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Molecular identification and species diversity of the microbiota associated with *Soumbara*, a traditional fermented food commonly consumed in Cote d'Ivoire

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ABSTRACT: The aim of this study was to investigate the species diversity of microbiota isolated from soumbara, a traditional alkaline-fermented food and the microbial distribution in six major producing regions in Côte d'Ivoire. Ribosomal gene sequencing revealed that the Ivorian soumbara is associated with a wide microbial diversity essentially composed of 10 *Bacillus* species representing more than 90% of total microbial isolates, 3 species of lactic acid bacteria (2.48%), 2 species of *Staphylococcus* (1.42%), 8 fungal species (4.61%) and 1.06% of other microorganisms. However, the PCR-RFLP revealed a very weak intraspecific diversity as strains belonging to the same species showed a single restriction profile. Moreover, the microbiota associated with Ivorian soumbara consisted in a core and autochthonous microbiota namely *B. subtilis* and *B. velezensis* that were consistently present in samples from all regions studied and an incidental microbiota that was variable through the regions. Among the variable microorganisms, the presence of potential pathogen opportunistic such as *Staphylococcus pasteuri*, *Staphylococcus sciuri* and toxinogen species such as *B. cytotoxicus* and *Aspergellus flavus* were particularly found. The core microbiota is believed to be involved in the normal process of African locust beans into soumbara and can serve as potential starter whereas the incidental and variable microbiota rather undesirable may be involved in the spoilage or food-born disease. In this study, many species were described for the first time in soumbara, and revealed a new biodiversity of microbiota associated with soumbara in Côte d'Ivoire.

Keywords: *Bacillus subtilis, Bacillus velezensis*, Côte d'Ivoire, microbial diversity, Soumbara, starter.

INTRODUCTION

Soumbara is a traditional fermented food commonly consumed in West and Central Africa (Mertz et al., 2001; Yagoub et al., 2004), essentially used as condiment to enhance food taste. One of the most important attributes of soumbara is its capability to lower blood pressure (Gutierrez et al., 2000; Diawara and Jakobsen, 2004). This attributes constitutes the main reason for the wide consumption of this food as it is recommended to persons suffering from hypertension (Gutierrez et al., 2000). However, this food is processed traditionally in compliance with no safety procedure and no standards, resulting in variable quality of soumbara. Moreover, this food is liable to be associated with undesirable microorganisms susceptible to cause food poisoning (Rainbaul, 1995; Somda et al., 2014). In Côte d'Ivoire, less is known about the microbiota associated with soumbara.

Practically, soumbara is obtained by traditional alkaline fermentation of African locust bean (*Parkia biglobosa*) also

known as ‘‘Néré’’ by the local population (Ouoba et al., 2005). This fermentation process takes place spontaneously with the development of the epiphytic microbiota that converts the compounds contained in the raw materials, enriching the nutritional value of soumbara, and impart health-benefits to the consumers (Anihouvi et al., 2007; Farhad et al., 2010). Among the main microbiota, *Bacillus* were reported to be commonly implicated in the soumbara fermentation (Savadogo et al., 2011). Previously, few studies showed that *Bacillus subtilis* and *Bacillus pumilus* from soumbara have not only different abilities to degrade the locust bean proteins, lipids and carbohydrates but also are able to inhibit and inactivate undesired bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* (Ouoba et al., 2003a; Ouoba et al., 2003b). Undesirable microorganisms generally alter food quality since they are associated with either food-borne disease or food spoilage. The species frequently found in soumbara and generally reported to be involved in food born-disease are *Bacillus cereus, Enterococcus faecium, Staphylococcus* spp., *Pseudomonas aeruginosa and Micrococcus spp*. from Nigeria, Benin, Senegal and Burkina Faso (N’Dir et al., 1997; Sanni et al., 2000; Omafuvbe et al., 2004; Ouoba et al., 2004; Azokpota et al., 2007). On the other hand, the fungal species, mostly *Aspergillus* and *Penicillium* present in soumbara were generally reported to be rather associated with food spoilage (Campbell-Platt, 1980).

