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## RESEARCH ARTICLE

# Molecular Identification of Yeasts and Lactic Acid Bacteria Involved in the Production of Beninese Fermented Food Degue

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### Abstract:

#### Background:

Traditional Beninese fermented food Degue is widely consumed in Benin and other countries in West Africa. It was originally made from milk and millet flour, but currently other cereals are used as well. Nowadays, Degue production occurs by spontaneous fermentation in individual households and information about the microorganisms involved is currently limited.

#### Objective:

The microbiota of Degue from Benin has not been studied so far, but its growing production in the country sets a demand for revealing the biodiversity of the microbial population involved in the fermentation process in order to take future steps for development of industrial technology and offer products with improved quality and safety.

#### Method:

In the present study, yeast and lactic acid bacteria from raw materials for Degue production and from several Degue products were isolated and identified by molecular methods including RFLP and ITS1-5.8S-ITS2 rRNA gene sequence analysis in yeasts, and 16S rRNA gene sequence analysis in lactic acid bacteria.

#### Results:

Lactic acid bacteria isolates were assigned to eight species within the genera *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus* and *Weisella*. Four species of yeasts were found in Degue: *Cyberlindnera fabianii*, *Candida glabrata*, *Kluyveromyces marxianus*, and *Meyerozyma caribbica*.

#### Conclusion:

The microbial population revealed is unique to Beninese Degue and needs further characterization for development of defined starter cultures.

**Keywords:** Degue, Benin, Identification, Yeast, Lactic acid bacteria, Molecular methods.

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## 1. INTRODUCTION

Fermentation processes are regarded as an important economical form of food production and preservation. Advantages of food fermentation include extended shelf-life of the products, improved palatability, digestibility, nutritive value, texture, taste and aroma resulting from the activity of the microorganisms involved [1 - 3]. The most common microorganisms found in fermented foods are yeasts and lactic acid bacteria (LAB). These organisms form stable mixed populations and the species composition depends on the raw materials used, geographical factors, preparation methods, production hygiene, *etc* [4 - 8]. Africa is regarded to be the continent with the richest variety of lactic acid fermented foods prepared from different raw materials [2, 9 - 12]. However, yeasts are also involved in these fermentations, but information on the dominant yeast species in African fermented foods is limited [13].

In Benin, a country in West Africa which is highly dependent on agriculture, fermented foods represent a major part of the daily diet and their production plays an important role in the Beninese domestic economy [14, 15]. Most fermented foods are prepared by spontaneous fermentations performed either at individual households or by small-scale companies using the locally available cereals maize, sorghum and millet, sometimes combined with milk [1, 4, 14 - 17]. Fermented products of these cereals are commonly consumed as refreshing beverages in the urban areas of Benin, especially appreciated during periods of extreme heat. One of these fermented beverages is Degue (Dèguè), which is also consumed in Burkina Faso and Mali. Degue varieties are produced by varying methods from mixtures of cereals with/or fermented milk [4, 8, 14, 15, 18, 19]. In Benin, due to the growth of street food consumption, production scale of this beverage has been rapidly increasing in the past few years. However, Degue production still occurs without any control of manufacturing parameters and hygiene practices [8, 15]. Information on the microbial species involved in the fermentation process is currently only available for Degue produced in Burkina Faso [4, 18, 20], while information related to microbiota of Beninese Degue extends mainly to enumeration of yeast and lactic acid bacteria populations [8, 14]. Therefore, an accurate identification of the microbial species involved in the production of different Degue types and understanding of their role in the mixed population is required in order to further develop defined starter cultures and industrial Degue products with improved quality and safety to meet the growing market demand. An industrial technology for the production of Degue may also be of interest to companies in Europe and North America aiming to diversify the food market by the introduction of new fermented products.

On this basis, the aim of the present study was to identify the naturally occurring yeast and LAB in cereal raw materials and milk used for the production of Degue, as well as in several types of Degue products.

## 2. MATERIALS AND METHODS

### 2.1. Samples for Isolation of LAB and Yeast

LAB and yeast strains were isolated from 26 samples of raw materials, combinations of them and Degue products, provided by the Laboratory of Microbiology and Food Technology, University of Abomey-Calavi (UAC), Benin (Table 1).

