2017). The aim of this study was therefore to obtain a deeper understanding of *lait caillé* processing by identifying the predominant microbiota involved in the spontaneous fermentation of *lait caillé* in the South-West of Burkina Faso by combining phenotypic and genotypic techniques and to study microbial successions taking place during the fermentation.

Materials and methods

Lait caillé processing, sampling and pH measurements

A survey of ten *lait caillé* end-products was performed by sampling (S1–S10) from three local open markets in Bobo-Dioulasso (in the South-West of Burkina Faso), i.e. S1–S6 at Ouezzin-ville market, S7–S8 at Grand marché market and S9–S10 at Farakan market. The *lait caillé* was processed by the vendor at the market, in covered plastic containers at ambient temperature, using part of the unsold cow milk originating from farms in outlying or rural areas of Bobo-Dioulasso. The milk was fermented until optimal characteristics of the product were reached, which was determined by the vendor. After purchase, the sample (approximately 250 mL) was transported to the laboratory in an ice-box (0–4 °C) for analysis.

A detailed study on *lait caillé* fermentation was subsequently performed by sampling throughout the traditional processing of *lait caillé* at a house-hold scale production site in the village Tolotama outside Bobo-Dioulasso (in the South-West of Burkina Faso). Approximately 3.5 L of fresh cow milk was roughly filtered (pore size of approximately 1 mm) into the fermentation container (plastic bucket covered with a straw-woven lid). The fermentation container was subsequently placed inside a closed room and left for spontaneous fermentation. The fermentation took place at ambient temperature 22.5–29.0 °C for 59 h until the optimal characteristics of the fermented product were reached, which was determined by the producer. Sampling was performed at 9 specific time points (0 h, 7 h, 13 h, 18 h, 28 h, 35 h, 41 h, 53 h and 59 h) during the fermentation process. At each previous time point the fermenting milk was homogenised then 100–150 mL of sample was collected aseptically in duplicate in sterile screw-capped bottles and transported in an ice box (0–4 °C) to the laboratory for analyses. The pH of *lait caillé* was measured using a pH-meter (Hanna, USA).

Enumeration and isolation of microorganisms

Ten grams of homogenized sample were mixed with 90 g of diluent [0.1% peptone (Cultimed, Spain), 0.85% NaCl (Scharlau, Spain), pH 7.0] to yield a 1:10 dilution. Microbial enumerations were done from appropriate tenfold dilutions. According to Akabanda et al. (2010), total lactic acid bacteria (LAB) were enumerated on Man, Rogosa and Sharpe

(MRS) agar (DIFCO, USA), incubated at 37 °C for 48 h in GasPak™ EZ containers with anaerobic container system sachets. Lactococcal counts were determined on M17 agar (Liofilchem, Italy), incubated aerobically at 37 °C for 48 h. Yeasts counts were determined on Sabouraud Chloramphenicol agar (Liofilchem, Italy) pH 5.6 ± 0.2, incubated aerobically at 30 °C for 72–96 h. Enterobacteriaceae counts were enumerated on Violet Red Bile Glucose (VRBG) agar (Liofilchem, Italy), incubated aerobically at 37 °C for 24 h. After enumeration, colonies were picked proportionally, based on their morphological differences (colour, shape, size, margin, surface) and purified by successive streaking on corresponding agar plates, aiming at 20 colonies per sampling time point. For long term storage, pure cultures of LAB were preserved at − 80 °C in corresponding broths (MRS, M17, respectively) with 19% (v/v) glycerol and yeasts in MYGP agar (3 g of yeast extract (Oxoid), 3 g of malt extract (Oxoid), 5 g of bactopeptone (Oxoid), 10 g of glucose (Sigma, Milan, Italy) and 20 g of agar (Oxoid) per liter of distilled water, pH 5.6) with 19% (v/v) glycerol.

Identification of LAB and yeasts by phenotypic- and biochemical characterisation

Micro- and macro morphological descriptions of the isolates were performed according to Akabanda et al. (2010). For bacteria and yeast colonies, colour, shape, size, surface and margin characteristics were examined from agar plates. All bacterial isolates were examined for Gram reaction (Gregersen 1978) and catalase activity (Taylor and Achanzar 1972). After genotypic identification, isolates of the *Enterococcus* genus were distinguished from isolates of the *Leuconostoc* genus by determination of CO_2 production from glucose, acid production from mannitol and the ability to grow at 45 °C (Facklam and Elliott 1995). Acid production from L-arabinose, mannitol, amygdalin and galactose was examined in order to differentiate between the species *Enterococcus durans*/*hirae*/*faecalis*/*lactis* and *faecium* (Manero and Blanch 1999). *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides* were differentiated by growth in NaCl 6.5% (w/v) (Facklam and Elliott 1995) and acid production from L-arabinose, mannitol, amygdalin and galactose (Björkroth et al. 2014).

