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Lactic Acid Bacteria and Yeasts Associated with Cassava Fermentation to *attiéké* in Burkina Faso and Their Technological Properties

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Abstract Attiéké is an appreciate cassava fermented food introduced in Burkina Faso some decade ago. It has been spread out in all the country. The production is still traditional using a spontaneous way of fermentation. The present study aims to evaluate the main microorganisms groups associated with attiéké production and to identify yeast and Lactic Acid Bacteria (LAB) species according to biochemical (API 20 C AUX and API 50 CHL) and molecular (PCR) methods. Ninety-six (96) samples were collected from three (3) producers during four successive productions. LAB (1.34 up to 6.94 log CFU/g) are the main microorganisms associated to cassava fermentation to attiéké followed by yeasts (1.31 up to 6.25 log CFU/g). Thermo-tolerant Coliforms (1.56 log CFU/g) have been counted in traditional starter only while total coliforms were numbered in both traditional starter and attiéké (1.94 and 0.98 log CFU/g). *Candida kruzei/incospicua, Candida norvegensis, Candida parapsilosis, Candida rugose, Candida boidinii, Candida tropicalis, Saccharomyces cerevisiae, Trichosporon ashii* were identified as the main yeast species. *Enterococcus spp, Lactobacillus brevis, Leuconostoc mesenroides spp mesenteroides/dextranicum, Lactococcus lactis spp lactis, Leuconostoc mesenteroides spp mesenteroides/dextranicum, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus buchneri* were identified as LAB species associated. A difference between the biochemical and the molecular identification has been observed. The technological properties of these microorganisms need to be highlight for a selection of starter culture. The pH varies from 6.05 to 4.21 and the acidity from 0.5 to 4.8. The final cyanogen content is very low. The cyanogenic potential decrease from 8.6 to 0.07, free cyanogenic acid from 1.7 to 0.064 and the no cyanogenic glucogenic from 3.84 to 0.046. For an efficient fermentation of cassava to attiéké, there is then a need to select appropriate bacterial strains with starch fermenting properties to reduce the production time with high biochemical properties.

Keywords: *Attiéké, yeasts, lactic acid bacteria, technological properties*

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1. Introduction

Attiéké is a cassava (*Manihot esculenta*, Crantz) semolina with an agglomerated appearence obtained from peeled fresh cassava tubers which are cut, cleaned, grounded, fermented, wrung, granuled, sifted, pre-dried, zonked, firing steam. It's then a cassava fermented food [1]. *Attiéké* is originate from Ivory Coast and was previously produced by Avikams, Alladjans, Adjoukrous and Ebriés people groups [2,3,4,5]. Its production and consumption are now spread in many countries in West Africa and beyond. Firstly imported from Ivory Coast, attiéké is now produced in several areas in Burkina Faso [6]. There is sometimes a difference between attiéké produced in Burkina Faso and the Ivory Coast one. The spontaneous fermentation of cassava roots into attiéké follows a traditional way of process [7]. During the grating operation unit, the cassava dough is mixed with a traditional inoculum as starter culture called *magnan*. The inoculum is prepared by storing boiled or roasted cassava

roots for three days in a jute bag [7]. The microorganisms associated in cassava fermentation include a wide lactic acid bacteria and *Bacillus* species. Yeasts and moulds are also part of this fermentation process [8]. Some of these microorganisms are highly desired for their biochemical compounds, which preserve the food, enhance aroma and flavour and reduce anti-nutrients as well as cyanogen. The main microorganisms involved in cassava-fermented food have been described in others countries. However, the associated microorganisms with attiéké production in Burkina Faso is still undescribed. The climatic and environmental conditions as well as the cassava varieties used may have an impact on this microflora loads and diversity. There is also a need for interesting strains from spontaneous fermentation to be select as starter culture in order to control the quality of attiéké. The present study aims then to evaluate the microbial charge of the main products of the process and to identify the dominant microorganisms species associated with cassava fermentation into attiéké in Burkina Faso.

2. Material and Methods

2.1. Sampling

The inoculum or traditional starter culture called *magnan*, the fermented cassava dough and the final product *attiéké* of four successive production were sampled. Ninety six (96) samples were then collected from three producing units of different localities in Burkina Faso: Gaoua (Poni province, Sud-Ouest region), Banfora (Comoé province, Cascades region) and Orodara (Kénédougou province, Houet region). Samples were collected using sterile stomacher bags placed in icebox and transported in the microbiology laboratory of Département Technologie Alimentaire (DTA/IRSAT/CNRST), where the main analyses were realized.

2.2. Enumeration of Lactic Acid Bacteria (LAB), Yeasts and Molds and Enterobacteria

The preparation of samples and tenfold dilutions for inoculation were carried out on peptone buffer water according to [9]. For all enumerations, 10 g of each sample were homogenized with 90 mL of sterile pepton bufferwater in a stomacher bag. The tenfold serials dilutions were prepared and spread-plated for microorganisms counts. Yeasts and molds were cultivated on Sabouraud-Chloramphenicol Agar (Oxoid LTD, Basingstore, Hamsphire, England) after incubation at 25°C for 4 - 5 days and counted according to ISO 7954 [10]. Lactic acid bacteria were cultivated on De Man, Rogosa and Sharpe Agar (MRS, Merck 10660, Merck, Darmstadt, Germany) incubated anaerobically (anaerobic jar) at 37°C for 2-3 days and counted according to [11,12]. Enterobacteria were cultivated on violet red bile glucose agar (VRBG) and after incubation for 24h ± 2h at 37°C and 44°C respectively for total and thermotolerant coliforms, counted according to ISO 4832 [13].

2.3. Isolation and Initial Characterization of LAB

Microorganisms colonies were randomly picked and purified by successive streaking onto the corresponding agar as described by [14]. Morphological characteristics such as colony and cell aspects, forms, grouping mode, mobility were registered. The phenotypical characterization was based upon Gram test, catalase reaction and salt tolerance. Gram test was carried out using KOH (3%) as described by [15]; catalase production was determined by adding to a colony on a glass slide a drop of H_2O_2 solution (30%). For long-term storage, purified isolates were stored at − 80°C. The aerobic mesophilic bacteria were stored in Nutrient broth (Oxoid, Basingstoke, UK) containing 20% glycerol (w/w) and LAB were stored in MRS broth (Merck, Darmstadt, Germany) containing 20% (w/w) glycerol. Working cultures were kept at 4°C on MRS agar or broth and were then conserved at -20°C.

2.4. Initial Characterization of Yeasts in Liquid Medium

Each isolated yeast colony was introduced in tubes containing 10 mL of nutrient broth and incubated at 25°C for 3 days up to a week. The grown of yeasts along tubes, the development of a ring in the surface as well as the presence of a deposit on the bottom of the tubes and the turbidity are then noted and used for a primary grouping of associated yeasts in the fermentation.

2.5. Biochemical Identification of LAB and Yeasts Isolates

All the isolates were firstly grouped based on theirs colonies aspects, their forms, mobility, Gram test, catalase. Isolates from each group were randomly picked for the biochemical identification. API 50 CH gallery and API 20 C AUX gallery were used for the biochemical identification of LAB and yeasts respectively according to BioMérieux [16]. For yeasts identification, a cell culture of 18 up to 24 hours is homogenized in the physiological water .The optical density (OD) of the culture was adjusted to have an equivalent of 1 McFarland. Cupules were filled avoiding bubble formation and the gallery are incubated at 30°C for 48 up to 72 hours. The interpretation was based on the turbidity of the cupule and then compare to a witness. For LAB identification, a cell culture of 18 up to 24 hours was homogenized in an API medium. The optical density (OD) of the culture was adjusted to 2 McFarland. The cupules were filled avoiding bubble formation. Cupules were covered with parafin oil to create anaerobic conditions and then incubated at 37 °C for 24 to 48 hours. Positive cupules were those that coloration was different to the witness.