Many studies in West Africa on the microorganisms fermenting African locust bean have been carried out mainly focusing on microbiota identification at biochemical and genotypic levels (Ouoba et al., 2004; Azokpota et al., 2007; Parkouda et al., 2009; Ouoba et al., 2010). However, the occurrence of microbiota strains involved in the soumbara production using molecular method in Côte d’Ivoire has not been investigated. The microbiota associated with Ivorian soumbara remain to be well described. This in turn should allow a selection of relevant species as valuable starter to control the fermentation of locust beans and improve the quality of soumbara.

The aim of this study was to investigate the species diversity of the microbiota associated with locust beans fermentation for soumbara production using molecular biology tools such as 16S and 5,8S gene sequencing to identify the isolates, and RFLP for strains typing.

MATERIAL AND METHODS

Sample collection

Samples of soumbara were obtained from six producing regions in north Côte d'Ivoire notably Poro (9° 25′ N, 5° 37′ W), Kabadougou (9° 30′ 0″ N, 7° 34′ 0″ W), Tchologo (9° 35′ 0″ N, 5° 11′ 0″ W), Bagoué (9° 31′ 0″ N, 6° 29′ 0″ W), Hambol (7° 41′ 0″ N, 5° 1′ 0″ W) and Béré (8° 3′ 0″ N, 6° 11′ 0″ W) In each region, two to three area were targeted depending on the availability of soumbara, where one sample of 100 g was collected per area. Samples of soumbara were collected in a Stomacher bags and sent to the laboratory for analysis within 24 hours in hermetically sealed coolers. Upon arrivals in laboratory, samples from areas belonging to the same region were mixed and homogenized to finally obtain 6 samples, each sample representing one region that was used for different microbiological analyzes.

Culture conditions and Isolation of microorganisms

25 g from each soumbara sample were withdrawn and added to 225 ml sterile buffered (pH 7.2) peptone water and homogenized using a Stomacher (AES labratoire) for 5 minutes. Then, 1 mL of the microorganisms enriched peptone water was diluted in 9 mL of tryptone salt solution from which a serial dilution was performed up to 10^{-5}. Next, strains isolation was performed using the plating technique, 0.1 mL from each diluted solution were plate onto specific media supplemented with 50 μg/mL of the antifungal compound nystatin for bacteria isolation. Incubation conditions were 30-37 ° C for 48 to 72 hours. Man-Rogosa-Sharpe (MRS) agar (CONDA, Madrid, Spain) was used for LAB whereas Nutrient Agar (CONDA, Madrid, Spain), Plate Count Agar (PCA) (CONDA, Madrid, Spain), Baird Parker Agar (CONDA, Madrid, Spain) were used respectively for *Bacillus*, Mesophilic aerobic germs (MAG) and *Staphylococci* identification. Oxytetracycline Glucose Yeast Extract Agar (OGYE) (Applichem, Darmstadt, Germany) supplemented with 1 mL/L of 10% oxytetracycline to inhibit bacterial growth (Mossel et al., 1970) was used for selective culture of fungal strains, incubation was performed at 25 to 30°C for 2 to 7 days. The bacterial and yeasts isolates were stored at -20°C in brain heart broth (BHB) (MERK, Darmstadt, Germany) supplemented with 20% (v/v) glycerol, in Eppendorf tubes and molds isolates were stored at 4°C in inclined test tubes containing OGYE agar for further investigation.

Total DNA extraction

1 ml of an overnight pre-culture was transferred into an Eppendorff vial and centrifuged at 13,000 rpm for 10 min, then the pelleted cells were used for total DNA extraction according to cetyltrimethylammonium bromide (CTAB) test protocol (Wagner et al., 1987). The quantity and quality of the DNA (1.5 µL) were estimated by Nanodrop spectrophotometer assay (Thermo Scientific NanoDrop2000 Spectrophotometer, USA).

PCR amplification of microbial ribosomal RNA genes

The bacterial 16S rRNA genes amplification was performed by PCR with the universal primers FGPS 1509 (5' AAGGAGGGGATCCAGCCGCA 3') and FSGP 6 (5

'GGAGAGTTAGATCTTGGCTCAG 3') (Normand et al., 1992). PCR reactions were performed with a thermocycler (APPLIED BIOSYSTEM model 2720 THERMAL) in a final volume of 10 µL containing 1.5 ng/µL of DNA extract as template, 1X OneTaq Quick-Load 2X Master Mix (NEB, South Africa), 0.1 µM of each primer and 3.8 µL ultra-pure water. An extraction control without template was systematically included in the procedure. The program used to run the PCR reactions was set as follow. After an initial denaturation at 94°C for 5 minutes, reactions were run for 35 cycles, each cycle comprising: denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72°C for 1 minute 30 seconds. Finally, extension at 7 minutes at 72°C was carried out.

