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Identification of yeasts present in artisanal yoghurt and traditionally fermented milks consumed in the northern part of Cameroon

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ABSTRACT

Bacteria generally ferment milk but, sometimes, yeasts are found in fermented milks. The presence of these yeasts in the microbial community of some fermented milks could be intentional or accidental. The Diversity of yeasts in the products was investigated using a molecular technique employing variable regions of 26S rDNA profiles generated by PCR-DGGE (Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis). Two types of samples: artisanal or handcraft yoghurt and traditionally fermented milks were collected in three towns of the three regions of the northern part of Cameroon. Firstly, a comparison was made between the 16 traditionally fermented milks collected in Maroua, Garoua and Ngaoundre each of the 3 regions. Secondly, it was between 26 artisanal fermented milks of each region and finally, between the two types of products. The different PCR-DGGE 26S rDNA profiles obtained were analyzed and DNA sequencing was used to compare yeasts from each method of production. Twelve (12) species of Yeasts were identified as: *Malassezia globosa, Hanseniaspora uvarum, Galactomyces candidum, Candida tropicalis, Aureobasidium pullulans, Torulaspora globosa, Saccharomyces cerevisiae/paradoxus, Pichia kluyveri, Candida parapsilosis, Torulaspora delbrueckii, Kluyveromyces marxianus, Candida orthopsilosis* and *Pseudozyma sp*. Yeast diversity was higher for artisanal fermented milks (yoghurt) with at least 10 species, while for traditionally non packed fermented milks only 5 species were identified with a predominance strain of *Galactomyces candidum, Candida parapsilosis, Torulaspora delbrueckii, Saccharomyces cerevisiae/paradoxus* and *Kluyveromyces marxianus*. The different species of yeast might be introduced accidentally in artisanal yoghurt; however, for traditionally fermented milks their presence might be associated to the starter.

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Introduction

In many African countries, foods are still frequently fermented on household scale through spontaneous fermentations at ambient temperatures, as system of food preservation [16]. A wide variety of traditionally fermented milks are prepared in small scale in various countries of Sub-Saharan Africa [42]. In the northern part of Cameroon, the inhabitants have secular culture based on the consumption of the milk and milk products. Because there is lack of cooling systems, the milk collected is fermented to obtain products such as "kidirmou" or "pendidam" to increase its shelf life [45]. The products obtained are a source of incomes for many families. Although the advantages of these fermented milks include the new and desirable tastes and textures, the quality of these traditionally prepared fermented foods is often poor as a result of neglected hygienic practices during preparation. [23].

Normally, yoghurts are produced using probiotics strains *Streptococcus thermophilus* and *Lactobacillus delbrueckii* susbsp. *bulgaricus* [30,37]. In the northern part of Cameroon with Maroua, Garoua and Ngaoundere as the mains towns, the producers of fermented milk products do not have pure starters, and use the back-slopping method to ferment pasteurized milk [38]. In back-slopping method, a part of the previous batch of a fermented product is used to inoculate the new batch. Moreover, in this region, there is no modern industry for dairy products, thus, milk fermentation is locally carried out in poor hygienic conditions. This might lied to the presence of microorganisms other than lactic acid bacteria.

Fermented milks have many advantages for the consumer health. It was shown that, lactic bacteria have can rebalance the intestinal flora and to fight against the pathogenic bacteria [26,32]. But preliminary studies [28] revealed that, for many samples of artisanal yoghurt, the bung of the bottles popped out when opened. This could be probably due to the presence of yeasts [15], which may cause digestive mycoses. The digestive mycoses can be manifested by chronic diarrheas [9,40]. Food associated with yeasts could also be a source of infections and adverse health responses [11].

Over recent years, because culture on petri dishes were too long, fastidious and not allow the growth of all the microorganisms the culture-independent methods were developed. Based on the molecular biology techniques, such as denaturing gradient gel electrophoresis (DGGE), have been developed to investigate yeast population dynamics in milk and many fermented products [4,33,44]. Investigations on the occurrence of yeasts and lactic acid bacteria in dairy products have been also carried out using traditional microbiological methods [12,24]. However, no investigation was focused on yeast diversity in yoghurt and traditionally fermented milks using non-culture dependent method PCR-DGGE in Cameroon. This technique allows the identification of the *non*-cultivable and cultivable fraction of microorganisms present in a sample.

This study aims to identify the dominant yeasts flora in artisanal yoghurts and traditionally fermented milks by using the DNA directly extracted from the fermented milk products and analyzed by PCR-DGGE which target the yeast 26 s rDNA, and then identifying it after sequencing.