2.6. Molecular Characterization of Yeasts and LAB

The protocol of [17] was used for the molecular identification of the isolates. The total DNA was extracted

from bacterial culture of 18 up to 24 h, according to the thermal shock method [18]. A loupful of isolated colony of each bacterial was added to 100 µL of sterilized demineralized water and then firstly frozen at -20°C for about twenty minutes. Then, they were introduced in a water bath at 100 °C for ten minutes. The separation of the DNA from the other cell components was obtained by a centrifugation at 13 000 rpm for one minute. The supernatant containing the DNA was collected in PCR tubes. A volume of 2.5 µL of each extracted DNA was homogenized with 4 µL of the Master Mix PCR (5x FIREPol® Mster Mix Solis BioDyne, Estonia), 0.5 µL of each primers (forward and reverse) and 12.5 µL of demineralized water. The primers used are showed in table I. DNA fragments were separated by applying 10 µL of each PCR product in 2% (v/p) of agarose gel (SIGMA, Germany) for electrophoresis using electrophorus curve (APPELEX, France). The gel was prepared in 0.5X Tris Borate EDTA (TBE) buffer containing 100 mM of Trizma base, 100 mM of boric acid, 2 mM EDTA and 0.5 µg µL-1 of ethidium bromide (BET). A DNA molecular marker (Solis BioDyne, Estonia) of 100 pb was included as standard for the determination of the size of the fragments. The gel was runned in 0.5 XTBE buffer for 90 minutes at 100 V and 100 mA. The visualization of the strips was done with an ultraviolet light illumination (SYNGENE 1295, UK) and photographed. Primers used are in following Table 1.

2.7. pH and Acidity Variation

Ten gram (10 g) of each sample were dissolved in 50 ml of sterile peptoned buffered water and mixed. The pH was directly measured with a numeric pH-meter (WTW multi line P4). For Total acidity, 10 g of each sample are mixed with 50 ml of distilled water in an erlenmeyer. 10 mL of the dilution were then titrated against 0.1 N KOH using phenolphthalein as indicator; the total acidity was then calculated as a percentage of lactic acid.

2.8. Reduction of Cyanogen Content Base on Enzymatic Method

Both cyanogenic potential content (CNp), free cyanogen content and non-glycosidic cyanogen content was concerned with the cyanogen determination. The enzymatic method is used. After extraction followed an addition of 0.1 ml of linamarase (SIGMA Aldrich, UK) solution with 5 $EUml^{-1}$ as activity. Free cyanide content was quantified using 0.1ml of each extract. Then 3.9 ml of phosphate buffer pH 4 and 0.1 ml Choramine T (SIGMA Aldrich, UK) reagent was added. The absorbance at 605 nm was measured after 10 min of incubation. Non-glycosidic cyanogens was calculated as the difference between the total cyanogenic potential and the free cyanide content.

2.9. Biochemical and Technological Properties of the Associated Microorganisms

The biochemical and technological properties concerned bile tolerance, evaluated using Bile Esculin Agar, acid production based on Klingberg *et al*. (2005), their capacity to degrade starch using modified MRS agar which contain starch as only source of carbon. The evaluation of the acidification power of some strains made by the dosage of the acidity of the medium each 6h during 24h. Exopolysaccharides (EPS) production of LAB strains has been putted in evidence using the method of sowing in dials on MRS agar containing 5% of sucrose according to Sawadogo-Lingani *et al*., (2010). Pathogen reduction in traditional starter, fermented dough and attiéké is evaluation by enumerating total coliforms and fecal coliforms using standard methods [11].

2.10. Statistical Analysis

The data collection as well as the diagrams were performed with Excel version 2010. Analysis of variance and average were done using XLSTAT 2014.5.03.

Table 1. List of the select primers

	Primer	Sequence (5' – 3')	References
Candida krusei	CkFKSfor359 CkFKSrev359	CATTGGCCGTTTCCATTGTGTTC CATCAAACCAAGCGTGATTCTTGC	[19]
Saccharomyces cerevisiae	SC-5fw SC-3bw	AGGAGTGCGGTTCTTTCTAAAG TGAAATGCGAGATTCCCCCA	[20]
Lactobacillus sp.	LbF LbR	GGA ATC TTC CAC AAT GGA CG CGC TTT ACG CCC AAT AAA TCC GG	[21]
Leuconostoc sp	LnF LnR	GAT CCA TCT CTA GGT GAC GCC G CAC CGC TAC ACA TGG AG	[22,23]
Lactococcus sp	LcF LcR	CTT TGA GTG ATG CAA TTG CAT C CAC CGC TAC ACA TGG AG	[22,23]
Pediococcus	PdF PdR	GTA AAG TGG CGT GTG TAC CTC AAG CAC CGC TAC ACA TGG AG	[22,23]
Streptococcus spp.	StF Str	AGA GTT TGA TCC TGG CTC AG GTA CCG TCA CAG TAT GAA CTT TCC	[24]
Enterococcus sp.	EnF EnR	TAC TGA CAA ACC ATT CAT GAT G AAC TTC GTC ACC AAC GCG AAC	[25,26]
Bactéries lactiques		fDl (AGAGTTTGATCCTGGCTCAG) rDl (TAAGGAGGTGATC-CAGCC)	[22, 23]

3. Results

3.1. Average Loads of Associated Microorganisms

Lactic acid bacteria are the main bacteria associated with cassava fermentation. The fermented cassava dough showed the high level of microbial charge followed by *attiéké* and *magnan*, the traditional starter. The count of yeast and molds varied from 1.31 log CFU/g (traditional starter) to 6.25 CFU/g for fermented cassava dough with an average of 4.33 log CFU/g. The counts of LAB ranged between 1.34 and 6.94 log CFU/g with an average of 4.66. Total coliforms count ranged between 0.98 and 1.94 log CFU/g with an average of 0.97 and the charge of thermos-tolerant coliforms varied from < 1 to 1.56 log CFU/g with an average of 0.52 log CFU/g. The count of yeast and molds in the traditional starter *magnan*, is low compare to those obtained in the fermented cassava dough and *attiéké*. Thermo-tolerant coliforms were found only in the traditional starter. The high level of fermenting bacteria in fermented cassava dough is correlated with an absence of coliforms. The microbiological counts varied according to the producer but also according to productions in the same producing unit. The count of microorganisms in traditional starter, fermented cassava dough and attiéké were shown in Figure 1.

3.2. Basically Characteristics of Yeasts Involve in Cassava Fermentation

About hundred and two (102) isolates of yeasts were picked out from traditional starter (25 isolates), fermented cassava dough (39 isolates) and *attiéké* (38 isolates) of the three producers. These isolates were grouped based on their aspects on plate count agar, their cell form in microscopy, and their properties in nutrient broth. The properties in nutrient broth include the growth on the top or in the bottom of the tube (oxygen affinity), the density of turbidity and deposit in the bottom. Based on these properties, twenty-five yeast isolates were selected for the biochemical characterization.