The fungal 5.8S rRNA gene was PCR amplified using universal primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') (Mitchell and Zuccaro, 2006). PCR reactions were performed in the same conditions as described above accepted that annealing happened at 55°C for 30 seconds. The presence and yield of specific PCR products was monitored using agarose 0.9% (w/v) gel electrophoresis at 100 V, for 50 minutes, in 1X Tris Borate EDTA buffer and visualized with ethidium bromide staining and UV transillumination.

Restriction of amplified rRNA genes

PCR product was digested with two restriction enzymes, HaeIII and RsaI, in separate reactions for bacterial 16S rRNA gene restriction, and then restriction enzymes HaeIII and AluI were used for fungal 5,8S rRNA gene restriction. The digestions were carried out for 2 hours at 37°C in water-bath in a final volume of 15 µL containing 5 µL of PCR product, 1.5 ng/µL of enzyme buffer, 8 µL of ultra-pure water and 10 U of restriction enzyme (NEB, South Africa). Digestion products were run on 2.5% agarose gel in 1X Tris-Borate EDTA buffer at 80 V for 3 hours using 100 bp DNA ladder (NEB, South Africa). Gels were stained with ethidium bromide and visualized by transillumination.

rRNA gene sequence analysis

PCR products were purified and then sequenced using the FGPS 1509 and FSGP 6 primer pair for bacterial strain and ITS1 primer for fungal strain. The basic local alignment search tool (BLAST, blastN) from the NCBI database site (blast.ncbi.nlm.nih.gov/) was used to find the closest sequences relative to the amplified 16S RNA and 5,8S RNA genes in order to identify respectively the bacterial and fungi strains. The phylogenetic tree was constructed from the partial sequences of RNA 16S and 5,8S genes alignment using the maximum likelihood method (Tamura et al., 2013).

RESULTS

Different groups of microorganism isolated from soumbara

A total number of 282 isolates composed the microbiota found in soumbara from six producing regions studied. This microbiota consisted essentially in four groups of bacteria notably Bacillus, lactic acid bacteria, *Staphylococcus* as well as other bacteria termed mixed group (MG) isolated from non-selective PCA medium and both groups of fungi namely yeasts and molds (Table 1). *Bacillus* isolates was so far, the dominant group with more than 90% of the total microbial isolates. The remaining groups of microorganism appeared to be the minor microbiota with LAB, *Staphylococcus* and fungi representing respectively 2.48, 1.42 and 4.61% of the total microbiota isolated (Figure 1). The mixed bacterial group MG composed of *Acinetobacter variabilis, Micrococcus yunnanensis, Paracoccus siganidrum,* represented 1.06% (Figure 1). The dominant group (*Bacillus*) was widely distributed in all the regions studied whereas LAB were found in soumbara from 4 regions (Béré, Kabadougou, Tchologo, Hambol) and *Staphylococcus* in 3 regions (Kabadougou, Bagoué and Hambol) (Table 1). Likewise, fungal strains were only isolated from soumbara in few regions such as Bagoué, Kabadougou, Poro and Tchologo (Table 1). The microbiota isolated from soumbara in Kabadougou region presented the widest diversity including the groups of *Bacillus*, LAB, *Staphylococcus*, Yeasts and Molds While the microbial diversity in the soumbara from Bagoué region also recorded similar diversity but did not contain LAB (Table 1). On the other hand, the region of Béré presented a weak microbial diversity with only 2 groups of microorganisms (Table 1). The sole non-fermentative microbial group was found to have a relative weak distribution with their presence in 3 regions (Table 1).