Genotypic characterisation

After the preliminary phenotypical characterisation, DNA was extracted using InstaGene (Bio-Rad Laboratories, Hercules, CA, USA) following the instructions of the manufacturer. The rep-PCR procedure was carried out as described by (Nielsen et al. 2007). In brief, the rep-PCR products were separated by 1.5% agarose gel electrophoresis (5 h, 120 V) in Tris–Borate–EDTA buffer (0.5× TBE) with a 1-kb DNA ladder as reference marker (Thermo Scientific, Lithuania). Afterwards, gels were stained with ethidium bromide and documented using a digital camera (Computer ® TV Zoom LENS, Japan). Cluster analysis of rep-PCR-profiles were performed using Bionumerics 7.1 (Applied Maths, Sint-Martens-Latem, Belgium) based on Dice's Coefficient of similarity with the unweighted pair group method with arithmetic averages clustering algorithm (UPGMA). Based on the clusters, representatives of each cluster (approximately the square root of the number of isolates within each cluster) were selected for sequencing of the 16S rRNA or 26S rRNA gene for LAB and yeasts, respectively. For 16S rRNA gene sequencing, the primers 27f and 1540r (Jensen et al. 2009) were used for amplification under the following conditions: initial denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 120 s, followed by a final extension at 72 °C for 10 min. For the D1/D2-region of 26S rRNA gene, primers NL-1 and NL-4 were used for amplification (Kurtzman and Robnett 1998) under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles at 95 °C for 90 s, 53 °C for 30 s, 72 °C for 90 s followed by a final extension step at 72 °C for 7 min. The PCR products were sent to a commercial sequencing facility (MacroGen, Germany). Subsequently, the LAB and yeasts sequences were manually corrected and assembled with CLC Genomics Workbench (version 8.5.1) software (CLC bio, Aarhus, Denmark). LAB sequences were aligned to 16S rRNA gene sequences in EzBioCloud (Yoon et al. 2017) and yeast sequences aligned to 26S rRNA gene sequences in NCBI GenBank database, using BLAST algorithm (Zhang et al. 2000). Nucleotide sequences obtained in this study have been assigned the GenBank Accession Nos. MH43170–MH431829 for LAB and MH447333–MH447352 for yeasts, as indicated in Tables 3 and 4. Isolates identified as *Lactobacillus plantarum* or *Lactobacillus pentosus* were differentiated by sequencing of multiplex PCR products targeting the *recA* gene according to Torriani et al. (2001) and identified by BLAST searches in NCBI GenBank database. *Candida parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis* were differentiated by sequencing of internal transcribed spacer (ITS)-regions with the primers ITS1 and ITS4, according to Esteve-Zarzoso et al. (1999) and identified by BLAST searches in NCBI GenBank database.

Results

Microbial counts of *lait caillé* end-products

In Table 1, pH and microbial counts are listed for the ten investigated *lait caillé* end-products obtained from the three local markets in Bobo-Dioulasso. The pH of the ten

Table 1 pH and microbial counts of *lait caillé* end-product samples from local open air markets in Bobo Dioulasso, South-West of Burkina Faso

	Samples (S)									
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
pH	3.7	3.8	3.7	3.5	3.9	4.2	4.2	3.8	4.0	3.8
Total LAB_{MRS} (log CFU/g)	7.8 ± 0.06	8.8 ± 0.04	10.0 ± 0.03	8.0 ± 0.01	7.9 ± 0.02	8.7 ± 0.05	9.2 ± 0.03	9.0 ± 0.01	8.6 ± 0.03	9.7 ± 0.01
$Lactococci_{M17}$ (log CFU/g)	7.9 ± 0.17	9.6 ± 0.01	8.9 ± 0.05	8.1 ± 0.04	8.0 ± 0.06	9.0 ± 0.04	10.1 ± 0.05	9.3 ± 0.02	8.6 ± 0.02	8.1 ± 0.05
Enterobacteriaceae (log CFU/g)	6.2 ± 0.10	< 3.0 ± 0.00	< 3.0 ± 0.00	–	–	7.9 ± 0.02	8.4 ± 0.14	6.8 ± 0.00	7.9 ± 0.02	3.3 ± 0.16
Yeasts (log CFU/g)	7.7 ± 0.08	6.7 ± 0.02	8.7 ± 0.01	6.2 ± 0.08	6.8 ± 0.04	5.5 ± 0.03	5.3 ± 0.06	5.8 ± 0.06	7.0 ± 0.04	7.1 ± 0.04

S1–S6 were collected at Ouezzin-ville market, S7–S8 at Grand marché market, S9–S10 at Farakan market

±, standard deviation; –, not determined

end-product market samples varied from pH 3.5 to 4.2. LAB were predominating all ten investigated samples with total LAB counts ranging from 7.8 ± 0.06 to 10.0 ± 0.03 log CFU/g and similar lactococcal counts of 7.9 ± 0.17 to 10.1 ± 0.05 log CFU/g. The Enterobacteriaceae counts ranged from < 3.0 log CFU/g and up to 8.4 ± 0.14 log CFU/g, hence in five of the samples comprising a significant part of the microbiota with 6.2 ± 0.10 to 8.4 ± 0.14 log CFU/g (S1, S6, S7, S8 and S9). For the yeasts, counts between 5.3 ± 0.03 and 8.7 ± 0.01 log CFU/g were obtained.

Microbial counts during *lait caillé* fermentation

Table 2 lists the pH and microbial counts from the detailed study of traditional *lait caillé* fermentation from the house-scale production site in the village Tolotama outside Bobo-Dioulasso. During the *lait caillé* fermentation, the pH decreased from 6.7 ± 0.01 in the fresh milk to 4.3 ± 0.08 at the end of the fermentation (59 h). Meanwhile, the total LAB counts increased during the fermentation from 5.4 ± 0.04 in fresh milk to 8.6 ± 0.02 log CFU/g in the end-product, with similar lactococcal counts of 5.4 ± 0.01 to 8.5 ± 0.15 log CFU/g throughout the fermentation. Enterobacteriaceae counts increased from 2.7 ± 0.01 log CFU/g at the onset of fermentation to high amounts of 6.7 ± 0.00 log CFU/g at the end of the fermentation. The counts for both total LAB and lactococci peaked at 53 h of fermentation at 9.0 ± 0.04 and 9.1 ± 0.06 log CFU/g, respectively, followed by a slight decrease towards the end of the fermentation. Enterobacteriaceae counts peaked at 28 h with 8.3 ± 0.13 log CFU/g and from this points decreased until the end of the fermentation. For yeasts counts, an increase throughout the fermentation was observed from 4.6 ± 0.08 log CFU/g (7 h) to a final load of 6.4 ± 0.11 log CFU/g.

Identification of LAB during *lait caillé* fermentation

From the production site in Tolotama, a total of 251 Gram positive, catalase negative, rod or coccoid isolates, obtained throughout the *lait caillé* fermentation, were presumptively identified as LAB and genotypically characterized by $(GTG)_5$-based rep-PCR fingerprinting followed by clustering. Table 3 shows the identities of representative LAB isolates from the eight clusters obtained, including GenBank Accession numbers. The representative LAB isolates were identified based on their 16S rRNA gene sequences and BLAST searches at EzBioCloud. Separate clusters for each identified LAB species, including all isolates from *lait caillé*, are presented in Online Resource 1 to 3.