**Table 1. Raw materials and product samples for the isolation of LAB and yeasts.**

Sample	Sample
Yogurt nature	White sorghum grains
Fermented milk powder	Millet flour
Degue from millet	Maize flour
Degue from red sorghum	White sorghum flour
Degue from white sorghum	Red sorghum flour
Degue from maize	Reconstituted milk powder (80%) mixed with soy milk (20%) and fermented
Millet pellets	Fermented cow's milk
Maize pellets	Degue from millet and cow's milk
Red sorghum pellets	Degue from red sorghum and cow's milk
White sorghum pellets	Degue from white sorghum and cow's milk
Millet grains	Degue from maize and cow's milk
Maize grains	Degue Abokpan
Red sorghum grains	Degue Akpan

The cereal raw materials were maize (*Zea mays L.*), millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) from the local market in the city of Abomey-Calavi, Benin. "Yogurt nature" is a starter mix of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (label information), which has been bought at a local pharmacy in Abomey-Calavi. Cow's milk has also been purchased at the local market. Milk powder (LACSTAR, Ireland) has been bought from Cotonou. The mixed products and the several types of Degue were prepared at the Laboratory of Microbiology and Food Technology, UAC, Benin.

## 2.2. Isolation of Yeast and Bacteria

For the isolation of yeasts, a decimal dilution method was employed. Diluted samples were plated on Wort agar (Sigma-Aldrich, USA) and incubated at 30°C for 3 days.

Bacteria, and specifically LAB were isolated using the reference ISO 15214:1998 method [21] with minor modifications. A total of 10 g of each sample was homogenized with 90 ml sterile peptone water, serially diluted and plated on the surface of MRS agar (Oxoid, UK). The plates were incubated anaerobically at 30°C for 48 to 72h prior to colony enumeration.

Single yeast and bacterial colonies with different morphologies were individually selected for purification on Malt Extract and MRS plates, respectively. Yeast isolates were maintained on Malt Extract agar (Sigma Aldrich, USA) at 4°C, while bacterial isolates were maintained in MRS at 4°C (sub-cultured once a month) and in 20% glycerol for long-term storage at -20°C.

## 2.3. Phenotypic and Biochemical Characteristics of Yeast and Bacterial Isolates

The screening for yeast and LAB strains was based on cultural characteristics, cell morphology and biochemical tests. Cell morphology was examined by microscope (Laboval 4, Carlzeiss, Jena, Germany). Each bacterial isolate was Gram-stained, and only the Gram-positive ones were further tested for catalase production by placing a drop of 3% hydrogen peroxide solution on bacterial biomass. Based on the results, 8 yeast isolates and 30 Gram-positive and catalase-negative putative LAB isolates were further subjected to molecular identification.

## 2.4. Yeast Identification by Sequencing of ITS1-5.8S-ITS2 Region

Amplification of the ITS1-5.8S-ITS2 region was carried out by transferring biomass from a fresh colony with the tip of a sterile toothpick into a PCR tube containing 10 µl sterile deionized water. Then, 40 µl PCR reaction mix was added to the cell suspension. The PCR reaction mix contained 1 µM of ITS4 (5'-TCCTC-CGCTTATTGATATGC-3') and 1 µM of ITS5 (5'-GGAAGTAAAAGTG-CTAACAAGG-3') primers (Metabion, Germany), 1 mM dNTPs (Thermo Scientific, USA), 0.8 U Taq polymerase and 1 x PCR buffer (Thermo Scientific, USA) [22]. The amplification was carried out in a PCR 2720 Thermal Cycler (Biosystems, Germany) using the following program: initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and final extension at 72°C for 7 min. PCR products were visualized in 2% agarose gel stained with SafeView (NBS Biologicals, UK) at 100 V for 50 min using VWR Mini Electrophoresis System (VWR, Germany) and MiniBis Pro (DNR Bio-Imaging Systems, Israel) for gel visualization. GeneRuler 1kb plus (Thermo Scientific, USA) was used as the molecular marker. Fragment sizes were calculated using image editing software ImageJ64 (freeware).

The PCR products were further subjected to RFLP analysis by digestion with restriction enzymes Hae III, Cfo I and Hinf I (Thermo Scientific, USA) at 37°C for 4h. Restriction fragments were visualized on 2% agarose gel stained with SafeView at 100 V for 55 min, and fragment sizes were calculated using ImageJ64. The obtained RFLP profiles were used for confirmation of yeast identification, which was carried out by DNA sequencing (MacroGen Europe, Inc.). The nucleotide sequences were compared to the GenBank database using the BLAST software [23].