16S rRNA gene PCR-RFLP and identification of bacterial isolates

Primers targeting the 16S rRNA gene in bacteria generated PCR amplicons of approximately 1500 bp (Table 2). The digestion of these PCR fragments, using restriction enzymes *Rsa*I and *Hae*III separately, allowed the classification of the 269 isolates into eighteen distinct groups based on Restriction Fragment Length Polymorphism (RFLP) Analysis (Table 2). Of the ten RFLP groups of *Bacillus*, the main species belonged to the RFLP groups I, II and III consisting in *Bacillus subtilis*, *Bacillus velezensis and Lysinibacillus macroides*, respectively. Moreover, the results show that *Bacillus subtilis* is the predominant species present in soumbara from all the regions studied representing 75% of the total microbiota

Table 1. Microorganism isolation of soumbara from the different producing region.

Microorganism	Groups	Regions					
		BERE	**PORO**	**KABADOUGOU**	**TCHOLOGO**	**BAGOUE**	**HAMBOL**
Bacteria	*Bacillus*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*
		Bacillus velezensis	*Bacillus velezensis*	*Bacillus velezensis*	*Bacillus velezensis*	*Bacillus velezensis*	*Bacillus velezensis*
		Lysinibacillus macroides	*Lysinibacillus macroides*	*Lysinibacillus macroides*	*Bacillus cytotoxicus*	*Lysinibacillus macroides*	*Bacillus drentensis*
		–	*Bacillus pumilis*	*Bacillus drentensis*	*Paenibacillus xylanilyticus*	*Rummeliibacillus stabekissi*	–
		–	*Bacillus endophyticus*	*Bacillus oleronius*	–	–	–
		–	*Rummeliibacillus stabekissi*	–	–	–	–
	LAB	*Streptococcus oralis*	–	*Enterococcus faecium*	*Enterococcus faecium*	–	*Enterococcus faecium*
		Enterococcus faecium	–	–	*Weissella cibaria*	–	–
	Staphyloccoci	–	–	*Staphylococcus pasteuri*	–	*Staphylococcus pasteuri*	*Staphylococcus pasteuri*
		–	–	*Staphylococcus sciuri*	–	–	–
	Other bacteria	–	*Acinetobacter variabilis*	*Micrococcus yunnanensis*	–	*Paracoccus siganidrum*	–
	Yeats	–	–	*Meyerozyma caribbica*	*Meyerozyma caribbica*	*Blastobotrys profilferans*	–
Fungi	Molds	–	*Fusarium oxysporum*	*Penicillium chrysogenum*	–	*Penicillium sclerotiorum*	–
		–		*Aspergillus flavus*	–	*Absidia corymbifera*	–
		–	–	–	–	*Cladosporium uredinicola*	–
		–	–	–	–	*Fusarium oxysporum*	–

- : no species.

Table 2 Bacterial species isolated and their RFLP group.

Microorganism groups	Strains isolated	16S gene length (Pb)	RsaI restriction fragments (Pb)	HaeIII restriction fragments (Pb)	Number of strains	RFLP group
Bacillus	*Bacillus subtilis*	1500	500+480+400+150	600+480+320+150	203	I
	Bacillus velezensis	1500	500+420+150	600+480+300+150	29	II
	Lysinibacillus macroides	1500	500+460+420+150	600+480+250+150	11	III
	Bacillus pumilis	1500	800+500+400+100	600+480+300+150	1	IV
	Bacillus drentensis	1500	480+420+350+150	500+250+150	2	V
	Bacillus endophyticus	1500	480+420+350+150	1200+300	3	VI
	Bacillus oleronius	1500	500+400+380+150	500+300+250+150	1	VII
	Bacillus cytotoxicus	1500	500+400+380+150	517+500+300+150	2	VIII
	Paenibacillus xylanilyticus	1500	500+400+380+150	900+300+250	1	IX
	Rummeliibacillus stabekissii	1500	480+460+380+150	600+480+250+150	2	X
Lactic acid bacteria	*Streptococcus oralis*	1500	700+400+280+180	600+480+300+150	1	XI
	Enterococcus faecium	1500	1000+400+150	600+480+250+150	5	XII
	Weissella cibaria	1500	500+440+150	1200+300	1	XIII
Staphylococci	*Staphylococcus pasteuri*	1500	500+420+150	1200+300	3	XIV
	Staphylococcus sciuri	1500	500+480+250+180+150	1200+300	1	XV
Mixed Group	*Acinetobacter variabilis*	1500	900+350+150	300+250+250+200	1	XVI
	Micrococcus yunnanensis	1500	500+400+300+200	480+300+250+150	1	XVI
	Paracoccus siganidrum	1500	500+420+380+200	500+180+150+100	1	XVIII