Representative isolates of each LAB species are shown in Fig. 1. In total eight bacterial species were identified. The predominant part of the LAB isolates from *lait caillé* (39% of the total LAB isolates) were identified as

Table 2 pH and microbial counts during *lait caillé* fermentation from a production site in Tolotama, Bobo Dioulasso, South-West of Burkina Faso

	Fermentation time (hour)								
	0	7	13	18	28	35	41	53	59
pH	6.7 ± 0.01	6.6 ± 0.02	6.2 ± 0.54	5.4 ± 1.05	4.9 ± 0.61	4.5 ± 0.25	4.5 ± 0.17	4.4 ± 0.09	4.3 ± 0.08
LAB_{MRS} (log CFU/g)	5.4 ± 0.04	5.7 ± 0.00	7.1 ± 0.13	8.0 ± 0.01	8.6 ± 0.06	8.6 ± 0.12	8.6 ± 0.08	9.0 ± 0.04	8.6 ± 0.02
$Lactococci_{M17}$ (log CFU/g)	5.4 ± 0.01	6.1 ± 0.12	8.9 ± 0.13	7.5 ± 0.08	8.8 ± 0.08	8.8 ± 0.04	8.6 ± 0.03	9.1[a]	8.5 ± 0.15
Enterobacteriaceae (log CFU/g)	2.7 ± 0.01	4.6 ± 0.09	6.7 ± 0.03	6.9 ± 0.10	8.3 ± 0.13	8.0 ± 0.05	7.3 ± 0.02	7.0 ± 0.05	6.7 ± 0.00
Yeasts (log CFU/g)	3.6[a]	4.6 ± 0.08	4.1 ± 0.02	4.4 ± 0.02	4.8 ± 0.01	4.9 ± 0.04	5.2 ± 0.11	6.1 ± 0.01	6.4 ± 0.11

±, :standard deviation

[a]Replicate sample lost

Lactococcus lactis (cluster VIII) with similarities between 99.8 and 100% to EzBioCloud sequences. The most abundant genus in *lait caillé* was *Enterococcus*, clearly separated into three clusters (III, IV and VI) with 99.1–99.8% similarity to EzBioCloud sequences of several *Enterococcus* spp. Based on biochemical tests the three clusters were identified to species level. Isolates in cluster IV being the predominant *Enterococcus* spp. (21% of the total LAB isolates) were identified as *Enterococcus lactis* as the isolates produced acid from arabinose but not from amygdalin (Manero and Blanch 1999). Isolates in cluster III (16% of the total LAB isolates) were identified as *Enterococcus hirae* as the isolates were not able to produce acid from either L-arabinose or mannitol but possessed α-galactosidase activity (Manero and Blanch 1999). Isolates in cluster VI (6% of the total LAB isolates) were identified as *Enterococcus faecium* as the isolates were unable to produce CO_2 from glucose but able to grow at 45 °C and to produce acid from arabinose, mannitol and galactose (Manero and Blanch 1999). Isolates in cluster II were identified as *Leuconostoc mesenteroides* (9% of the total isolated LAB) based on biochemical tests since the 16S rRNA gene sequences could not differentiate between *L. mesenteroides* and *Leuconostoc pseudomesenteroides* with 99.1–99.3% similarity to EzBioCloud sequences. Hence, isolates in cluster II had no growth at 45 °C but could grow in the presence of 6.5% NaCl (w/v). Further, no acid was formed from L-arabinose, mannitol and amygdalin, but acid was formed from galactose and CO_2 was produced from glucose (Facklam and Elliott 1995). Isolates in cluster V were identified as *Weissella paramesenteroides* (4% of the total LAB isolates) and had 99.1–99.4% similarity to EzBioCloud sequences. Isolates in cluster VII were identified as *Pediococcus pentosaceus* (2% of the total LAB isolates) with 99.9% similarity to EzBioCloud sequences. Isolates in cluster I (1% of the total LAB isolates) were identified as *Lactobacillus plantarum* or *Lactobacillus pentosus* with 99.7–99.8% similarity to EzBioCloud sequences. By multiplex PCR of *recA* gene (Torriani et al. 2001) cluster I isolates were identified as *L. plantarum* with 100% similarity to EzBioCloud sequences (results not shown).

Identification of yeasts during *lait caillé* fermentation

From the production site in Tolotama, 169 isolates obtained during *lait caillé* fermentation were presumptively identified as yeasts following phenotypic characterisation. Table 4 shows the identities of representative yeast isolates from the four clusters obtained by analysis of $(GTG)_5$-based rep-PCR fingerprinting, including GenBank Accession numbers. Separate clusters for each identified yeast species with all isolates from *lait caillé* are presented in Online Resource 4 and 5.

Representative isolates of each yeast species are shown in Fig. 2. In total four yeast species were identified in *lait caillé*. The predominant part of the yeast isolates (52% of the total isolated yeasts) were identified as *Saccharomyces cerevisiae* (cluster C) with 99.5–100% similarity to GenBank sequences. The second most abundant group of yeasts were found to belong to the *Candida parapsilosis* group (comprising *Candida parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*) with 99.2–100% similarity to 26S rRNA gene sequences in GenBank, clearly separated into two clusters (A and D). Based on sequencing of ITS, isolates in cluster A (43% of the total isolated yeasts) were identified as *C. parapsilosis* with 100% similarity to GenBank sequences and isolated in cluster D (1% of the total isolated yeasts) were identified as *C. orthopsilosis* with 100 similarity to GenBank sequences (results not shown). Isolates in cluster B were identified as the genus *Coniochaeta* spp. (anamorph: *Lecythophora*) (3% of the total yeast isolates) with 99.4–100% similarity to 26S rRNA gene sequence in GenBank.