Materials and methods

Study area and sampling

Among the dairy products the most consumed in the northern part of Cameroon, there are: yoghurts, “kindirmou”, “pendidam” and cheeses [28]. Generally, fermented milks are particularly cheap. “Kindirmou” is produced from the fresh boiled cow milk poured in a container and incubated without any further stirring for 12 h by adding “kindirmou” from the previous batch [14,28]. The “kindirmou” is simply consumed after addition of sugar but, the “pendidam”, used as acidifying porridges, contains less milk fat. In most cases, a small amount of water is added in the “pendidam”. The two fermented milks are very close together, considering the technological and microbiological aspects. These products are generally sold the next days and can be kept at room temperature during several days. The yoghurt is artisanal produced either with reconstituted powdered milk or raw milk. It is prepared by adding one cup of yoghurt from the previous day to approximate 20 L of boiled milk in a pot. The milk is then left to ferment for a day at room temperature. The products obtained are packaged in plastic flasks and kept in refrigerators and sometimes in freezers.

Two types of samples (artisanal yoghurt and traditionally fermented milks) were collected in the northern part of Cameroon, in the town of Maroua, Garoua and Ngaoundere. Maroua, a city found in Far North Region is located 10.59 latitude and 14.32 longitude and it is situated at elevation 406 m above sea level. Garoua is found in North Region, located 9.30 latitude and 13.40 longitude and is situated at 199 m above sea level. Ngaoundere is a city found in Adamaoua Region, located 7.33 latitude and 13.58 longitude and it is situated at elevation 1128 m above sea level.

Forty-two samples of yoghurts (26) and fermented milk (16) were respectively collected in Maroua (15 samples), Garoua (15 samples) and Ngoundere (12 samples), three regions of the North Cameroon. The samples were collected according to their availability. Among these samples, 10, 9 and 7 samples of artisanal yoghurts packed in plastic flasks of 250 mL were collected as they were sold in Maroua, Garoua and Ngaoundéré respectively. On the other hand, 5, 6 and 5 samples of fermented milk ("Kidirmou" and "pendidam"), generally contained in calabash, were collected in 250 mL sterile bottles respectively in Maroua, Garoua and Ngaoundéré. All these samples were transported in iceboxes containing ice blocks to the laboratory for the different analyses.

Analysis

DNA extraction

The DNA extraction was done according to El Sheikha et al., [13] method. Two (2) mL of sample were introduced in 1,5 mL Eppendorf tubes containing 0.3 g of glass marbles, homogenized, vortexed for 2 min (Vortex Genie 2 SI-A256, USA) and centrifuge for 15 min at 12,000 × g at 4 °C. The supernatant containing fat layer, was discarded and the cell pellets were suspended again in 100 µL TE (Tris-Ethylenediamine Tetraacetic Acid) solution [10 mM Tris–HCl; 1 mM EDTA, pH 8.0 (Promega, France)]. After homogenization, 100 µL lysozyme solution (25 mg/mL, Eurobio, France) was added and kept at room temperature (25 ± 2 °C) for 5 min and 100 µL proteinase K solution (20 mg/mL, Eurobio, France) were added and the mixture was incubated at 42 °C for 20 min. The lusozyme is a glycoside hydrolase that catalyzes the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-Dglucosamine residues in peptidoglycan, which is the major component of some bacterial cell wall. The Proteinase K is an enzyme that digests proteins acids). Then 50 µL of 20% Sodium Dodecyl Sulfate (SDS) were added to each tube, and incubated at 42 °C for 10 min, then 400 µL of a sulfactant Cetyltrimethyl ammonium bromide (CTAB; Sigma-Aldrich, USA) in 20% NaCl 3 M were added to each tube, and then incubated at 65 °C for 10 min. Extraction was done with 700 µL phenol/chloroform/isoamyl alcohol (25/24/1; v/v/v; Carlo Erba, France). The mixture was homogenized manually for 1 min until it became whitish and centrifuged at 12,000 × *g* for 20 min. The aqueous layer was transferred to a new Eppendorf tube. This step of extraction was repeated. The residual phenol was removed by extraction with 600 µL chloroform/isoamyl alcohol (24/1; v/v), and centrifuged for 15 min at 12,000 × *g*. The aqueous phase was transferred into a new tube; the DNA was stabilized with 30 µL of sodium Acetate (3 M, pH 5) and well mixed. The samples were precipitated by adding a volume (500 µL) of ice-cold isopropanol (100%, −70 °C) and stored at −20 °C for 12 h. After centrifugation at 12,000 × *g* for 30 min, the supernatant was eliminated, 500 µL of 70% ethanol was added to wash the DNA pellets and the tubes were centrifuged at 12,000 × *g* for 5 min. After discarding the ethanol, the DNA pellets were air-dried at room temperature, resuspended in 100 µL of ultrapure water and stored at 4 °C until analysis.