isolated. Lactic acid bacteria (LAB) clustered into three groups with *Enterococcus faecium* as the main species whereas *Staphylococcus* strains fallen in two RFLP groups with *Staphylococcus pasteuri* being the main species. The last group isolated from non-selective agar was composed of mixed species namely *Micrococcus yunnanensis, Acinetobacter variabilis*, *Paracoccus siganidrum,* each species characterized by a particular restriction profile. *Bacillus* recorded the widest species diversity followed by lactic acid bacteria and *Staphylococcus*. The phylogenetic tree shows the relationship between the *Bacillus* strains representing the main microbiota isolated (Figure 2).

Bacterial species distribution in the different soumbara producing regions

The isolated microbiota from soumbara was composed of a core microbiota consisting in *Bacillus subtilis* and *Bacillus velezensis* that were consistently found in all the regions studied and a variable microbiota unequally distributed in the different regions (Figure 1). *Lysinibacillus macroides* also presented a wide distribution through the regions studied although this species was not found in samples from two regions notably Hambol and Tchologo (Figure 1). Moreover, beside the fermentative and dominant microbiota notably

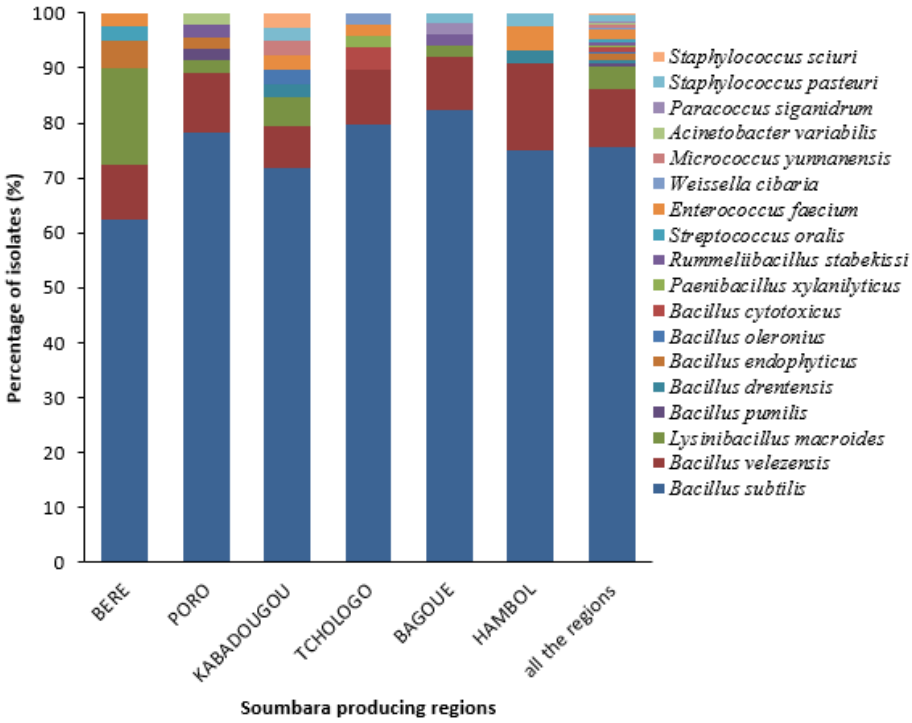

Figure 1. Bacterial species distribution in the different Soumbara producing regions.