Table 3 Species identification of representative LAB isolated during *lait caillé* fermentation from the production site in Tolotama, Bobo Dioulasso, South-West of Burkina Faso, by sequencing of the 16S rRNA gene

Cluster	Isolate code[a]	Identities EzBioCloud	Similarity to EzBioCloud sequence (%)	GenBank accession number	Identified LAB species
I	RTIX-7.2.7	945/947	99.8	MH431790	*Lactobacillus plantarum*
I	RTVIII-7.2.1	1030/1033	99.7	MH431791	*Lactobacillus plantarum*
II	RTIX-7.2.6	899/907	99.1	MH431792	*Leuconostoc mesenteroides*
II	RTVI-7.1.4	971/980	99.1	MH431793	*Leuconostoc mesenteroides*
III	MTVIII-7.1.11	995/998	99.7	MH431794	*Enterococcus hirae*
III	MTIII-6.1.6	929/932	99.7	MH431795	*Enterococcus hirae*
III	MTVIII-7.2.1	990/996	99.4	MH431796	*Enterococcus hirae*
III	MTVIII-7.1.7	1004/1007	99.7	MH431797	*Enterococcus hirae*
III	RTIII-5.1.5	1000/1003	99.7	MH431798	*Enterococcus hirae*
III	RTV-7.3.6	891/983	99.8	MH431799	*Enterococcus hirae*
III	RTIII-5.2.2	707/709	99.7	MH431800	*Enterococcus hirae*
III	RTV-7.3.7	994/997	99.7	MH431801	*Enterococcus hirae*
III	MTVIII-7.2.4	867/871	99.5	MH431802	*Enterococcus hirae*
IV	RTIII-5.2.3	956/965	99.1	MH431803	*Enterococcus lactis*
IV	MTV-7.3.1	1000/1004	99.6	MH431804	*Enterococcus lactis*
IV	MTV-7.1.8	922/924	99.8	MH431805	*Enterococcus lactis*
IV	RTIV-6.2.7	996/1000	99.6	MH431806	*Enterococcus lactis*
IV	MTV-7.3.2	950/954	99.6	MH431807	*Enterococcus lactis*
IV	RTIX-7.2.4	1134/1138	99.7	MH431808	*Enterococcus lactis*
IV	RTIII-5.1.6	923/930	99.3	MH431809	*Enterococcus lactis*
IV	RTVIII-7.3.4	992/999	99.3	MH431810	*Enterococcus lactis*
IV	RTIV-6.5.1	889/891	99.8	MH431811	*Enterococcus lactis*
V	RTII-4.3.3	919/927	99.1	MH431812	*Weissella paramesenteroides*
V	RTII-4.3.4	937/944	99.3	MH431813	*Weissella paramesenteroides*
V	RTI-4.1.1	900/905	99.4	MH431814	*Weissella paramesenteroides*
VI	RTIII-5.1.3	1030/1032	99.8	MH431815	*Enterococcus faecium*
VI	RTVII-7.1.4	999/1004	99.6	MH431816	*Enterococcus faecium*
VII	RTI-4.3.1	993/994	99.9	MH431817	*Pediococcus pentosaceus*
VII	RTI-4.3.2	1017/1018	99.9	MH431818	*Pediococcus pentosaceus*
VIII	RTV-7.3.3	1013/1013	100	MH431819	*Lactococcus lactis*
VIII	RTVIII-7.3.2	981/981	100	MH431820	*Lactococcus lactis*
VIII	RTVII-7.1.10	868/868	100	MH431821	*Lactococcus lactis*
VIII	MTVIII-7.3.1.1	917/917	100	MH431822	*Lactococcus lactis*
VIII	RTVII-7.2.4	878/878	100	MH431823	*Lactococcus lactis*
VIII	RTIX-7.1.9	979/981	99.8	MH431824	*Lactococcus lactis*
VIII	MTIII-6.1.4	920/921	99.9	MH431825	*Lactococcus lactis*
VIII	RTVII-7.2.1	877/877	100	MH431826	*Lactococcus lactis*
VIII	RTIII-5.1.8	959/959	100	MH431827	*Lactococcus lactis*
VIII	RTVI-7.1.10	911/911	100	MH431828	*Lactococcus lactis*
VIII	RTVIII-7.1.7	968/969	99.9	MH431829	*Lactococcus lactis*

[a]Isolate codes beginning with: “R” originates from MRS agar plates and “M” from M17 agar plates

LAB and yeasts succession during *lait caillé* fermentation

Microbial successions of the identified LAB and yeasts were observed during *lait caillé* fermentation at the production site in Tolotama (Table 5). At the onset of the fermentation (0 h) the isolated LAB were dominated by *P. pentosaceus* comprising 50.0% of the isolated LAB, followed by *W. paramesenteroides* and *L. mesenteroides* accounting for 30.0% and 20.0% of the isolated LAB, respectively. After