PCR amplification and DGGE analysis

Two µL of the total DNA extracted were pipetted directly onto an optical measurement surface of the Thermo Scientific™ NanoDrop 2000, a full-spectrum, UV–Vis spectrophotometers used to quantify and assess purity of DNA. After verification of the presence DNA extracted, its quantity and quality, a fragment of the D1 region of the 26S rDNA gene amplification was carried out with two eukaryotic universal primers: GC NL1f (5′ - CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC ATA TCA ATA AGC GGA GGA AAA G - 3′) and LS2 r (5′ - ATT CCC AAA CAA CTC GAC TC - 3′). The 50 µL reaction mixtures were constituted as follows: controlled DNA (5 µL); 5X Gotaq buffer (10 µL), dNTP (5 µL); Primer 1 GCNL1 (10µL); Primer 2 NL2 (10 µL) and Gotaq polymerase (0.25 µL). For amplification, the cycle was carried out at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 15–30 s, annealing at 52 °C for 15–30 s and extension at 72 °C for 15–30 s and a single final extension at 72 °C for 5 min. In Order to verify the quality of the PCR products, Aliquots (5 µL) of PCR products were analyzed first by electrophoresis in 2% w/v agarose gel with Tris-Acetate-EDTA (TAE) 1 × buffer (40 mM Tris–HCl, pH 7.4, 20 mM sodium acetate, 1.0 mM Na_2EDTA), stained with ethidium bromide (Promega, France) 50 µg/mL in TAE 1 × and quantified by using a standard (DNA mass ladder 100 bp; Promega). PCR products were analysed by DGGE, by using a Bio-Rad code universal mutation detection system (Bio-Rad, USA), using first the procedure described by Muyzer et al. [34]. The PCR products were then analyzed by denaturing gradient gel electrophoresis (DGGE), a technique using DCode system apparatus (BioRad, Hercules, CA, USA). The Polyacrlamide gels (8% w/v, Acrylamide/Bisacrylamide 37.5/1 of 0.8 mm thickness) were prepared using 40–60% Urea-formamide denaturing gradients (Promega, France). For the preparation of the gel, 16 mL of each of the cold solutions (stored at 4 °C) of 40 and 60% (100% corresponded to 7 M urea and 40% v/v formamide; Promega, France). In this mixture, 40 mg of ammonium persulfate (Promega, France) and 40 µL of Tétraméthylenediamine (TEMED) (Promega, France) were added. A volume of 9 µL of blue/orange coloring agent (Promega, France) was added to 35 µL of PCR products and poured in each well. The electrophoresis was proceeded in two steps: at 20 V for 10 min and 80 V for 12 h. The gels were stained for 30 min with Ethidium Bromide, rinsed and photographed in a transilluminator UV at 318 nm and the image obtained was proceeded using gel smart system 7.3 (Clara vision, Ulis, France). Imaging and statistical analysis of Individual lanes of the gel images were straightened using Image Quant TL software V. 2003 (Amersham Biosciences, USA). Banding patterns were standardized with two reference patterns included in all gels, the rDNA amplification products from the pure bacterial strains of *Candida apicola* and DNA of *Pichia galeiformis*. This software permitted to identify a band and compare its position with standard patterns. The DGGE fingerprints were scored by the presence and absence of co-migrating bands, independent of intensity. Pairwise community similarities were quantified using the Dice similarity coefficient (SD) [22].

$$SD = 2Nc/Na + Nb \quad (1)$$

Where Na represents the number of bands detected in sample A, Nb the number of bands in sample B, and Nc the number of bands common to both samples.

The similarity index was expressed within a range of 0 (completely dissimilar) to 100 (perfect similarity). The data generated by the calculation of similarity coefficients were exploited for the main component analysis (CPA) by the Statistica software (Version 7.1), and for the hierarchical ascending classification CAH by XLStat (Version 2014).

For the identification of yeast community, the DNA bands on DGGE gels were then excised with a sterile scalpel, eluted with 50 µL sterile ultrapure water and stored overnight at 4 °C. These extracted DNA was amplified again with non

GC-clamp primers. The PCR products were sent to GATC Biotech (Germany) for a second DNA purification and sequencing. Searches for similar sequences of 26S rDNA were performed using the basic local alignment search tool (BLAST) in GenBank (http://www.ncbi.nlm.nih.gov/) after editing and trimming the sequences by BioEdit sequence alignment editor, version 7.0.0 in order to determine the closest known relative species [2].