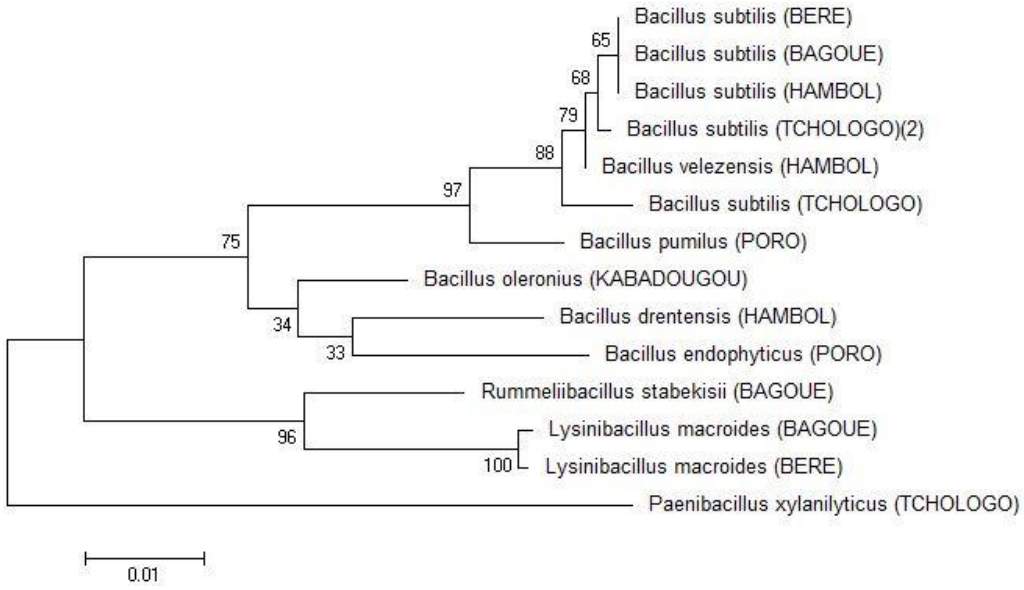

Figure 2. Phylogenetic tree of Bacillus strains isolated from Ivorian Soumbara. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-1369.2283) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 14 nucleotide sequences. All positions with less than 100% site coverage were eliminated. That is, fewer than 0% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were total of 512 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

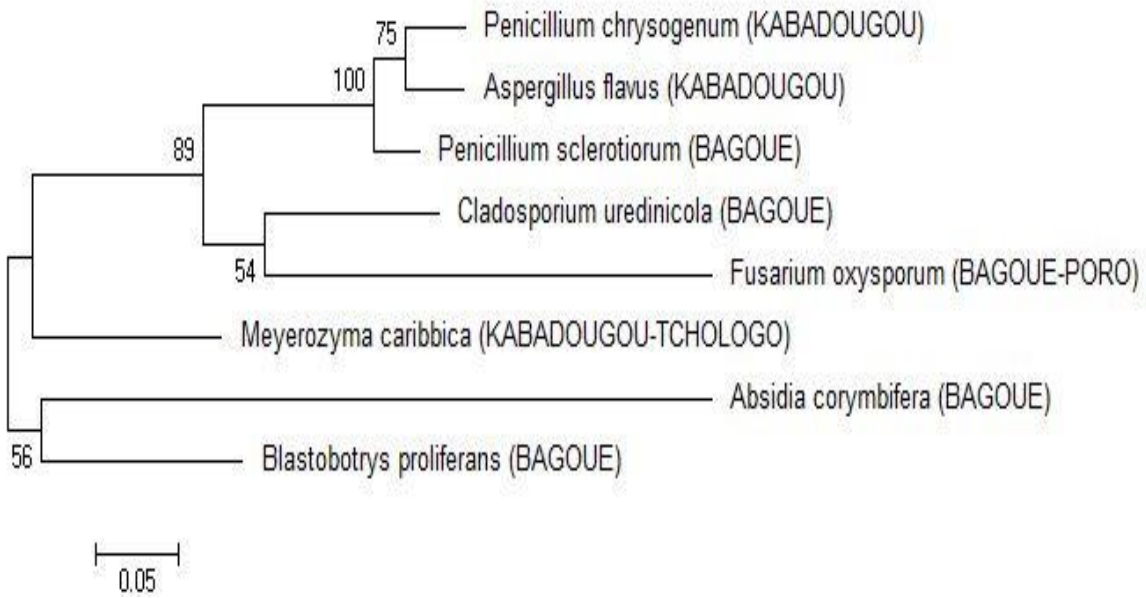

Figure 3 Phylogenetic tree of fungal strains isolated from Ivorian soumbara. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-2037.7490) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 8 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 100% site coverage were eliminated. That is, fewer than 0% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were total of 344 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Bacillus and LAB, a non-fermentative microbiota was found namely *Staphylococci*, *Acinetobacter*, *Micrococcus* and *Paracoccus*. Among the fermentative strains the particular presence of the toxinogenic *Bacillus cytotoxicus* (from Tchologo) and the undesirable *Enterococcus faecium* (from Tchologo, Hambol and Béré) were observed. Moreover, the samples presented different microbial diversity or community depending on the region. Hence, the sample from Kabadougou region included the widest diversity with 9 different microbial species while samples from Hambol regions presented only 5 species (Table 1).