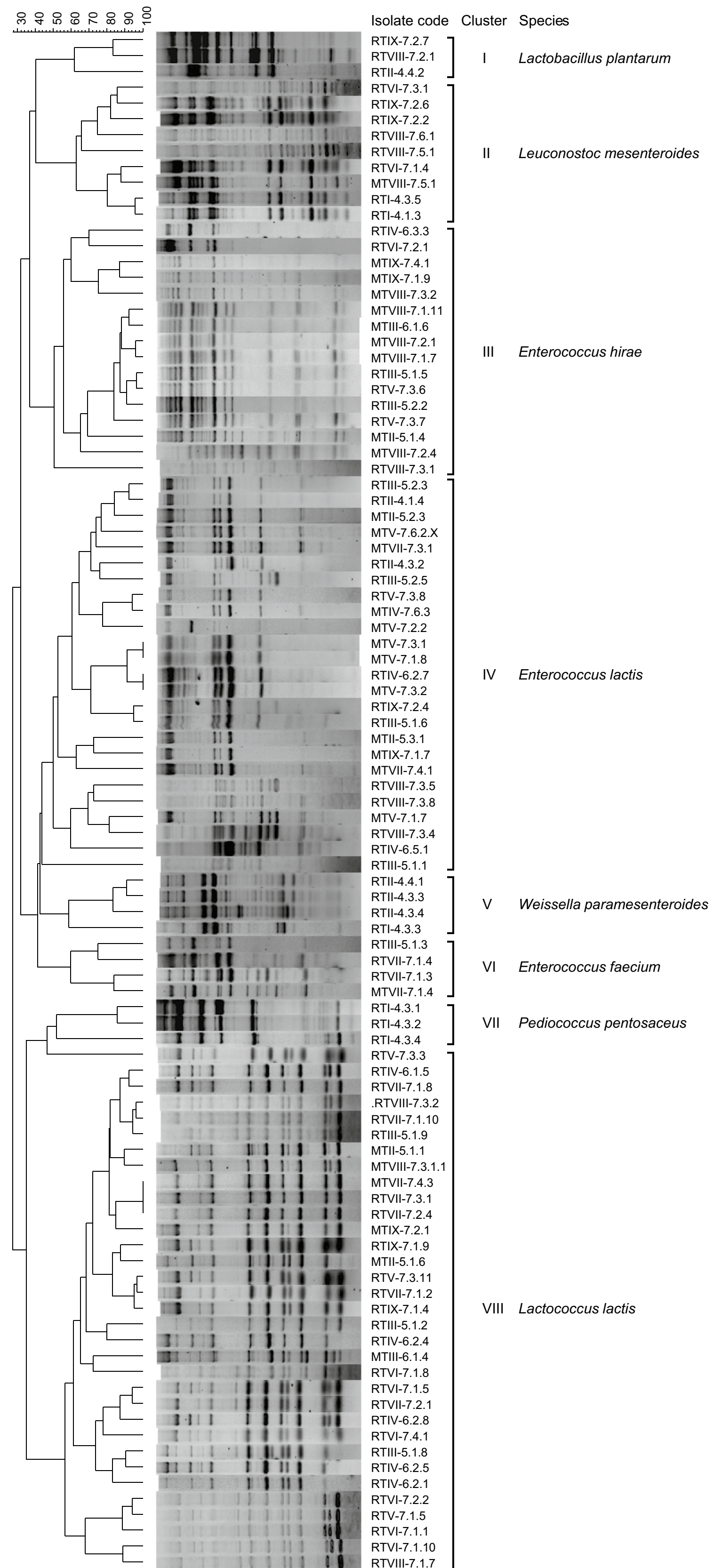

Fig. 1 Dendrogram of rep-PCR cluster analysis of LAB isolates from the *lait caillé* fermentation, based on Dice's coefficient of similarity with the unweighted pair group method with arithmetic average clustering algorithm (UPGMA). A representative sub-sample of LAB isolates is shown

Table 4 Species identification of representative yeasts isolated during *lait caillé* fermentation from the production site in Tolotama, Bobo Dioulasso, South-West of Burkina Faso, by sequencing of the 26S rRNA gene

Cluster	Isolate code	Length D1/D2 sequence (bp)	Identities GenBank	Similarity to GenBank sequence (%)	GenBank accession number	Identified yeast species
A	STII-3.1.5	586	585/586	99.8	MH447333	*Candida parapsilosis*
A	STI-2.2.6	585	585/585	100	MH447334	*Candida parapsilosis*
A	STII-3.1.3	584	584/584	100	MH447335	*Candida parapsilosis*
A	STVI-3.2.2	592	592/592	100	MH447336	*Candida parapsilosis*
A	STV-3.5.1	592	592/592	100	MH447337	*Candida parapsilosis*
A	STVII-4.5.1	586	586/586	100	MH447338	*Candida parapsilosis*
A	STIV-3.1.4	583	583/583	100	MH447339	*Candida parapsilosis*
B	STII-3.3.2	551	551/551	100	MH447340	*Coniochaeta* spp.
B	STII-3.3.1	518	516/518	99.6	MH447341	*Coniochaeta* spp.
B	STV-3.8.1	552	552/552	100	MH447342	*Coniochaeta* spp.
B	STIII-2.9.1	552	552/552	100	MH447343	*Coniochaeta* spp.
C	STIX-5.1.2	517	517/517	100	MH447344	*Saccharomyces cerevisiae*
C	STIV-3.4.1	566	566/566	100	MH447345	*Saccharomyces cerevisiae*
C	STIII-2.1.4.1	516	516/516	100	MH447346	*Saccharomyces cerevisiae*
C	STIII-2.3.4	587	587/587	100	MH447347	*Saccharomyces cerevisiae*
C	STVII-4.1.5	588	587/588	99.8	MH447348	*Saccharomyces cerevisiae*
C	STIV-3.2.2	591	591/591	100	MH447349	*Saccharomyces cerevisiae*
C	STVII-4.6.1	585	585/585	100	MH447350	*Saccharomyces cerevisiae*
C	STVI-3.3.1	565	564/565	99.8	MH447351	*Saccharomyces cerevisiae*
D	STVI-3.7.2	591	591/591	100	MH447352	*Candida orthopsilosis*

7 h of fermentation the highest LAB species diversity was observed with seven identified species. At this time point (7 h), *E. lactis*, *L. lactis* and *E. hirae* were the most abundant species (21.3–31.9% of the isolated LAB) followed by *E. faecium*, *W. paramesenteroides*, *L. mesenteroides* and *L. plantarum* (2.5–10.6% of the isolated LAB). Throughout the rest of the fermentation, *W. paramesenteroides* and *P. pentosaceus* were not observed. From 13 h to the end of the fermentation (59 h), *L. lactis* was generally dominating ranging from 20.2 to 55.2% of the isolated LAB with an additional high abundance of *E. lactis* (8.6–64.9%) and *E. hirae* (7.2–47.2% of the isolated LAB). At the end of the fermentation *lait caillé* was predominated by *L. lactis* (31.4%) followed by *E. hirae, L. mesenteroides* and *E. lactis* (15.7–22.9% of the isolated LAB). Less frequently isolated LAB, were *L. plantarum* (2.5–5.2%) and *E. faecium* (3.4–16.6% of the isolated LAB) both occurring at the early stages and at the end of the *lait caillé* fermentation.