## 2.5. Extraction of Bacterial DNA

Total genomic DNA from putative LAB strains was extracted from overnight cultures, grown in MRS by using a previously optimized Chelex-100 method. Briefly, 18 h-culture of each isolate was centrifuged at 10 000 x g for 10 min (4°C), washed twice with DNase-free deionized water and re-suspended in 100 µl 6% Chelex (Bio-Rad Laboratories, Foster City, CA). The suspension was incubated at 56°C for 20 min, vortexed and subjected to boiling and freezing cycles for 8 min and 5 min, respectively. After centrifugation at 14 000 x g for 5 min (4°C), 80 µl of the supernatant was incubated with 20 µl Proteinase K (20 mg/mL) (Thermo Scientific, USA) at 65°C for 60 min. Cell debris was then

collected by centrifugation at 14 000 x g for 5 min (4°C), and the supernatant was stored at -20°C. Concentration and purity of the extracted DNA ( $A_{260}/A_{280}$ ) was measured by a spectrophotometer (Shimadzu UV-1800, Japan).

## 2.6. Molecular Identification of Bacteria by Sequencing of 16S rRNA Gene

PCR amplification of 16S rRNA gene for sequence analysis was performed in thermal cycler (Bio-Rad Laboratories Inc., Hercules, USA). Each PCR contained 10µM of each 27F – AGAGTTTGATCMTGGCTCAG and 1492R – TACCTTGTACGACTT primer [24], 200 ng DNA, 2.5 mM of each dNTP, and 1.25 U Takara Ex taq™ DNA polymerase (Takara Biotechnology Co. Ltd., China). Cycling conditions were set at an initial denaturation at 95°C for 3 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, elongation at 72°C for 90 s, and final elongation at 72°C for 5 min. PCR products were then visualized in 1% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen, USA) and 1 kb DNA ladder (Invitrogen, USA) was used as molecular marker. PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and sequenced with universal primer 27F. The nucleotide sequences were analyzed by BLAST, ClustalW and BioEdit software packages [25].

## 3. RESULTS AND DISCUSSION

A total of 42 yeast and bacterial isolates were obtained from cereal and milk samples used for the preparation of Beninese Degue, as well as from several product types. Isolates selection was based on colony morphology, microscopic cell observation and biochemical tests aiming to obtain different yeast and lactic acid bacteria. Eight yeast isolates were selected based on differences of cultural and morphological characteristics, and thirty isolates were tentatively assigned as LAB based on colony and cell characterization, Gram-positive and catalase-negative reactions. The yeast and LAB isolates were further subjected to molecular identification to the species level.