Fungal strains identification and distribution in the different soumbara producing regions

Sequence analysis show that the isolated fungal microbiota was essentially composed of molds species namely *Penicillium sclerotiorum*, *Penicillium chrysogenum*, *Absidia corymbifera*, *Cladosporium uredinicola, Fusarium oxysporum* and *Aspergillus flavus* whereas yeasts consisted in only two species notably *Meyerozyma caribbica and Blastobotrys proliferans.* The phylogenetic tree shows the relationship between these fungal strains (Figure 3). Regarding their distribution, molds were found only in 4 regions (Bagoué, Kabadougou, Poro and Tchologo) with a variation from a region to another (Figure 4). An unequal distribution of these microorganisms was observed, as no molds species was consistently isolated in all regions. The widest distributed species *Meyerozyma caribbica* (Kabadougou and Tchologo) and *Fusarium oxyporium* (Bagoué and Poro) were found in only two regions (Figure 4), The soumbara from Bagoué regions displayed the greatest mold diversity with four distinct species (*Blastobotrys proliferans, Penicillium sclerotiorum, Absidia corymbifera*, *Cladosporium uredinicola*, *Fusarium oxysporum*) while the soumbara from Kabadougou region presented three molds species (*Meyerozyma caribbica, Penicillium chrysogenum, Aspergillus flavus*). On the other hand, the soumbara from Poro and Tchologo regions showed the weakest mold diversity with respectively one species *Fusarium oxysporum* et *Meyerozyma caribbica* (Figure 4).

DISCUSSION

In this study, the microbiota associated with soumbara from six producing regions in north Côte d'Ivoire was

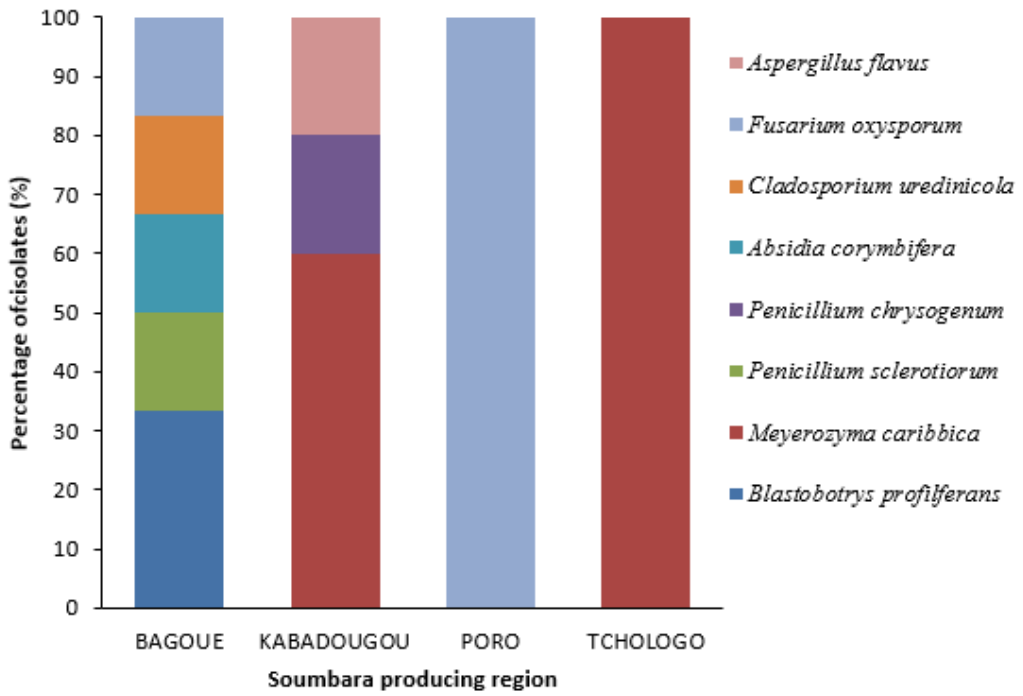

Figure 4. Fungal species distribution in the different Soumbara producing region.

isolated and analyzed. Most of studies concerning the microbiology of soumbara in Cote d'Ivoire were focused on routine analysis of the microbiological quality that does not involve molecular analysis. Hence, the genetic diversity of the microorganisms from soumbara has not been investigated in Cote d'Ivoire.