The yeasts in the *lait caillé* samples was dominated by *C. parapsilosis* during the first 18 h of fermentation ranging from 48.5 to 80.0%, followed by *S. cerevisiae* comprising 10.5–45.5% of the isolated yeasts. From 28 h and throughout the rest of the fermentation, yeast species diversity changed and *S. cerevisiae* was the predominating yeast, ranging between 47.4 and 100.0% of the isolated yeasts. From 53 h until the end of the fermentation (59 h) the only identified yeast species was *S. cerevisiae*. Other less frequently isolated yeasts during the fermentation were *Coniochaeta* spp. (3.0–10.5%) and *C. orthopsilosis* (3.0% to 5.9% of the isolated yeasts), occurring from 7 h to 35 h in the *lait caillé* fermentation.

Discussion

The samples from the local markets differed from those sampled at the end of fermentation in the house-hold production site in terms of fermentation conditions. In addition, the market samples were already in a state of stored end-products under ambient conditions, which may explain the differences in pH and e.g. Enterobacteriaceae counts. The pH of *lait caillé* from market samples (3.7 to 4.2) and from production site (4.3 ± 0.08) are comparable to the pH reported for other African fermented milk products, i.e. *kivuguto* (pH 4.5) from Rwanda (Karenzi et al. 2012) and *nyarmie* (pH 3.5 to 4.2) from Ghana (Obodai and Dodd 2006), with fermentation times of 24–48 h. Further, the results on LAB counts (7.8 ± 0.06 to 10.0 ± 0.03 log CFU/g) in the present study of both market samples of *lait caillé* end-products and from the production site in Tolotama showed predominance of LAB and are comparable to the findings for other African spontaneous fermented

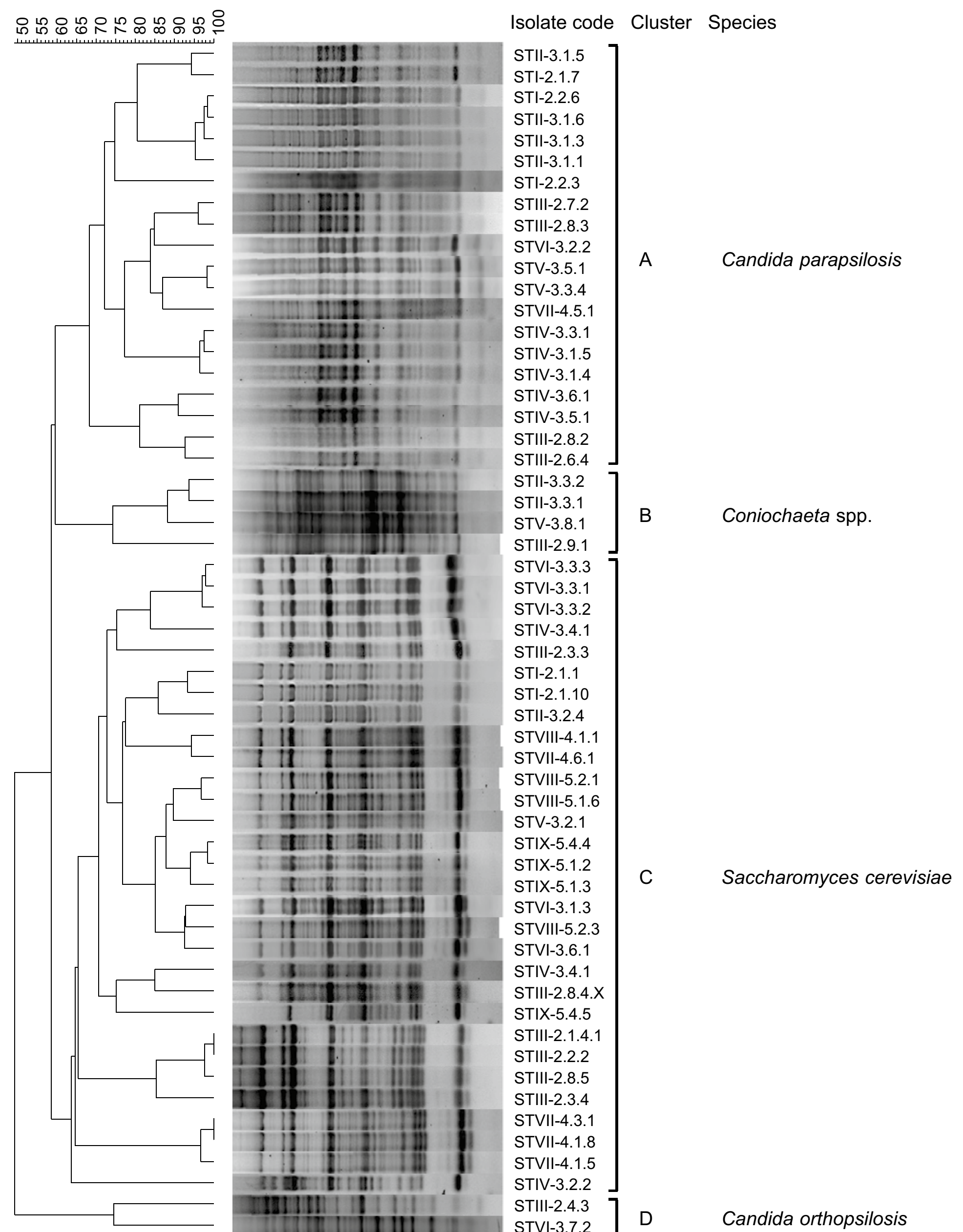

Fig. 2 Dendrogram of rep-PCR cluster analysis of yeast isolates from the *lait caillé* fermentation, based on Dice's coefficient of similarity with the unweighted pair group method with arithmetic average clustering algorithm (UPGMA). A representative sub-sample of sequenced yeast isolates is shown

milk products where 8.1 to 9.0 log CFU/mL of LAB was reported in *nyarmie* (Obodai and Dodd 2006) and 8.0 to 8.7 log CFU/mL in *nunu* (Akabanda et al. 2013), both from Ghana. The high level of the presumptive LAB count (5.4 ± 0.04 log CFU/g), recorded at the beginning of the fermentation, may arise from the hygienic practices used at the farm. Previously, especially milk containers have been associated with relatively high total bacterial counts (Bonfoh et al. 2003), while also cow's skins have been associated with noticeable lactobacilli counts (Monsallier et al. 2012). Similarly, the variability of microbial counts and pH observed among the samples from market could be explained by the variable, uncontrolled fermentation conditions; particularly the handling of the milk before and after the spontaneous fermentation.