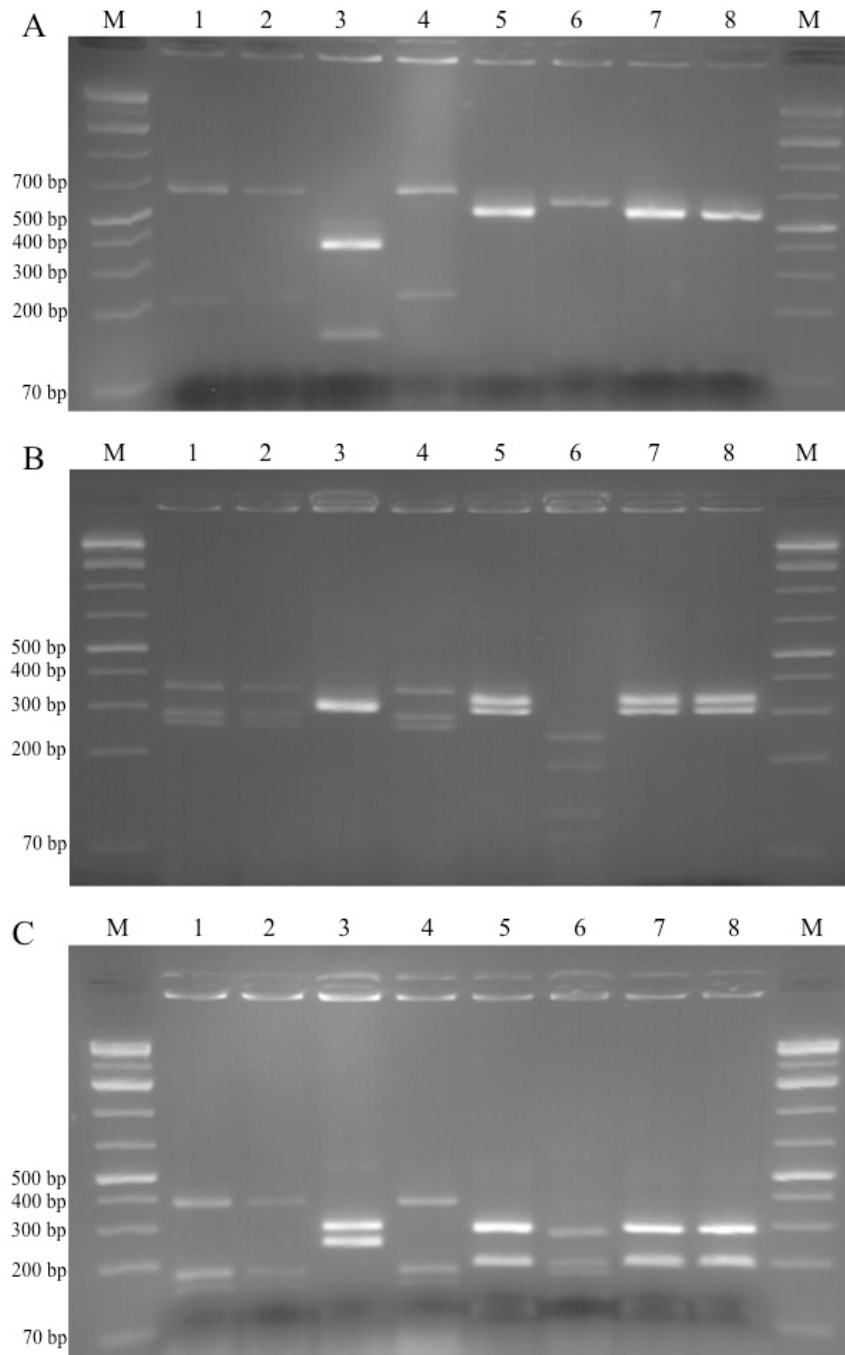
Molecular identification of the yeast isolates was carried out by PCR amplification of the ITS1-5.8S-ITS2 rRNA region [22]. The amplicons obtained were between 660 to 940 bp. Because of the differences in analytical conditions, none of the yeast isolates could be identified by RFLP profile comparison with previously published patterns of common yeast species [22, 26]. Strain identification based on RFLP profiles alone would require a pattern database obtained with a unified analytical method, as well as more detailed digestion with additional restriction endonucleases, such as AluI, DdeI, ScrFI, and TaqI as strain related pattern variability could occur within a species [26].

The obtained RLFP profiles of the yeast isolates were used to confirm identification performed by DNA sequencing. Results from the DNA sequencing showed 4 yeast species belonging to 4 genera - *Cyberlindnera*, *Candida*, *Kluyveromyces* and *Meyerozyma* (Table 2). Three of the isolates were identified as *Cyberlindnera fabianii* (formerly *Pichia fabianii*, *Hansenula fabianii*, and *Lindnera fabianii*). These yeast originated from corn and red sorghum pellets and from Degue prepared from millet pellets. *Kluyveromyces marxianus* was found in the Degue sample prepared from millet, and an isolate obtained from millet flour was identified as *Meyerozyma carribica* (formerly *Pichia carribica*).

**Table 2. Identification of yeasts from different raw materials and Degue products by RFLP and sequencing of ITS1-5.8S-ITS2 rDNA region.**

Product	Number of Isolates	PCR Product (bp)	Restriction Fragment Length (bp)			PCR Identification	Nearest Neighbour	Identity (%)
			Hae III	Hinfl	CfoI			
Degue from millet	2	660	560	320+300	315+230	<i>Cyberlindnera fabianii</i>	<i>C. fabianii</i> CNRMA8.1492	100%
		800	630	240+180+110+85	310+230+210	<i>Kluyveromyces marxianus</i>	<i>K. marxianus</i> WM 03.289	99%
Corn pellets	1	660	560	320+300	315+230	<i>Cyberlindnera fabianii</i>	<i>C. fabianii</i> CNRMA8.1492	99%
Red sorghum pellets	1	660	560	320+300	315+230	<i>Cyberlindnera fabianii</i>	<i>C. fabianii</i> CNRMA8.1492	99%
Millet grains	1	940	675+210	380+290+270	395+195+175	<i>Candida glabrata</i>	<i>C. glabrata</i> PUMY010	99%
Red sorghum grains	1	940	675+210	380+290+270	395+195+175	<i>Candida glabrata</i>	<i>C. glabrata</i> PUMY010	99%
Millet flour	1	675	380+145	320	325+280	<i>Meyerozyma carribica</i>	<i>M. carribica</i> PMM10-912L	100%
Degue from millet and cow's milk	1	940	675+210	380+290+270	395+195+175	<i>Candida glabrata</i>	<i>C. glabrata</i> PUMY010	99%