3.3. Yeasts Identities Based on API 20 C AUX System

From the twenty one (21) isolates used for API 20 C AUX test and identification, height yeast species were found. They were seven *Candida kruzei/incospicua* (33.33% of isolates), seven *Candida norvegensis* (33.33%), two *Candida parapsilosis* (9.52%), one *Candida rugosa* (4.76%), one *Candida boidinii* (4.76%), one *Candida tropicalis* (4.76%), one *Saccharomyces Cerevisiae* (4.76%), one *Trichosporon ashii* (4.76%). The percentage of similarity ranged between 62.7 and 99.8%. *Candida krusei* and *Candida norvegensis* were the main yeasts species associated with cassava fermentation into *attiéké*. The characterized isolates are summarized in Table 2 and Table 3. Sugars are differently appreciate by yeasts. D-Glucose is the most used while pectin sugar, D-cellulobiose and D-Melezitose are less used.

The percentage of similarity of the isolates is ranged between 62.7 and 99.8 as it showed in table III. *C. kruzei/incospicua* (n=7) and *C. norvegensis* (n=7) are the most represented of yeasts species associated in cassava fermentation to attiéké.

There is a difference in the diversity of yeasts associated to attiéké production according to the nature of the samples. *Attiéké* was found to have the most diversity in yeasts compared to cassava fermented dough and the traditional starter. Some species was specific to the traditional starter (*T. ashii)*, some was specific to the fermented dough (*C. tropicalis*) and other to *attiéké* (*C. parapsilosis*, *C. rugose*, *C. boidinii*). This difference is highlight in the following Table 4.

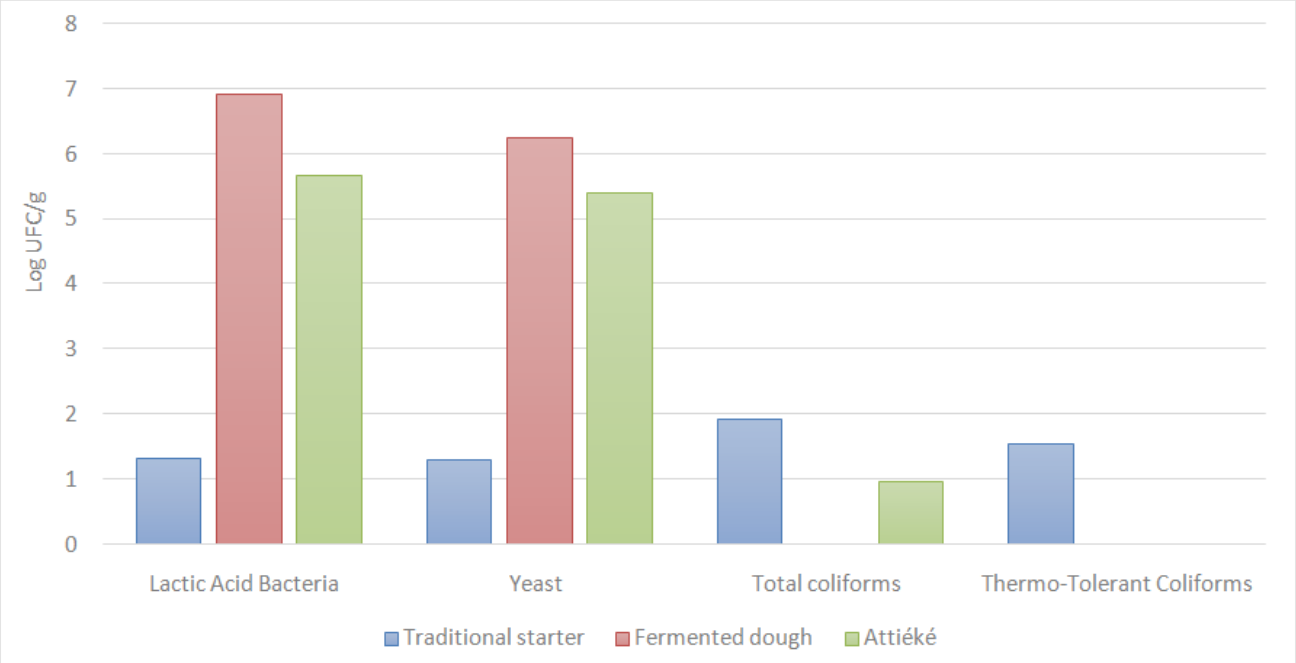

Figure 1. Microbial loads of cassava products

Table 2. Sugar assimilation profile of yeasts isolates based on API 20 C AUX

	L1370, L2366, L2130, L3307, L1357, L2314, L2199	L3243, L2318, L2194, L3301, L3308, L2215, L3306	L3312, L3309	L3300	L0014	L0011	L0013	L1355
D-Glucose	+	+	+	-	+	+	+	+
Pectin sugar	-	-	-	+	-	-	-	+
Xylose	+	+	-	+	+	+	-	+
Galactose	+	+	-	-	+	-	-	-
D-Cellulobiose	-	-	-	?	-	?	-	?
D-Lactose	-	-	-	-	-	-	-	-
D-Maltose	-	?	-	+	-	+	+	+
D-Sucrose	-	-	-	+	-	+	-	+
D-Trehalose	-	+	-	-	?	-	-	-
D-Melezitose	-	+	-	-	-	-	-	-
D-Rafinose	-	?	+	-	-	-	+	-
	Candida kruzei/incospicua	*Candida norvegensis*	*Candida parapsilosis*	*Candida Rugosa*	*Candida boidinii*	*Candida Tropicalis*	*Saccharomyces Cerevisiae*	*Trichosporon ashii*

Table 3. Yeasts identities according to API 20 C AUX test

Isolates code	Corresponding species	Similarity (%)	Percentage of isolates
L1370, L2366, L2130, L3307, L1305, L2314, L2199 (n = 7)	*Candida kruzei/incospicua*	*>98.60*	33.33
L3243, L2318, L2194, L3301, L3308, L2215, L3306 (n=7)	*Candida norvegensis*	*62.7- >98*	33.33
L3312, L3309 (n=2)	*Candida parapsilosis*	*99.80*	9.52
L3300 (n=1)	*Candida rugosa*	*96.20*	4.76
L3014 (n=1)	*Candida boidinii*	*98.20*	4.76
L2011 (n=1)	*Candida tropicalis*	*84.80*	4.76
L3013 (n=1)	*Saccharomyces cerevisiae*	*98*	4.76
L1355 (n=1)	*Trichosporon ashii*	*63*	4.76

Table 4. Yeast diversity according to the nature of the products

Species	Repartition (%)		
	Traditional starter	Cassava fermented dough	*Attiéké*
Candida kruzei/incospicua	28.57	57.14	28.57
Candida norvegensis	0	50	50
Candida parapsilosis	0	0	100
Candida rugosa	0	0	100
Candida boidinii	0	0	100
Candida tropicalis	0	100	0
Saccharomyces cerevisiae	0	0	100
Trichosporon ashii	100	0	0

Figure 2. Electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with Candida krusei Group Specific Primer, Lane M: 100-bp DNA molecular mass marker (hyperLadder IV), Lane T-: negative control, Lxxxx: strains code

3.4. Molecular Characteristics of the Yeasts Isolates Associated to the Fermentation of Cassava

The molecular characteristics aims to certificate previous characterization of isolates. The primers of *Candida krusei* and *Saccharomyces cerevisiae* used revealed that isolates L1370 L2366, L2130, L3307, L1355, L2314 and L2199 belong to *Candida krusei* species and isolate L0013 belongs to *Saccharomyces cerevisiae* specie. The hybridization of the primers and presumed *Candida krusei* DNA strain (359bp) are showed in the Figure 2.