Results

The total DNA extracted from artisanal yoghurt and traditionally fermented milks was amplified and the PCR products analyzed by DGGE. The DGGE profiles for the yeast community of these different products were obtained. The DGGE fingerprinting of artisanal yoghurt are presented on Fig. 1 The analysis of this figure reveals the presence of 20 bands, but we numbered only the identified 10 main bands. Among all the sample sites, the samples from Garoua (G) has more bands (16 with 9 of the 10 identified for the samples of artisanal yoghurt) thus has more species of yeast than the samples from the others regions. The samples of Garoua were followed by those from Maroua (15 with 9 of the 10 identified yeasts) and those of Ngaoundere (9 with 7 of the 10 identified yeasts). *Candida apicola* used as a reference pattern was found in the samples of Maroua (M1 and M2) and those of Garoua (G9).

Among these bands, the DGGE patterns also revealed the presence of some common bands on almost all the samples sites (bands 4, 5, 6, 7, 8, 9). The DGGE profile varies from one sample to another but, the samples of the same locality have generally high similarity. The dendrogram obtained Fig. 1b) shows that in 45% of similarity, there are two main clusters: ((1) the first cluster included the samples of Maroua and Garoua while the second cluster contained the samples of Ngaoundere. High similarity exists between the yeast profiles of the artisanal yoghurts of Maroua and those of Garoua.

The DGGE fingerprinting of traditionally fermented milks is presented on Fig. 2. The analysis of this figure reveals the presence of 8 different identified bands over 20 bands present. The DGGE patterns revealed the presence of some common bands to almost all the samples sites. Among these are bands 1, 4, 7 and 9. Considering the sample origins, the samples from Ngaoundere (N) have the highest number of bands (15 with 7 of the 8 identified in the samples of traditionally fermented milks), followed by those from Maroua (13 with 7 of the 8 identified yeasts) and those from Garoua (12 with 6 of the 8

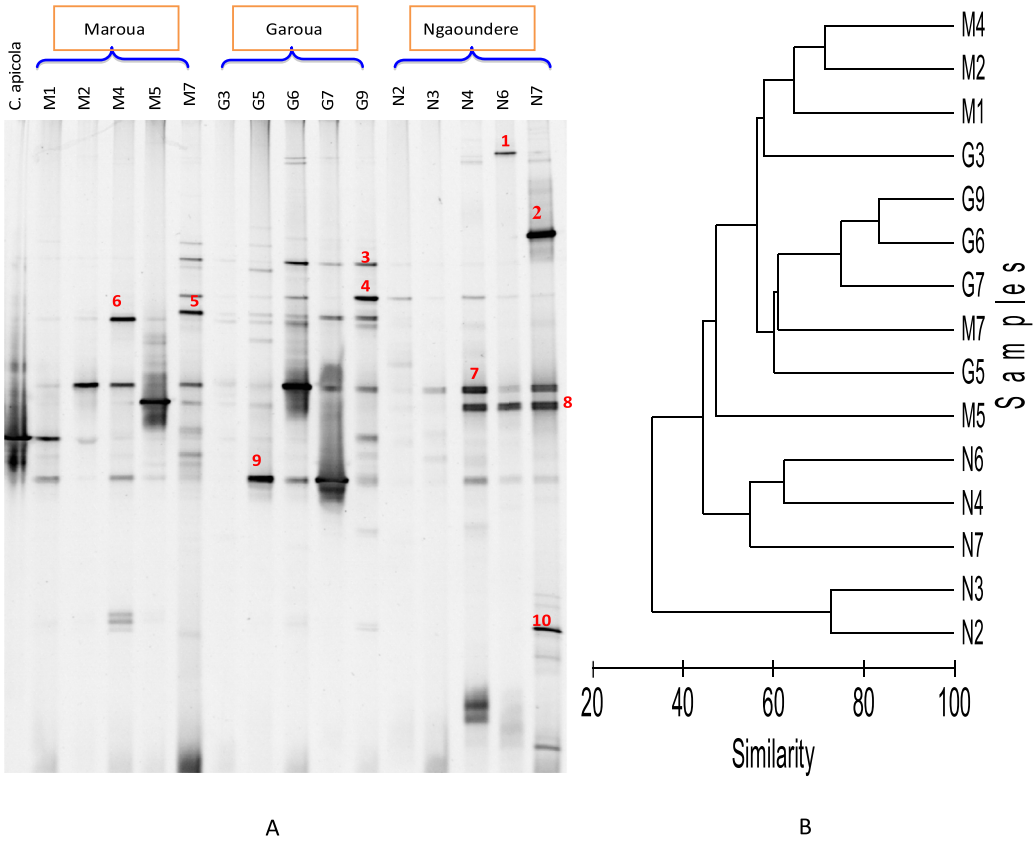

Fig. 1. (A): DGGE profiles of PCR amplicons of the domain D1 of 26S rDNA representing the yeasts biodiversity in samples artisanal yoghurt (B): Cluster analysis of 26S rDNA banding profiles of yeasts from artisanal yoghurt. Maroua: M1-M7, Garoua: G3-G9 ; Ngaoundere: N2-N7.