*Candida glabrata* isolates were recovered from millet and red sorghum grains and from Degue prepared with millet and cow's milk. The three isolates had identical PCR product size (940 bp), and the RFLP profile comparison showed identical patterns with all three restriction endonucleases (Figs. 1A, 1B, 1C - lanes 1, 2 & 4). This confirmed that the isolates were of the same species. Fig. (1) shows similar results with regards to *C. fabianii* isolates (lanes 5, 7 and 8).



**Fig. (1).** Restriction fragment length polymorphism of ITS1-5.8S-ITS2 region of rDNA using restriction enzymes HaeIII (A), HinfI (B), CfoI (C). Lanes 1, 2, 4 - *C. glabrata*, Lane 3 - *M. carribica*, Lanes 5, 7, 8 - *C. fabianii*, Lane 6 - *K. marxianus*.

The yeast species isolated in this study are commonly found in cereal and dairy products, as well as other food matrices from various regions in the world. *Kluyveromyces marxianus* was found in other traditional cereal-based fermented foods from Benin - the sorghum-based “gowe”, and in “mawe”, prepared from maize. In mawe it was identified along with *C. glabrata* in the initial fermentation stage of the product [6, 27]. It is interesting to note that *C.*

*glabrata* was not recovered from samples of the final products. *K. marxianus* has been predominant in dairy products such as the Spanish “Cabralés” cheese [22] and Central Asian “Koumiss” [28]. Lopandic *et al.* [29] isolated 71 strains of *K. marxianus* from dairy products available on the Austrian market. These findings show that *K. marxianus* is not limited to cereal-based fermented foods only, and same applies to *C. glabrata*. Padonou *et al.* [30] found *C. glabrata* in the African fermented cassava food “Lafun”, whereas Nyanga *et al.* [31] identified this yeast species during fermentation of masau fruits in Zimbabwe. *C. glabrata* was also identified in Bulgarian wheat-based fermented beverage “boza” [5]. *C. fabianii* was previously isolated from traditional Indian rice wine starter “hamei” [32], and Mukisa *et al.* [33] isolated *C. fabianii* from Ugandan traditional food “Obushera”, prepared from sorghum and millet.

In the present study *C. fabianii* was isolated along with *K. marxianus* from Degue from millet pellets. Pedersen *et al.* [34] also found both species in the Ghanaian food “Fura”, which, similarly to Degue, was prepared by spontaneous fermentation of millet and milk. *M. carribica* found in the present study in millet flour has previously been isolated by Leong *et al.* [35] from maize during storage in Cameroon. This species was also found in a variety of other matrices, such as the Brazilian fermented beverage “cachaça” [36], Ghanaian cocoa bean heap fermentation [37], phylloplane of sugarcane in Thailand [38], and olive fruits, paste, and pomace from Spain [39].

It is interesting to note that some yeast species, such as *Candida krusei*, *C. tropicalis* and *Saccharomyces cerevisiae*, commonly found in other cereal-based fermented foods from Benin and other geographical locations countries were not recovered from Degue [5, 6, 33, 34].

Sequencing of the 16S rRNA gene from each of the selected putative LAB isolates from raw materials and Degue samples revealed the presence of five genera. Specifically, eight species of *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus* and *Weissella* were identified Table (3). The predominant species was *Lactobacillus fermentum* (37% of all LAB isolates). It was isolated from the fermented milk powder, red sorghum flour and pellets, and maize flour, as well as in 6 of the 8 analyzed Degue types. An earlier study of Asmahan and Muna [39] focused on API 20 CHL identification of LAB involved in the preparation of the sorghum-based fermented food “kisra” in Sudan also revealed dominance of *Lb. fermentum*, and the same applied for African pearl millet slurries [40].