3.5. Basically Characteristics of LAB Isolates

The morphological and physiological characterization of the LAB isolates collected from the traditional starter *magnan*, the fermented cassava dough and the *attiéké* samples showed that all the cells were Gram positive, catalase negative, no motile, spherical or rod (short or long) and grow at different temperatures (10°C, 25°C, 37°C and 45°C). Three hundred twenty (320) LAB isolates were collected.

3.6. LAB Identity According to API 50CHL

The biochemical characterization of the selected LAB using API 50 CHL revealed height (8) different species: *Lactobacillus brevis 1* (16.67%), *Lactobacillus fermentum 2* (5.55%), *Leuconostoc mesenroides spp mesenteroides/dextranicum2* (5.55%), *Lactococcus lactis spp lactis1* (27.77%), *Leuconostoc mesenteroides spp mesenterides/dextranicum1* (16.67%), *Lactobacillus fermentum* (15.55%), *Lactobacillus plantarum1* (11.11%), *Lactobacillus buchneri* (11.11%). The more representative LAB species according to the biochemical properties are *Lactococcus lactis spp lactis1* followed by *Leuconostoc mesenteroides spp mesenterides/dextranicum1* and *Lactobacillus brevis 1*. The percentage of similarity of these strains ranged between 75% and 99.90%. The biochemical profile of each strain according to their carbohydrates metabolism is showed in Table 5. L-Fucose, D-Fucose, L-Sorbose, L-Rhamnose, L-Xylose and D-Arabinose are not use as carbon source by any bacterial strain. L-Arabinose, D-Ribose, D-Galactose, D-Glucose, D-Maltose, D-Melibiose are used by all bacterial strain while other sugars are used differently (Table 5).

Table 5. Lactic acid bacteria profile according to their carbohydrates metabolism

	Bb24,	Bc17, Bg07, B119, B219, Bg01	B02b, B03b, B279	B255	B292	Bb25, Bb31, Bb28	B166-1, B102	Bb18, Bb12
D-Arabinose	-	-	-	-	-	-	-	-
L-Arabinose	+	+	+	+	+	+	+	+
D-Ribose	+	+	+	+	+	+	+	+
D-Xylose	+	-	+	+	+	+	-	+
L-Xylose	-	-	-	-	-	-	-	-
D-Galactose	+	+	+	+	+	+	+	+
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	?	+	+	+	+	+	+	+
D-Mannose	-	+	+	+	+	+	+	+
L-Sorbose	-	-	-	-	-	-	-	-
L-Rhamnose	-	-	-	-	-	-	-	-
D-Cellobiose	-	+	+	-	-	+	+	-
D-Maltose	+	+	+	+	+	+	+	+
D-Lactose	?	+	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+	+	+
D-Saccharose	+	+	+	+	+	+	-	+
D-Trehalose	-	+	+	+	+	?	-	-
D-Mélézitose	-	-	-	-	-	+	+	-
D-Raffinose	+	-	+	+	+	+	+	+
Amidon	-	-	-	-	-	-	-	-
D-Turanose	-	-	+	+	+	+	+	+
D-Lyxose	-	-	-	-	-	-	-	-
D-Tagatose	-	+	+	-	-	+	-	-
D-Fucose	-	-	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-	-	-
	Lactobacillus fermentum 1	*Lactococcus lactis spp lactis1*	*Leuconostoc mesenteroides spp mesenteroides/ dextranicum1*	*Leuconostoc mesenteroides spp mesenteroides/ dextranicum2*	*Lactobacillus fermentum 2*	*Lactobacillus brevis1*	*Lactobacillus plantarum1*	*Lactobacillus buchneri*

Table 6. LAB identity according to API 50 CHL

Isolates	corresponding species	Similarity (%)	Isolate (%)
Bb24,	*Lactobacillus fermentum 1*	*>81.20*	*5.55*
Bc17, Bg07, B119, B219, Bg01	*Lactococcus lactis spp lactis1*	*>79*	*27.77*
B02b, B03b, B279	*Leuconostoc mesenteroides spp mesenteroides/ Dextranicum1*	*>79*	*16.67*
B255	*Leuconostoc mesenteroides spp mesenteroides/ dextranicum2*	*>76*	*5.55*
B292	*Lactobacillus fermentum 2*	*75%*	*5.55*
Bb25, Bb31, Bb28	*Lactobacillus brevis1*	*99.70%*	*16.67*
B166-1, B102	*Lactobacillus plantarum1*	*99.90*	*11.11*
Bb18, Bb12	*Lactobacillus buchneri*	*92.90*	*11.11*

The identity of the selected LAB isolates according to API 50 CHL is as presented in Table 6. The percentage of similarity is ranged between 75 and 99.90 percent.

3.7. Molecular Characteristics and Identity of LAB Associated in Cassava Fermentation into Attiéké

The molecular identification was carried on to certificate the identity of the associated microorganisms previously performed according to API, based on biochemical properties. The LAB strains is firstly used to confirm that all isolates LAB are really LAB. Then, specific species strains are used for the identification. Figure 3 shows the molecular identification of LAB strains. LAB strains previously characterize reveal themselves to really LAB as it showed in Figure 3.

For *Lactobacillus spp*. Identification, *Lactobacillus spp* group specific primer was used. The isolates Bb25, Bb31, B102, Bb28, Bb12, Bb24 that are been formally identified as LAB were found to belong the genus *Lactobacillus spp*. They was previously identify as *Lactobacillus* in biochemical level (API 50 CHL). Figure 4 shows the electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with *Lactobacillus spp*.

Enterococcus spp. group specific primer is used for *Enterococcus spp* identification. Two isolates (B258 and B214) were identified as *Enterococcus spp*. They was previously identify as *Enterococcus spp* according to API 50 CHL results. The electrophoresis gel analysis of PCR-amplified 16S rDNA of *Enterococcus spp*. Is showed in Figure 5.

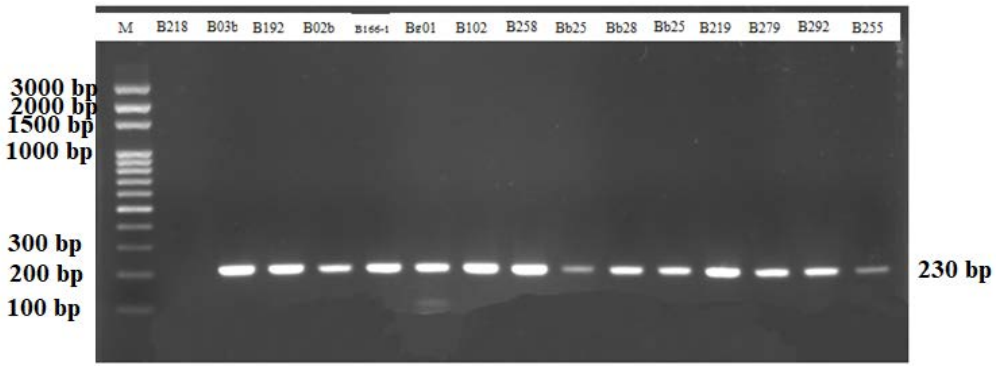

Figure 3. Electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with LAB Group Specific Primer, Lane M: 100-bp DNA molecular mass marker (hyperLadder IV), Bxxx: strains code