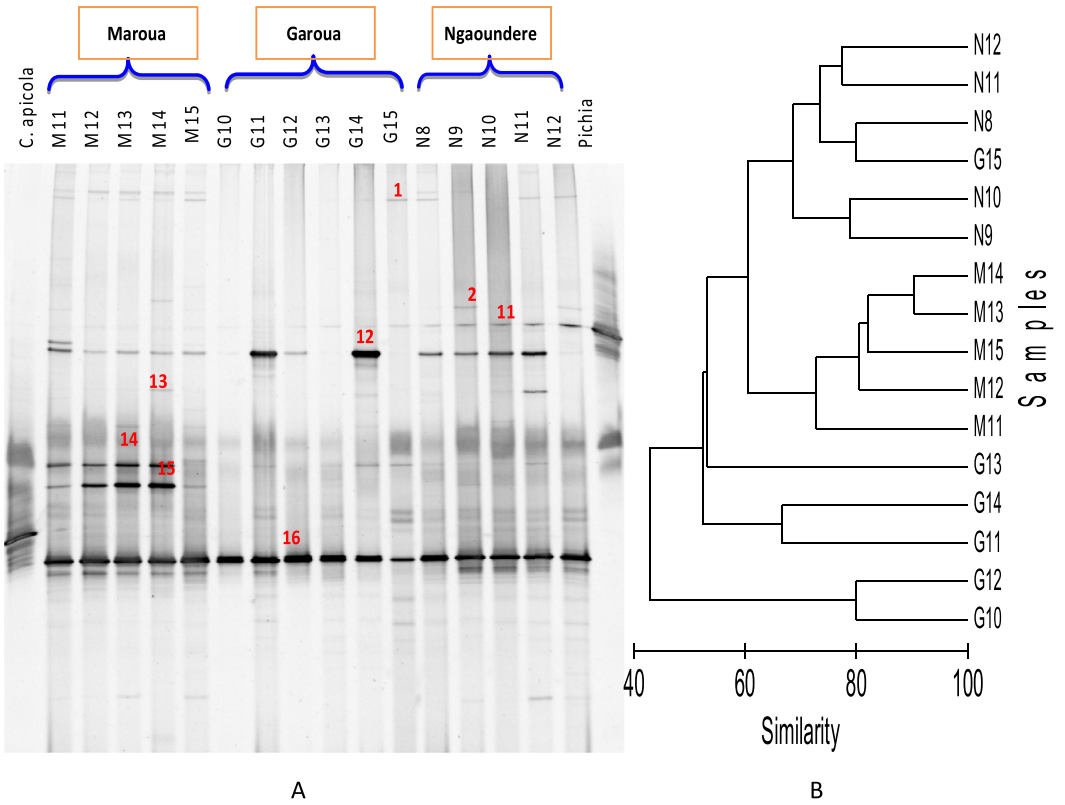

Fig. 2. (A): DGGE profiles of PCR amplicons of the domain D1 of 26S rDNA representing the yeasts biodiversity in samples traditionally fermented milks (B): Cluster analysis of 26S rDNA banding profiles of yeasts from traditionally fermented milks. Maroua: M11-M15, Garoua: G10-G15, Ngaoundere: N8-N12.

identified yeasts). The band 3 is common to four samples from Garoua and six samples from Ngaoundere. The band 1 is common to the samples from the 3 localities: Ngaoundere (N11 and N12), Garoua (G15) and Maroua (M15, M13, M11).

The band 6 is present on the samples M14, G14, N12 Fig. 2). Like the samples of artisanal yoghurts, the profiles are specific to the different localities and there is a great similarity between the samples from the same area. The dendrogram obtained (Fig. 2. B) reveals that, at 55% of similarity, there are two main clusters: ((1) the first cluster includes the samples from Garoua while the second cluster comprises the samples of Ngaoundere and Maroua. There was not a similarity of 100% between samples.

A comparison between the traditionally fermented milks and the artisanal yoghurts of the different sampling localities was done and the results presented on Fig. 3. Thus:

- For a given locality, there was a great similarity between the yeast profile of Traditionally Fermented Milks (TFM): Ngaoundere (N10, N9, and N8), Maroua (M12, M13, and M14) and Garoua (G11, G12 and G11).
- There is not a great similarity between the profiles of the artisanal yoghurt of the same site.

Among all these bands, three, represented by 4, 7 and 9 are found both in artisanal yoghurts and in traditionally fermented milk independently of the sample location.

DNA bands were excised from the denaturing gels, amplified and sequenced. The following species (13) of yeast, were grouped into 10 genera and identified as (Table 1): *Malassezia globosa, Hanseniaspora uvarum, Galactomyces candidum, Candida tropicalis, Aureobasidium pullulans, Torulaspora globosa, Saccharomyces cerevisiae/paradoxus, Pichia kluyveri, Candida parapsilosis, Torulaspora delbrueckii, Kluyveromyces marxianus, Candida orthopsilosis and Pseudozyma sp*.