**Table 3. Identification of LAB from from different raw materials and Degue products by sequencing of 16S rRNA gene.**

Product	Number of Isolates	PCR Identification	Nearest Neighbour	Identity, %
Yogurt nature	3	<i>Lactobacillus plantarum</i>	<i>Lb. plantarum</i> BGG05-3	99
		<i>Lactobacillus plantarum</i>	<i>Lb. plantarum</i> SH5	97
		<i>Lactobacillus plantarum</i>	<i>Lb. plantarum</i> S4	98
Fermented milk powder	1	<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> strain TW27-2	98
Degue from millet	2	<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> JCM 7776	99
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> TW21-6	98
Degue from white sorghum	2	<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> LG1	99
		<i>Lactobacillus plantarum</i>	<i>Lb. plantarum</i> UNIFG122	98
Degue from maize	2	<i>Lactobacillus pentosus</i>	<i>Lb. pentosus</i> MP-10	97
		<i>Lactobacillus plantarum</i>	<i>Lb. plantarum</i> BGM40	99
Millet pellets	1	<i>Enterococcus mundtii</i>	<i>E. mundtii</i> APUIK-47	97
Maize pellets	1	<i>Enterococcus mundtii</i>	<i>E. mundtii</i> APUIK-47	97
Red sorghum pellets	2	<i>Weissella paramesenteroides</i>		99
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> TW27-5	100
Maize flour	3	<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> E10-15	98
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> TW27-2	99
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> TW27-2	98
White sorghum flour	1	<i>Pediococcus acidilactici</i>	<i>P. acidilactici</i> EL8-2	99
Red sorghum flour	2	<i>Pediococcus acidilactici</i>	<i>P. acidilactici</i> EL8-2	99
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> 21DCCH01MX	98
Reconstituted milk powder (80%) mixed with soy milk (20%) and fermented	1	<i>Lactobacillus rhamnosus</i>	<i>Lb. rhamnosus</i> AY675253	99
Fermented cow's milk	1	<i>Streptococcus thermophilus</i>	<i>S. thermophilus</i> TH1435	98
Degue from millet and cow's milk	2	<i>Lactobacillus pentosus</i>	<i>Lb. pentosus</i> MP-10	99
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> 17DCCH01MX	97

(Table 5) contd.....

Product	Number of Isolates	PCR Identification	Nearest Neighbour	Identity, %
Degue from red sorghum and cow's milk	1	<i>Lactobacillus rhamnosus</i>	<i>Lb. rhamnosus</i> SSU5	10
Degue from white sorghum and cow's milk	1	<i>Pediococcus acidilactici</i>	<i>P. acidilactici</i> EL8-2	98
Degue Abokpan	2	<i>Weissella paramesenteroides</i>		100
		<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> 17DCCH01MX	97
Degue Akpan	2	<i>Lactobacillus fermentum</i>	<i>Lb. fermentum</i> TW27-2	98
		<i>Weissella paramesenteroides</i>		99

The other species found in Degue products from Benin were *Lb. plantarum*, *Lb. pentosus*, *Lb. rhamnosus*, *P. acidilactici* and *W. paramesenteroides*. It is interesting to note that *Lb. fermentum* was the only species found in common with the bacterial microbiota of millet Degue from Burkina Faso [4]. This shows that despite the similarity in raw materials and production methods, the geography-related factors play a key role for the microbiota formation in Degue.