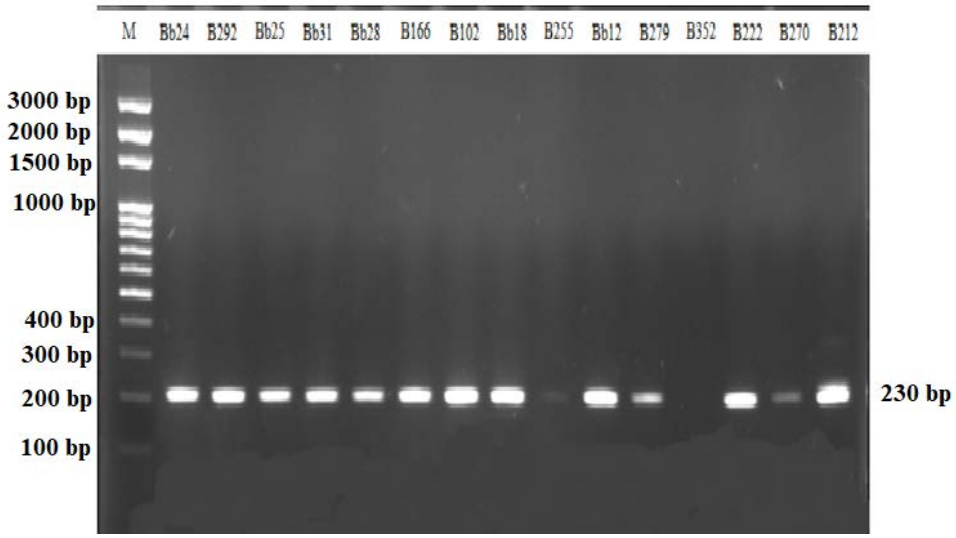

Figure 4. Electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with *Lactobacillus spp* Group Specific Primer, Lane M: 100-bp DNA molecular mass marker (hyperLadder IV), Lane T-: negative control, Bxxx: strains code

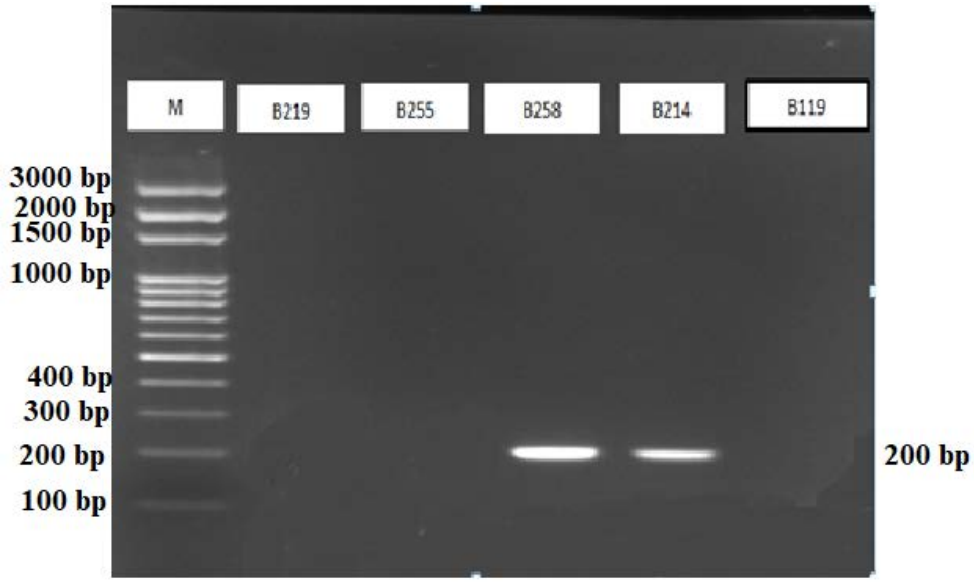

Figure 5. Electrophoresis gel analysis of PCR-amplified 16S rDNA fragments obtained with Enterococcus spp. Group Specific Primer, Lane M: 100-bp DNA molecular mass marker (hyperLadder IV), Lane T-: negative control, Bxxx: strains code

3.8. The Technological Properties of the Associated Microorganisms

Some physiological properties such as Gram, catalase, form, mobility, grouping mode, growth at 25°C, 37°C and 45°C was firstly use for a basic grouping of associated microorganisms in cassava transformation to attiéké. It from those groups that forty (40) lactic acid bacterial isolates was picked up for the technological properties evaluation.

Table 7. Technological properties of some bacterial strains

Strains code	EPS Production	Starch Fermentation	Sucrose Fermentation	Bile tolerance	Gas Production	NaCl 6.5%	NaCl 18%	pH<5.3 at 6h	pH< 4.8 at 12h	pH<4.2 at 18h	pH<3.85 at 24h
B413	-	-	+	+	-	+	+	-	-	+	+
B415	-	-	+	+	-	+	+	+	-	-	-
B424	-	-	+	-	-	+	+	+	+	+	-
B119	-	-	+	-	+	+	+	-	-	+	+
B272	-	-	+	-	-	+	+	+	+	+	+
B102	-	-	+	-	-	+	+	-	-	-	-
B192	-	-	+	-	-	+	+	-	-	+	+
B222	-	-	+	-	-	+	+	+	+	+	+
B228	-	-	+	-	-	+	+	+	+	+	-
B466	-	-	+	±	-	+	+	-	+	+	-
B464	-	-	+	+	-	+	+	-	-	-	-
B462	-	-	+	+	-	-	-	-	+	+	+
B480	-	-	+	+	-	+	+	-	+	+	-
B402	-	-	+	-	-	-	-	-	-	-	-
B012	-	-	+	-	-	+	+	-	+	-	+
B707	-	-	+	-	-	+	+	-	-	-	+
B025	-	-	+	±	-	+	+	-	-	+	-
B701	-	-	+	+	-	+	+	-	-	-	-
B102	-	-	+	-	+	+	+	+	+	-	-
B018	-	-	+	-	+	+	+	+	-	+	+
B337	-	-	+	-	-	+	+	-	-	+	-
B383	-	-	+	+	-	+	+	-	+	-	-
B389	-	-	+	+	-	+	+	-	+	-	-
B385	-	-	+	+	-	+	+	-	-	-	+
B339	-	-	+	+	-	+	+	+	-	+	+
B373	-	-	+	+	+	+	+	+	-	+	-
B378	-	-	+	-	-	+	+	+	+	-	-
B352	-	-	+	-	+	+	+	+	+	+	-
B350	-	-	+	-	+	+	+	-	+	-	+
B501	-	-	+	-	+	+	+	+	-	+	+
B384	-	-	+	+	+	+	+	-	-	+	-
B351	-	-	+	+	+	+	+	-	+	-	-
B017	-	-	+	+	-	+	+	+	+	+	+
B219	-	-	+	-	-	+	+	-	-	-	-
B220	-	-	+	-	+	+	+	-	-	-	+
B511	-	-	+	-	+	+	+	+	+	-	+
B513	-	-	+	±	+	+	+	+	+	+	-
B516	-	-	+	-	+	+	+	-	+	+	+
B504	-	-	+	±	+	+	+	+	-	+	+

The technological properties concerned are their capacity to use the main carbohydrates in cassava as carbon source, their capacity to reduce cyanogen content level in the final product, to growth in particular conditions, to produce EPS and to preserve food spoilage throughout antimicrobial activity. All the selected microorganisms are EPS negative, use sucrose as carbon source but none use starch. The others properties depend on each isolate as presented in following Table 7.