Microbial successions are generally reported for spontaneous fermented food products (Akabanda et al. 2013; Greppi et al. 2013; Jespersen 2003; Nielsen et al. 2007; Wullschleger et al. 2013). In our study, eight different LAB species were identified in *lait caillé* from the production site in the village Tolotama, namely *L. lactis*, *L. mesenteroides*, *E. lactis*, *E. hirae*, *E. faecium*, *P. pentosaceus*, *W. paramesenteroides* and *L. plantarum*. At the end of the *lait caillé* fermentation *L. lactis* was the dominating LAB identified, which is similar to findings from end-products of e.g. *kivuguto* from Rwanda, the Masai fermented milk from Tanzania

Table 5 Microbial succession during *lait caillé* fermentation from the production site in Tolotama, Bobo Dioulasso, South-West of Burkina Faso

Relative abundance (%) of each species	Fermentation hour								
	0	7	13	18	28	35	41	53	59
LAB									
Lactococcus lactis	–	21.3	33.1	55.2	21.9	34.3	54.0	20.2	31.4
Leuconostoc mesenteroides	20.0	2.5	0.6	4.2	6.6	4.3	5.5	14.4	20.9
Enterococcus lactis	–	31.9	24.9	25.5	64.9	15.3	16.6	8.6	15.7
Enterococcus hirae	–	21.3	24.9	15.1	6.6	46.0	7.2	47.2	22.9
Enterococcus faecium	–	10.6	16.4	–	–	–	16.6	3.4	3.8
Pediococcus pentosaceus	50.0	–	–	–	–	–	–	3.4	–
Weissella paramesenteroides	30.0	9.9	–	–	–	–	–	–	–
Lactobacillus plantarum	–	2.5	–	–	–	–	–	2.9	5.2
Yeasts									
Saccharomyces cerevisiae	20.0	10.5	45.5	19.0	47.4	70.6	86.7	100.0	100.0
Candida parapsilosis	80.0	78.9	48.5	76.2	42.1	23.5	13.3	–	–
Candida orthopsilosis	–	–	3.0	–	–	5.9	–	–	–
Coniochaeta sp.	–	10.5	3.0	4.8	10.5	–	–	–	–

For LAB the highest count on either Man, Rogosa and Sharpe or M17 agar were used for calculating relative abundancies. All yeasts were counted on Sabouraud Chloroamphenicol agar

–: not detected

and Fulani fermented milk from Burkina Faso (Isono et al. 1994; Karenzi et al. 2012; Savadogo et al. 2004). In fact, *L. lactis* is typical of most of African fermented milk products, and commonly used as the acidifying starter culture for most industrialised fermented milk products. It has been reported as the most widely detected species in a survey covering up to 25 African fermented dairy products and raw milk (Jans et al. 2017). However, sometimes *L. lactis* is not the predominant LAB most probably due to differences in the environment and the processing. Thus, in *nunu*, a fermented milk from Ghana, *Lactobacillus fermentum* was reported as the dominant LAB (Akabanda et al. 2013) and in a recent study, Parker et al. (2018) reported *Streptococcus* and *Lactobacillus* as the dominant genera in another type of *lait caillé* (curdle milk based on pasteurised milk) from Northern Senegal. From the present study, *L. lactis* may be considered as the main acidifier of *lait caillé*. Furthermore, some strains of *L. lactis* have been reported to produce exopolysaccharides (Cerning 1995; Ruas-Madiedo et al. 2002) functioning as bio-thickening agents (Duboc and Mollet 2001). Hence, *L. lactis* might also improve the rheological properties of *lait caillé* (Kleerebezem et al. 1999). *L. mesenteroides* was isolated throughout the fermentation in the present study. This heterofermentative LAB has previously been reported among the dominant LAB of the Tunisian *leben* and the Rwandese *kivuguto* (Karenzi et al. 2012; Samet-Bali et al. 2012). *Leuconostoc* spp. are generally associated with production of aroma compounds in dairy products, by conversion of citrate into flavour compounds like diacetyl (Lore et al. 2005). Thus, *L. mesenteroides* may add to the development of the characteristic aroma of *lait caillé*. Furthermore, a noticeable amount of *E. lactis* (9–65%) and *E. hirae* (7–47% of the isolated LAB) were obtained throughout the *lait caillé* fermentation. *Enterococcus* spp. has been reported in other fermented milk products e.g. *leben* from Tunisia (Samet-Bali et al. 2012) and *nunu* from Ghana (Akabanda et al. 2013), as well as in many traditional cheeses from the Mediterranean countries (Moreno et al. 2006). Only few studies have dealt with the ability of enterococci as milk acidifiers, however, it has been reported that some species of *E. faecium* and *E. faecalis* grown in camel, ovine or caprine milk could produce relatively high amounts of lactic acid (El Hatmi et al. 2018; Freitas et al. 1999). Additionally, it has been reported that certain species of enterococci exhibit probiotic properties (Moreno et al. 2006, Quirós et al. 2007). On the contrary, the perceived safety of enterococci is affected by the fact that some are opportunistic pathogens, especially, *E. faecalis*, whereas *E. faecium* appears to pose a lower risk (Franz et al. 2003) and further antibiotic resistance and virulence factors have been reported for both enterococci species (Ogier and Serror 2008).