Among these yeast species, 4 can be found in milk (*Galactomyces candidum, Pichia kluyveri, Kluyveromyces marxianus, Saccharomyces cerevisiae/paradoxus),* 5 were from the environment (*Malassezia globosa, Hanseniaspora uvarum, Aureobasidium pullulans, Torulaspora globosa, Torulaspora delbrueckii),* 4 from the hands workers (*Malassezia globosa, Galactomyces candidum, Candida tropicalis, Candida parapsilosis)* and 6 of them were among the potential pathogens for humans (*Malassezia globosa, Hanseniaspora uvarum, Candida tropicalis, Candida parapsilosis, Kluyveromyces marxianus, Candida orthopsilosis).*

Among all these species identified, three, represented by *Galactomyces candidum, Torulaspora delbrueckii and Saccharomyces cerevisiae/paradoxus* were present in artisanal yoghurts and in traditionally fermented milk independently of the sampling location.

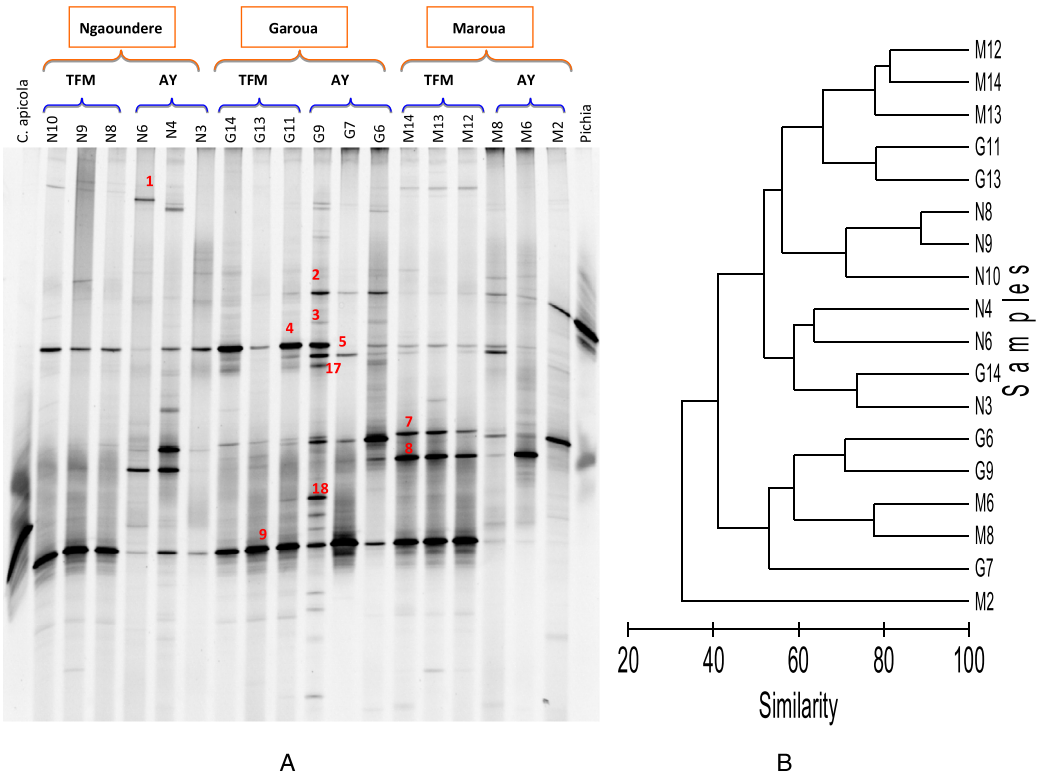

Fig. 3. (A): DGGE profiles of PCR amplicons of the domain D1 of 26S rDNA representing the yeasts biodiversity in both artisanal yoghurts and traditionally fermented milks (B): Cluster analysis of 26S rDNA banding profiles of yeasts. TFM: Traditionally fermented milks; AY: Artisanal yoghurts.

Table 1
Different yeasts identified on artisanal yoghurts and traditional fermented milks from Garoua, Maroua and Ngaoundere.