3.9. Acid Production during Fermentation

During cassava fresh root fermentation to attiéké, pH decrease significantly when acidity increase. The pH vary from 6.05 to 4.21 and the acidity from 0.5 to 4.8 as showed in the following Figure 6.

3.10. Cyanogen Reduction

The cyanogen content is also significantly reduce during the fermentation. The final cyanogen content is very low. The cyanogenic potential (CNP) decrease from 8.6 to 0.07 ppm, free cyanogenic acid (HCN) from 1.7 to 0.064 ppm and the no cyanogenic glycogenic (NGC), from 3.84 to 0.046 ppm. The decrease curve is as showed in the following Figure 7.

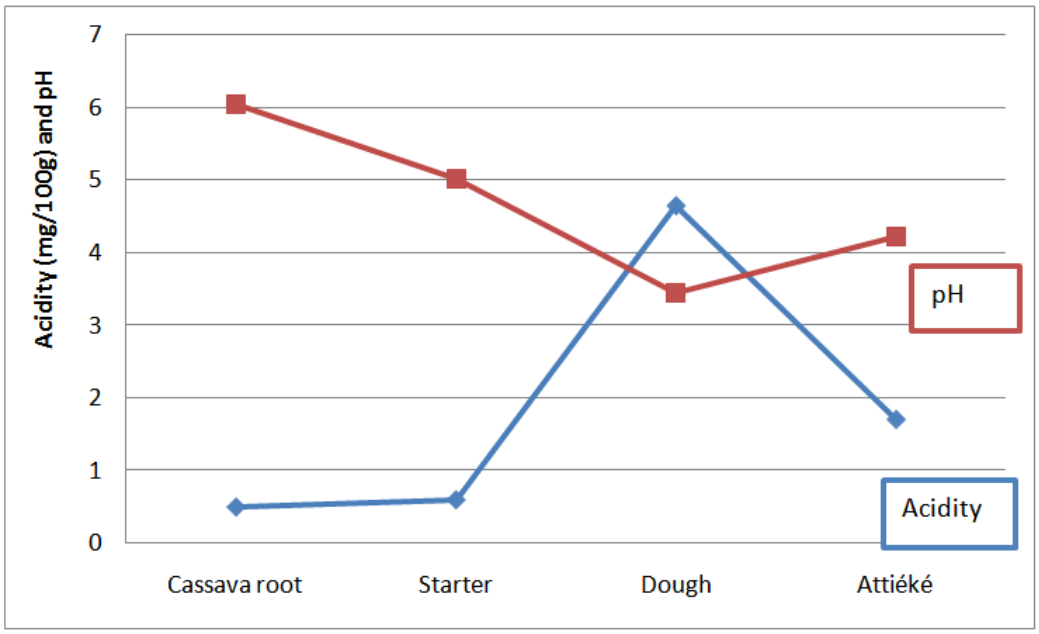

Figure 6. Variation on pH and acidity during attiéké fermentation

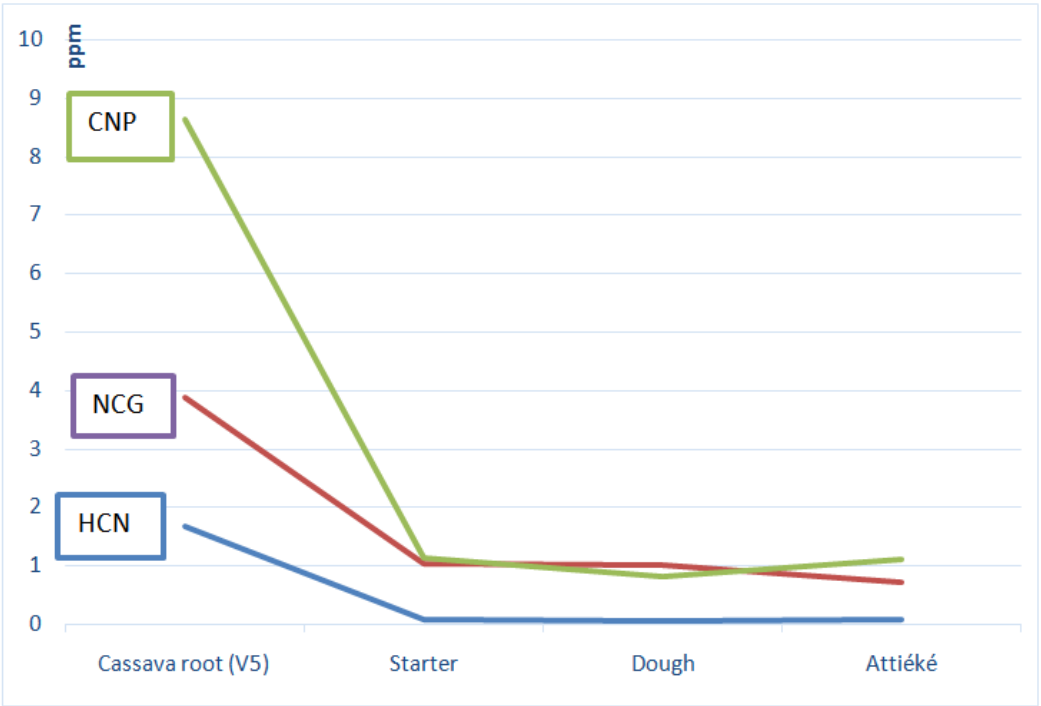

Figure 7. Cyanogen content reduction during cassava fermentation to attiéké

4. Discussion

The microorganisms counts, varied according to the type of products (Traditional starter, cassava fermented dough and *attiéké*). It may be tribute to the hygienic conditions of the process, their nutrient content, their water activity and their physicochemical parameters. The traditional way of the process may be the main factor of the variation of microorganisms counts according to both production and producer. These factors may also explain the difference in the diversity according to the products. The absence of thermo-tolerant coliforms in the final products attiéké could be the result of the fermentation effect that increase the acidity of the product, avoiding undesirable bacterial growth. The results are similar to those found previously by [7]. The content of microorganisms in the inoculum (the traditional starter) is less than those obtained in Ivory Coast by [2] which was 7.75 to 9.23 log CFU for LAB and 7.74 to 8.30 log CFU for yeasts. . There is a diversity of the microorganisms associated to attiéké production. Spontaneous fermentation is the main way of cassava fermentation to produce attiéké even in Ivory Cost [2,27,28,29]. Yeasts species identified in this study are diversified (*Candida kruzei/incospicua, Candida norvegensis, Candida parapsilosis, Candida rugose, Candida boidinii, Candida tropicalis, Saccharomyces cerevisiae, Trichosporon ashii*). There is also a difference in the identities of the species between the biochemical and the molecular methods used. The difference between molecular and biochemical identification has been also found in previous study [30]. However, the few number of specific yeast primers of molecular identification could also explain this difference. The spontaneous nature of the fermentation influences on the diversity of yeasts involved in cassava fermentation into attiéké. Yeasts contribute to synthetize volatile organic compounds and unicellular protein in fermented food [31,32,33,34]. But, yeasts also contribute to food spoliation [35]. Some yeasts species such as *Candida validated, Candida holmii, Candida krusei, Kloeckera japonica, Saccharomyces cerevisiae* were previously identified in traditional starter of *attieke* made in three villages in Ivory Coast. However *Candida tropicalis* was the only yeast species identified to be associated to *attiéké* fermentation according to Coulin *et al*., (2006). This difference in the diversity of yeast strains in *attiéké* production may be attribute to many factors. The main factors are the environmental and climatic conditions of production zones [36,37], the mastery of the fermentation process by producers as well as the spontaneous nature of fermentation [7,38,39] the variety of cassava used. On average, yeasts were more representative in fermented doughs and in attiéké than in the traditional starter because of the water activity.