The yeast counts obtained in the present study, from the end-products of *lait caillé* at the production site in Tolotama (6.4 ± 0.11 log CFU/g), were for some samples higher than previously reported for nunu i.e. 5.0 to 5.8 log CFU/mL (Akabanda et al. 2013). Considering the level of yeasts in *lait caillé*, it could be assumed that the yeasts might play a role in the fermentation process, probably in the sensorial acceptability of the product by the consumer. The yeasts identified in *lait caillé* from Tolotama included *S. cerevisiae*, *C. parapsilosis*, *C. orthopsilosis* and *Coniochaeta* spp. Among these, *S. cerevisiae* was the only yeast detected at

the end of the *lait caillé* fermentation. *S. cerevisiae* has often been isolated from African traditional fermented milk products (Akabanda et al. 2013; Gadaga et al. 2000; Obodai and Dodd 2006). Sudun et al. (2013) suggested that during the fermentation of *airag*, an alcoholic fermented milk product containing a co-culture of LAB and yeasts, the galactose resulting from degradation of lactose by the LAB promoted the growth of yeasts able to utilise galactose, such as *S. cerevisiae* (Sudun et al. 2013). A higher production of aroma compounds, such as ethanol, acetaldehyde and malty compounds has been reported to occur for some co-cultures of LAB and yeasts during fermentation of milk (Gadaga et al. 2001). Consequently, the authors suggested a possible interaction between LAB and yeasts during the fermentation process. Such interactions may also exist in *lait caillé* fermentation. *Coniochaeta* spp. has been isolated from wood or bark from different trees as well as from dung of various mammals (Damm et al. 2010), hence the *Coniochaeta* spp. identified in the *lait caillé* samples in the present study, were most likely introduced into the fermentation from the traditional straw-woven lids used to cover the fermentation containers or during the milking. It is though clear that the *Coniochaeta* spp. does not play a major role in the *lait caillé* fermentation.

Identifications of microorganisms based on 16S or 26S rRNA gene sequences pose, in some cases, difficulties due to high similarities between sequences of closely related species (Schleifer 2009). In the present study, it was not possible to differentiate between the isolated *Enterococcus* spp. based on the 16S rRNA sequences due to high similarity between sequences for these species (Švec and Franz 2014). Contrary, the rep-PCR fingerprinting profiles clearly separated the different *Enterococcus* spp. contributing to the identification of the isolated microorganisms from *lait caillé*. These findings are supported by previous studies, where rep-PCR was used to differentiate *Enterococcus* spp. (Pangallo et al. 2008; Švec et al. 2005). For *C. parapsilosis* and *C. orthopsilosis* it has previously been reported that only few nucleotides differ and that multi-gene analysis or ITS sequencing could be applied as techniques for identifying to species level (Tavanti et al. 2005). When studying the rep-PCR fingerprints obtained in the current study, clearly separated profiles were obtained for the two species, indicating that rep-PCR likewise could aid in the separation of *C. parapsilosis* and *C. orthopsilosis*.

In the present study it became clear that high amounts of Enterobacteriaceae occurred both in the ten *lait caillé* end-products sold at markets in Bobo-Dioulasso (up to 8.4 ± 0.14 log CFU/g) and at the production site in Tolotama (6.7 ± 0.00 log CFU/g), making these bacteria a considerable part of the microbiota in some of the *lait caillé* samples. Similar levels of Enterobacteriaceae have been reported in a previous study on hygienic quality of raw and sour milk products from Burkina Faso, sampled at different markets, with total coliform counts of 5.6 ± 0.59 log CFU/mL (Tankoano et al. 2016). Other traditional fermented milk products from Namibia and South Africa, sampled at household level, reported mean counts of coliforms to 6.5 log CFU/mL (Beukes et al. 2001). In a study on the microbiota in nunu, high levels of Enterobacteriales including *Escherichia coli* (7–13% relative abundance) and *Klebsiella pneumoniae* (3–71% relative abundance) were detected by shotgun amplicon sequencing (Walsh et al. 2017). The high level of Enterobacteriaceae in *lait caillé* can be explained by the poor hygienic conditions surrounding the milking step and milk storage, as observed during sampling. In addition, the manufacturing practices did not include pasteurisation or back-slopping and relied on spontaneous fermentation, which are major risk factors that enable various microorganisms, including potential pathogenic microorganisms, to grow during the fermentation (Broutin et al. 2007; Fondén et al. 2006). Moreover, *C. parapsilosis* was the dominant yeast until 28 h of fermentation. *C. parapsilosis* is a common spoilage yeast in fermented dairy products due to production of lipolytic and proteolytic enzymes (Fröhlich-Wyder 2003) and it is moreover recognised as an opportunistic pathogen (Silva et al. 2012). The high abundance of *C. parapsilosis* could indicate poor hygiene and ineffective cleaning procedures. Importantly, this yeast species decreased during the *lait caillé* fermentation in our study. This could be due to inhibitory compounds produced during the fermentation including possible antagonism from other yeasts present during the fermentation, e.g. through killer toxin production (Viljoen 2006).

Conclusion

Our results showed that *lait caillé* end-products sold at markets were partly dominated by LAB and yeasts along with considerable amounts of Enterobacteriaceae in some of the samples. These results were confirmed in the detailed study of the *lait caillé* fermentation. Further, microbial successions occurred during *lait caillé* fermentation with *L. mesenteroides*, *P. pentosaceus* and *W. paramesenteroides* being present at the onset of the fermentation. After 7 h the LAB diversity changed and *L. lactis*, *E. lactis* and *E. hirae* became the predominant LAB for the remaining of the fermentation. For yeasts *C. parapsilosis* was dominating the first 18 h of *lait caillé* fermentation. After 35 h *S. cerevisiae* became the most abundant yeast species and after 53 h the only yeast species identified. The deeper understanding of the microbiota involved in the *lait caillé* fermentation obtained in this study and the fact that high levels of especially Enterobacteriaceae were detected, point out the need to upgrade the *lait caillé* process by introducing pasteurisation combined with the use of acidification cultures, specifically made for *lait caillé*, to enhance the quality and food safety.

Acknowledgements The authors are grateful to the local producers and retailers who contributed to the study. The authors would like to acknowledge Ministry of Foreign Affairs of Denmark (Danida) for funding through the project *Preserving African food microorganisms for Green Growth* (Project Number DFC No. 13-04KU).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals Not applicable.

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