Bands	Yeast identified	Percentage	Accession
1	*Malassezia globosa*	90	KC415075
2	*Candida parapsilosis*	100	HE605209.1
3	*Hanseniaspora uvarum*	100	KP171581.1
4	*Galactomyces candidum*	100	KC967683.1
5	*Candida tropicalis*	97	AB741055.1
6	*Aureobasidium pullulans*	99	MH870594.1
7	*Torulaspora delbrueckii*	99	MG773365.1
8	*Kluyveromyces marxianus*	99	JF715179.1
9	*Saccharomyces cerevisiae/paradoxus*	99	KY109393.1
10	*Pichia kluyveri*	100	MF788149.1
11	*Galactomyces candidum*	99	MF431584.1
12	*Candida parapsilosis*	100	MG271798.1
13	*Torulaspora delbrueckii*	99	MG773365.1
14	*Kluyveromyces marxianus*	99	JF715179.1
15	*Saccharomyces cerevisiae/paradoxus*	100	KY109407.1
16	*Candida orthopsilosis*	90	KJ817125.1
17	*Pseudozyma*	90	KC845938.1

Discussion

Our studies were carried out on traditionally fermented milks and artisanal yoghurts collected in Maroua, Garoua and Ngaoundere. The analysis of yeast profiles of the artisanal yoghurts revealed that there is a higher similarity between the samples collected in Maroua and Garoua. This could be due to the proximity of the two towns with similar climate, geographical proximity. There are 209 Km between Maroua and Garoua while 270 Km separate Garoua from Ngaoundere. A perfect similarity (100%) was not observed; this may be due to the fact that the process of production of these traditional yoghurts varies from one artisan to another and the absence of a standard starter for fermentation. Many authors [10,27]

reported that the fermented milks sold in Ngaoundere were of poor microbiological quality due to the fact that at any time and step, the product can be contaminated by yeast. This may justify the high load of yeast and molds ranging from 1×10^3 to 1×10^9 UFC/mL in the *pendidam* (a traditional fermented milk) collected in Ngaoundere [31]. The fact that the yoghurts from Ngaoundere have better quality than those from Garoua and Maroua could be due to the proximity with the politic and the economic capitals (respectively Yaounde and Douala) where it is easy to find good starters or industrial yoghurt of good quality that are used as a starter.

The analysis of yeast profiles of the traditionally fermented milk revealed that even if the profiles were specific to the different localities, some common bands were observed on the profile. The bands 1, 4, 7 and 9 (Figs. 1 and 2) represented respectively by *Malassezia globosa, Galactomyces candidum, Torulaspora delbrueckii* and *Saccharomyces cerevisiae/paradoxus* are common to almost all the sample sites. These species of yeast, naturally found in the environment can be brought into milk by contact during production. The yeast communities of traditionally fermented milks are greatly linked to the production environment.

The comparison of the traditionally fermented milks and artisanal yoghurts on the same PCR-DGGE banding patterns reveals that for a given location, there was a great similarity between the samples. This can be explained by the fact that the manufacturing processes almost the same with little variations due to locality. Usually, the cooled-pasteurized milk is poured in calabash for the fermentation. It is possible that the biofilm formed on porous wall of the calabashes influence greatly the composition of the yeast flora. Moreover, for this product instead of the use of a specific starter, the back-slopping method is usually used during fermentation.

It was also shown that there is not a great similarity between the DNA profiles of the artisanal yoghurts for a given locality and might be due to the variation in the processing methods used for this product between producers. Similar results were reported on fermented traditional milks in South Africa [5]. Moreover, the quality and the concentration of the starter might be not constant. The containers might also influence the presence of yeasts in the samples of fermented milks and yoghurts [31]. It might also be due to the acidophilic character of yeast with low sensitivity to the antagonistic activity of lactic acid bacteria. In many samples, the bands were revealed traducing that the yeast concentration was between 10^2 and 10^4 UFC/mL in the samples [6]. These concentrations corresponded to the value recommended by the standards ($<10^4$ CFU/mL) for some fermented milks like Kefir and Koumis, not for yoghurt [7].

Ten (10) genera with 12 species of yeast were identified from all the different excised bands of the PCR-DGGE gel patterns. Among these yeast species, some are naturally found in milk, in environment, some came from the hands of human workers. Some of these yeast species were among the potential pathogens for humans. These results are in agreement with those of Gadaga et al. [17] who reported that yeasts were present in various traditionally fermented milks from Sub-Saharan Africa. From 30 traditionally prepared Zimbabwean amasi, 20 different yeast species were isolated, predominantly including *Saccharomyces dairenensis, S. cerevisiae, Candida lusitaniae* and *C. colliculosa.* The majority (8/12) of these yeast species is considered as contaminants in these fermented milks analyzed because they are not added intentionally [18,35,46].