There is also a diversity in LAB among the microorganisms associated with cassava fermentation into attiéké. However, the primers used in the molecular identification were not so specific to species. There is a need of DNA sequencing for a formal identification. Previous studies on the main microorganisms associated with cassava fermentation into attiéké in Ivory Coast found the following species: *L. plantarum, L. fermentum, L. cellobiosus, L. brevis,L. mesenteroides, P. acidilactici, W. cibaria,; Lactobacillus sp; E. faecium* [2,40,41]. A spontaneous fermentation of cassava in Nigeria revealed *L. plantarum, L. rhamnosus, L. hilgardii, L. paracasei, W. paramesenteroides, L. mesenteroides, E. faecium, E. casseliflavus, P. acidilactici*, as main lactic acid bacteria [42]. There is then a difference in the microbial diversity during cassava fermentation according to areas. The climatic and environmental conditions have an impact on the microbial diversity in cassava fermentation. Djeni *et al.* (2015) have also confirmed this hypothesis during their investigation among attiéké producers in three localities in Ivory Coast. The concerned communities have some specificities in the production process of *attiéké*. The same cassava variety was used for a spontaneous fermentation. The mastery of the production process especially the fermentation step has an impact on the microbial diversity, biochemical, nutritional and sanitary parameters as it has been already observed (Guira *et al*., 2016a). A high level of *attiéké* production then requires the quality management in units, the mastery of the all production process and specifically the fermentation one. A selection of specific microorganisms with pectinolytic, polygalacturonase enzyme activity for cassava fermentation into attiéké is necessary for more valorization of this fermented food [31,43,44,45,46,47].

The fermentation lead to a great reduction of cyanogen content in the final product (*attiéké*). The acidity of the environment and the linamarase activity contribute to the reduction of the cyanogen content. The impact of the fermentation on the cyanogen reduction have been already proved in previous studies [8,31,48,49,50,51]. The residual content of cyanogen in attiéké is neglected. The variation in the acidity between cassava root and attiéké is important. But the decrease on the acidity is low compare to the Ivorian and the Beninese one [29,52]. The technological properties of the associate microorganisms explain the kinetic profile. In MRS nutrient broth the pH decrease in to lower than in cassava dough. Not all the isolated LAB strains are fermenting starch and more of them don't ferment sucrose. On the other hand, starch in the main carbohydrate in cassava. The no fermentation of cassava lead to a long time of the fermentation. Ivorian producer spend few time in the fermentation process than burkinabé because some of the isolated strains in Ivorian *attiéké* production process can use starch and sucrose as carbohydrate source [2,40]. For an efficient fermentation of cassava to *attiéké*, there is then a need to select appropriate bacterial strains with starch fermenting properties to reduce the production time with a high biochemical properties [8,53,54]

5. Conclusion

A diversity of microorganisms including yeasts and LAB are associated to the fermentation of cassava into attiéké. *Candida* spp. is the most dominant yeast whereas *Lactobacillus spp, Lactococcus spp, Enterococcus spp, Leuconostoc spp* are the main LAB. The environmental conditions and the physicochemical parameters may have been influenced the microbial diversity. A formal identification throughout DNA sequencing is needed as well as the technical and nourishing profile of each strain. The fermentation process contribute to reduce

significantly le cyanogen content of the cassava fresh root. The spontaneous nature of the process leads to uncontrolled results of the final product. The unfermentation of starch by associate spontaneous microorganisms require the use of starter with specific bacterial strains which have high technological properties for quality attiéké production.

Conflict of Interest

The authors declare any conflict of interest.

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Ethical Statements

This study does not involve any human or animal testing.

References

[1] CODINORM, *Attiéké-spécifications.*, NI4511, Editor. 2006, CODINORM: Abidjan, Côte d'Ivoire. p. 5.

[2] Assanvo, J., et al., *Microflora of traditional starter made from cassava for "attiéké" production in Dabou (Côte d'Ivoire).* Food control, 2006. 17(1): p. 37-41.

[3] Firmin, A., *Optimum conditions for cooking attiéké.* Tropical science, 1998.

[4] Tchekessi, C.K., et al., *Production and microbiological evaluation of three types of "Dèguè", a local fermented drink made from milk in Benin* International Journal of Multidisciplinary and Current Research 2014. 2: p. 714-720.

[5] Tetchi, F.A., *Effect of cassava variety and fermentation time on biochemical and microbiological characteristics of raw artisanal starter for attiéké production.* Innovative Romanian food biotechnology, 2012. 10: p. 40.

[6] Guira, F., et al., *Origins, production, and utilization of cassava in Burkina Faso, a contribution of a neglected crop to household food security.* Food Science & Nutrition, 2016a.

[7] Guira, F., et al., *Hygienic Quality and Nutritional Value of Attiéké from Local and Imported Cassava Dough Produced with Different Traditional Starters in Burkina Faso.* Food and Nutrition Sciences, 2016b. 7(07): p. 555.

[8] Guira, F., A. Tankoano, and A. Savadogo, *African cassava Traditional Fermented Food: The Microorganism's : Contribution to their Nutritional and Safety Values-A Review.* International Journal of Current Microbiology and Applied Sciences, 2016c. 5(10): p. 664-687.

[9] 6887, I., *Microbiologie des aliments- Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique. Partie 1: règles générales pour la préparation de la suspension mère et des dilutions décimales*. 1999: France. p. 5.

[10] Norme, N., *ISO 7954 (1988).* Directives générales pour le dénombrement des levures et moisissures.

[11] ISO 11290-2, O.I.d.N., *Microbiology of food - horizontal Method for the enumeration of the mesophilic lactic acid bacteria - Technical by counting of the colony at 30°C.*, in *French standard* 1998, ISO. p. 7.

[12] ISO, O.I.d.N., *Cereales, legumineuses et produits derives. Dosage du taux de cendres par incineration*. 2007, ISO. p. 8.

[13] ISO 4832, O.I.d.N., *Microbiology of food*, in *Horizontal method for the enumeration of the coliform. Method by counting of the colonies*. 2006, ISO. p. 6.

[14] Padonou, S.W., et al., *The microbiota of Lafun, an African traditional cassava food product.* International journal of food microbiology, 2009. 133(1): p. 22-30.

[15] Gregersen, T., *A rapid method for distinction of Gram-negative from Gram positive bacteria.* Eur. J. Appl. Microbiol. Biotechnol., 1978. 5: p. 123-127.

[16] BioMérieux, *Api 20 C AUX: sysrtème d'identification des levures.* 2007.

[17] Giraffa, G. and E. Neviani, *Molecular identification and characterization of food-associated lactobacilli.* Italian journal of food science, 2000. 12(4): p. 403-423.

[18] Cattoir, V. *Identification moléculaire des mycobactéries et détection de la résistance aux antibiotiques*. in *Annales de Biologie Clinique*. 2004.

[19] Brillowska-Dąbrowska, A. and A. Siniecka, *Molecular detection of Candida krusei.* RED. ZAGR. ANGIELSKI, 2012. 3: p. 275-277.