Similar microbial population of LAB was identified by conventional phenotypic and biochemical methods in other spontaneously fermented cereal-based foods from Africa [41]. These results also partly confirmed the findings of Adimpong *et al.* [42] and Ouoba *et al.* [43] studying indigenous African cereal-based fermented foods. Similar LAB species have been identified by biochemical tests in combination with AFLP analysis in South African sourdoughs [44]. Members of *Lactobacillus* and *Weissella* have also been found in Zambian fermented products based on maize, millet and/or sorghum by amplification of the V1 to V4 hypervariable regions on the 16S rRNA gene [7]. Owusu-Kwarteng *et al.* [45] and Soro-Yao *et al.* [19] reported predominance of *Lb. fermentum*, *Lb. plantarum*, *Lb. reuteri*, *Lb. salivarius*, *P. acidilactici*, *W. confusa* and *W. cibaria* in millet-based spontaneously fermented products in West Africa (Ghana and Cote d'Ivoire). By applying a combination of phenotypic and genotypic methods (API CHL kits) Nwachukwu *et al.* [46] and Oyedeji *et al.* [47] identified *Lb. plantarum*, *Lb. pentosus*, *Lb. cellobiosus*, *Ln. mesenteroides*, and *P. pentosaceus* in maize, millet and cassava used for the production of indigenous Nigerian cereal-based fermented foods "fufu" and "ogi", while lactic acid populations in sorghum-based drinks from Zimbabwe ("chibuku", a sorghum beer) and Uganda ("bushera") were found to consist of *Lb. plantarum*, *Lb. paracasei subsp. paracasei*, *Lb. fermentum*, *Lb. brevis* and *Lb. delbrueckii subsp. delbrueckii*, *Streptococcus thermophilus*, *Lactococcus lactis subsp. lactis*, *Lc. raffinolactis*, *Ln. mesenteroides subsp. mesenteroides*, *Ln. mesenteroides subsp. dextranicum* and *W. confusa* [48, 49].

The present study showed that the predominant microbiota in the fermented milk samples from Benin was presented by *Lb. plantarum*, *Lb. fermentum*, *Lb. rhamnosus* and *S. thermophilus*. These results partly comply with the findings of Nyambane *et al.* [50] who identified mainly *S. thermophilus*, *Lb. plantarum* and *Ln. mesenteroides* in traditional Kenyan fermented milk "amabere amaruranu". A larger species diversity was revealed by Mohammed and Ijah [51] using biochemical and phenotypic methods for identification of LAB in yogurt and milk products in Nigeria. They reported the presence of *Lb. bulgaricus*, *Lc. lactis*, *Lb. acidophilus*, *S. thermophilus*, *S. cremoris*, *Lc. lactis*, *P. halophilus* and *P. cerevisiae*. Similar results were obtained by Savadogo *et al.* [52] for identification of LAB from Burkina Faso fermented milks by using spacer region between 16S and 23S rRNA genes.

The comparison of LAB microbiota found in raw materials and Degue products from Benin with those of other similar products show that although individual species may be found in common, the combination of species in Beninese Degue is unique for this product.

## CONCLUSION

The present study is the first to explore the yeast and lactic acid bacteria diversity in Degue - a traditional cereal-based fermented beverage produced in Benin. Four yeast species were found in the samples of raw materials and various types of Degue - *C. fabianii*, *C. glabrata*, *K. marxianus* and *M. caribbica*, and identification of the LAB isolates revealed 8 species: *Lb. fermentum*, *Lb. plantarum*, *Lb. pentosus*, *Lb. rhamnosus*, *E. mundtii*, *P. acidilactici*, *S. thermophilus* and *W. paramesenteroides*. The differences of species diversity in comparison to other similar fermented foods suggest that microbial community structure in Degue is not a simple consequence of the nature of the raw materials used, but it is a result from a variety of abiotic and biotic factors affecting the natural selection of specific microbial populations - origin of the raw materials, climatic and geographical factors, preparation method and work environment, *etc.* Therefore, further studies on the microbiota of Degue products from various locations in Benin on the microbial dynamics throughout the various stages of Degue preparation and on the role of each species in the mixed population involved in Degue fermentation will form the basis for future development of defined starter cultures and for

establishment of an industrial technology for Degue production to meet the growing consumption in Benin by offering products with improved quality, safety and nutritional value.

#### LIST OF ABBREVIATIONS

AFLP	=	Amplified Fragment Length Polymorphism
C.	=	Candida
DNA	=	Deoxyribonucleic Acid
dNTP	=	Deoxynucleotide Triphosphate
E.	=	Enterococcus
K.	=	Kluyveromyces
LAB	=	Lactic Acid Bacteria
Lb.	=	Lactobacillus
Ln.	=	Leuconostoc
M.	=	<i>Meyerozyma</i>
MRS	=	de Man, Rogosa and Sharpe
P.	=	<i>Pediococcus</i>
PCR	=	Polymerase Chain Reaction
RFLP	=	Restriction Fragment Length Polymorphism
RNA	=	Ribonucleic Acid
rRNA	=	Ribosomal Ribonucleic Acid
S.	=	Streptococcus
W.	=	Weisella

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

#### HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

#### CONSENT FOR PUBLICATION

Not applicable.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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