After using the culture dependant method of identification, [24] reported that many yeast species were identified on the locally Made Yoghurt (Shalom) Marketed in Dschang, Bafoussam (West) and Bamenda respectivelly in the West and Northwest Regions of Cameroon. Among these species, there are : *Candida kruzei/inconspicua, Candida boidinii, Candida dubliniensis, Candida zeylanoides, Candida lusitaniae, Candida albicans 1, Candida albicans 2, Rhodotorula mucilaginosa 1, Rhodotorula mucilaginosa 2, Stephanoascus ciferrii Trichosporon asahii Kodamae aohmeri, Pichia angusta, Cryptococcus laurentii, Cryptococcus humicola* and *Kloeckera* sp. These species are different from those obtained in our study because geographically, the sampling sites and the technique of identification used were not the same.

Normally, fungi less contaminates dairy products than other products, because they are refrigerated and are often produced with heated milk, and some of them are fermented products. Many authors like Prado et al. [36]; Akabanda et al. [1] and Desmasures [8] have shown that, some yeast are able to survive and grow in dairy products. Among them, *Candida, Galactomyces, Kluyveromyces* and *Saccharomyces* can be added as technological adjunct cultures to manufacture dairy products.

Fungal contamination of dairy milk products can occur at different stages, from dairy farms to dairy processing units and in consumers' homes. Independent of the animal species, raw milk generally contains between 3 and 5 log 10 CFU/mL fungi with higher number of yeast cells than fungal spores [25]. Raw milk collected by traditional technique is generally contaminated by yeast [29]. Normally, milk used is pasteurized both for traditionally fermented milks and artisanal yoghurts. If the milk is not well pasteurized, the product will be subjected to yeast contamination. The sources of yeast in milk and milk products are numerous. It begins with the teat surface, to the stable and milking parlor environments [43]. If the pasteurization is well done, a contamination by yeast may occur during manufacturing due to the dairy plant environment, equipment, ingredients and air [28,41]. Packaging materials can also be a source of yeast in milk and milk products (fermented milks and yoghurts).

Candida species have already been reported as responsible for dairy product spoilage. *Candida parapsilosis* has been the most frequently isolated species. Besides *Candida, Kluyveromyces, Galactomyces*, and *Saccharomyces* are frequent spoilers of fresh dairy products (fresh cheese, cream, and yoghurt) and heat-treated products. Yeast species can utilize lactose or galactose and assimilate lactic, citric and succinic acid, metabolize proteins and fats and can grow well at low temperatures [3].

Many of these yeasts are able to have an impact on human organisms so, the genus Candida is incriminated in more than 80% of yeast infections. It ranges from superficial infections, especially respiratory, digestive, and genital mucous membranes,

to deep localizations (pulmonary mycosis) and disseminated (septicemic mycosis). Among these yeasts, *C. albicans* is more involved, but *C. tropicalis* and *C. parapsilosis* are found with a lower incidence [20]. The presence of spoilage yeasts in the analyzed products is probably due to post-heat-treatment contaminations. These results are in agreement with those of Robinson [39]. The genera *Candida, Pichia* and *Torulaspora* are considered as dairy spoilage agents [19]. *Pseudozyma sp,* one of the identified yeasts was reported by some authors as pathogenic for humans [21,35]. It usually occurs in patients with some type of immunosuppression that predisposes opportunistic infections.

The three species of yeast represented by *Galactomyces candidum, Torulaspora delbrueckii and Saccharomyces cerevisiae/paradoxus* present in all samples of the 3 sampling localities, can be used as traceability markers for the traditional fermented milks from the northern part of Cameroon.

These results showed that it is interesting to use this molecular method (PCR-DGGE) to bring out the yeast strains found in some fermented milks in the northern part of Cameroun. This study is the first that describes yeasts associated with the production of fermented milks by using the molecular DGGE method. This study, performed by PCR-DGGE, describes the dominant species of yeast in the fermented milks. It could be strengthened by supplementing the DGGE method with analysis based on culture-dependent method to obtain a complete ecology of yeast strains in the fermented milks. Further research could be also done on some strains (*Galactomyces, Kluyveromyces* and *Saccharomyces*) that could be used as starter cultures for the benefit of the fermented milks, for Cameroonian producers.

Conclusion

The aim of this study was to investigate the diversity of dominant yeast flora in artisanal yoghurts and traditionally fermented milks in northern Cameroon by PCR-DGGE. The results revealed the presence of the genera *Malassezia, Hanseniaspora, Galactomyces, Candida, Aureobasidium, Torulaspora, Saccharomyces, Pichia, Kluyveromyces and Pseudozyma.* The diversity of yeasts in artisanal yoghurt was higher than in traditionally fermented milks. The presence of yeasts introduced accidentally in the different products and those potentially pathogen to human means that the analyzed products did not have a good microbial quality. To complete this study, it will be necessary to isolate the viable yeast flora from these fermented milk. It will also be important to know exactly at which moment the product is contaminated by yeast and to study the technological potential of these yeasts naturally found in fermented milk and, and their use as a starter for traditionally fermented milks.

Authorship conformation

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

Declaration of Competing Interest

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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