[20] Perez-Gallardo, R.V., et al., *Reactive oxygen species production induced by ethanol in Saccharomyces cerevisiae increases because of a dysfunctional mitochondrial iron–sulfur cluster assembly system.* FEMS yeast research, 2013. 13(8): p. 804-819.

[21] Abdulamir, A., et al., *Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities.* African Journal of Biotechnology, 2010. 9(10): p. 1481-1492.

[22] Heilig, H.G., et al., *Molecular diversity of Lactobacillus spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA.* Appl. Environ. Microbiol., 2002. 68(1): p. 114-123.

[23] Savadogo, A., et al., *Identification of exopolysaccharides-producing lactic acid bacteria from Burkina Faso fermented milk samples.* African Journal of Biotechnology, 2004. 3(3): p. 189-194.

[24] Karsidani, S.H., et al., *Molecular epidemiology of zoonotic streptococcosis/lactococcosis in rainbow trout (Oncorhynchus mykiss) aquaculture in Iran.* Iranian journal of microbiology, 2010. 2(4): p. 198.

[25] Ammor, M.S., A.B. Flórez, and B. Mayo, *Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria.* Food microbiology, 2007. 24(6): p. 559-570.

[26] Jamaly, N., et al., *Characterization of Enterococci isolated from Moroccan dairy products.* African Journal of Microbiology Research, 2010. 4(16): p. 1768-1774.

[27] Djeni, N., et al., *Quality of attieke (a fermented cassava product) from the three main processing zones in Côte d'Ivoire.* Food Research International, 2011. 44(1): p. 410-416.

[28] Djéni, T.N., et al., *Process of attieke production in Côte d'Ivoire: new trends, updates and effects on quality and preference of the food.* International Journal, 2014. 2(8): p. 644-653.

[29] Djéni, T.N., et al., *Assessment of knowledge, attitudes and practices of food handlers in Attieke production units in relation to food hygiene and safety in Côte d'Ivoire in 2012.* Food and Nutrition Sciences, 2014. 5(10): p. 896.

[30] Dušková, M., et al., *Identification of lactobacilli isolated from food by genotypic methods and MALDI-TOF MS.* International Journal of Food Microbiology, 2012. 159(2): p. 107-114.

[31] Freire, A.L., C.L. Ramos, and R.F. Schwan, *Microbiological and chemical parameters during cassava based-substrate fermentation using potential starter cultures of lactic acid bacteria and yeast.* Food Research International, 2015. 76: p. 787-795.

[32] Panda, S.K. and R.C. Ray, *Fermented Foods and Beverages from Tropical Roots and Tubers.* Tropical Roots and Tubers: Production, Processing and Technology, 2016: p. 225.

[33] Rai, A.K., et al., *Production of bioactive protein hydrolysate using the yeasts isolated from soft chhurpi.* Bioresource Technology, 2016. 219: p. 239-245.

[34] Tamang, J.P., N. Thapa, and T.C. Bhalla, *Ethnic Fermented Foods and Beverages of India*, in *Ethnic Fermented Foods and Alcoholic Beverages of Asia*. 2016, Springer. p. 17-72.

[35] Oro, L., et al., *Evaluation of damage induced by Kwkt and Pikt zymocins against Brettanomyces/Dekkera spoilage yeast, as compared to sulphur dioxide.* Journal of applied microbiology, 2016(121): p. 207-214.

[36] Drumonde-Neves, J., et al., *Yeast Biodiversity in Vineyard Environments Is Increased by Human Intervention.* PloS one, 2016. 11(8): p. e0160579.

[37] Jara, C., et al., *Microbial terroir in Chilean valleys: Diversity of non-conventional yeast.* Frontiers in microbiology, 2016. 7.

[38] Ramos, C.L., et al., *Microbiological and chemical characteristics of tarubá, an indigenous beverage produced from solid cassava fermentation.* Food microbiology, 2015. 49: p. 182-188.

[39] Rebouças, K.H., et al., *Evaluating Physicochemical and Rheological Characteristics and Microbial Community Dynamics during the Natural Fermentation of Cassava Starch.* Journal of Food Processing & Technology, 2016. 2016.

[40] Coulin, P., et al., *Characterisation of the microflora of attieke, a fermented cassava product, during traditional small-scale preparation.* International journal of food microbiology, 2006. 106(2): p. 131-136.

[41] Djeni, N., et al., *Biochemical and microbial characterization of cassava inocula from the three main attieke production zones in Côte d'Ivoire.* Food Control, 2015. 50: p. 133-140.

[42] Anyogu, A., et al., *Molecular characterisation and antimicrobial activity of bacteria associated with submerged lactic acid cassava fermentation.* Food Control, 2014. 39: p. 119-127.

[43] Assamoi, A.A., et al., *Isolation and screening of Weissella strains for their potential use as starter during attiéké production/Isolement et sélection de souches de Weissella comme de potentiels ferments pour la production d'attiéké.* Biotechnologie, Agronomie, Société et Environnement, 2016. 20(3): p. 355.

[44] Rosales-Soto, M., C. Ross, and F. Younce, *Physico-chemical and sen-sory evaluation of cooked fermented protein fortified cassava (Manihot es-culenta Crantz) flour.* ADVANCES IN FOOD TECHNOLOGY AND NUTRITIONAL SCIENCES, 2016. 2(1): p. 9-18.

[45] Rosales-Soto, M.U., et al., *Microbiological and physico-chemical analysis of fermented protein-fortified cassava (Manihot esculenta Crantz) flour.* LWT-Food Science and Technology, 2016. 66: p. 355-360.

[46] Thonart, P., et al., *Isolation and screening of Weissella strains for their potential use as starter during attiéké production.* Base, 2016. 20 (3): p. 355-362.

[47] Wakil, S. and I. Benjamin, *Starter developed pupuru, a traditional Africa fermented food from cassava (Manihot esculenta).* International Food Research Journal, 2015. 22(6): p. 2565-2570.

[48] Essers, A.A., R.M. Van Der Grift, and A.G. Voragen, *Cyanogen removal from cassava roots during sun-drying.* Food Chemistry, 1996. 55(4): p. 319-325.

[49] Heuberger, C., *Cyanide content of cassava and fermented products with focus on attiéké and attiéké garba*. 2005, Diss., Naturwissenschaften, Eidgenössische Technische Hochschule ETH Zürich, Nr. 16247, 2006.

[50] O'Brien, G.M., et al., *Cyanogenic potential of fresh and frozen cassava on retail sale in three Irish cities: a snapshot survey.* International Journal of Food Science & Technology, 2013. 48(9): p. 1815-1821.

[51] Yeoh, H. and F. Sun, *Assessing cyanogen content in cassava-based food using the enzyme-dipstick method.* Food and Chemical Toxicology, 2001. 39(7): p. 649-653.

[52] Sahoré, D. and G. Nemlin, *Effect of technological treatments on cassava (Manihot esculenta Crantz).* Food and Nutrition Sciences, 2010. 1: p. 19-23.

[53] Darman, R.D., J.J.E. NGANG, and F.-X. ETOA, *AMELIORATION DU ROUISSAGE DU MANIOC PAR UTILISATION D'UN STARTER MICROBIEN DE TROIS SOUCHES [AMELIORATION OF CASSAVA RETTING BY A STATER CULTURE OF THREE STRAINS]*. 2015.

[54] Kimaryo, V., et al., *The use of a starter culture in the fermentation of cassava for the production of "kivunde", a traditional Tanzanian food product.* International Journal of Food Microbiology, 2000. 56(2): p. 179-190.