The present study revealed a wide diversity of microorganisms associated with soumbara in Cote d'Ivoire, *Bacillus subtilis* being the major species whatever the studied region. Previously, Fatoumata et al. (2016) using biochemical approach also reported that *B. subtilis* was the dominant microbial species in soumbara from three Ivorian regions (Korhogo, Bouaké and Abidjan). However, these authors found a weak diversity of *Bacillus*, only two species namely *B. subtilis* and *B. pumilus* in comparison with this study that allowed the detection of ten *Bacillus* species notably *B. subtilis*, *B. pumilis*, *B. velezensis*, *B. drentensis*, *B. endophyticus*, *B. oleronius, B. cytotoxicus, Paenibacillus xylanilyticus*, *Rummeliibacillus stabekissi* and *Lysinibacillus macroides*. This clearly show the usefulness and the accuracy of the molecular approach used in this study. Furthermore, *Bacillus* species, particularly *B. subtilis* and *B. velezensis* seems to be the most well adapted microorganism to the soumbara ecosystem since these bacteria were also found in soumbara from many other west African countries such as Benin, Nigeria, Burkina Faso, Mali, Senegal and Ghana (N'Dir et al., 1997; Omafuvbe et al., 2004; Ouoba et al., 2004; Amoa-Awua et al., 2006; Azokpota et al., 2007; Meerak et al., 2008). Both *Bacillus* species are probably the main and crucial actor responsible for African locust bean fermentation and the basic organoleptic characteristic of soumbara. Supporting this assertion, *B. subtilis* was previously reported to be the initiator of locust bean fermentation through the degradation of proteins, lipids and carbohydrates (Ouoba et al., 2003a; Ouoba et al., 2003b; Ouoba et al., 2005) and identified as high producer of methyl butanoic acid, the typical aroma of soumbara (Akanni et al., 2018). *Bacillus subtilis* was used as starter culture in locust bean fermentation for soumbara production (Ogbadu and Okagbue, 1988; Ouoba et al., 2004), whereas the exact role of *Bacillus velezensis* remains to be elucidated.

The dominant microbial species that were consistently found in all the six regions studied, is indicative of the typical core microbiota directly involved in the fermentation and manufacturing process of African locust bean into soumbara. However, beside this core microbiota, a variable and minor microbiota composed of fermentative and non-fermentative species was found. The variable fermentative species composed of *Bacillus* (*Bacillus drentensis*, *Bacillus endophyticus*, *Bacillus oleronius*, *Paenibacillus xylanilyticus*, *Rummeliibacillus stabekissi*, *Lysinibacillus macrolide*), lactic acid bacteria (*Weissella cibaria*) and yeasts (*Blastobotry sproliferans, Meyerozyma caribbica*) although being minor, may be involved in particular aroma resulting in a variation of flavor from a region to another region as it was generally observed during the sampling. On the other hand, the non-fermentative microbiota essentially consisting of

Staphylococcus pasteuri, Staphylococcus sciuri, *Streptococcus oralis, Micrococcus yunnanensis*, due to their pathogenic potential as opportunistic microorganism are known to be undesirable in food system and associated with serious diseases such as skin, soft tissue and rhino pharyngeal infections (Stepanovic et al., 2003; Shittu et al., 2004; Carretto et al., 2005). These hand-carried microorganisms are generally indicative of a human contamination probably occurred during the production or the marketing process under unacceptable hygienic conditions. Thus, the presence of non-fermentative and potentially pathogen microbiota could be an excellent indicator of contamination and hygienic conditions related to the preparation and conditioning of soumbara. Regarding the distribution of this contaminating microbiota that was extremely variable through the regions, one could assume that this is also indicative of the difference in hygienic conditions of soumbara preparation. Additionally, *Bacillus cytotoxicus,* a newly described thermotolerant member of the *Bacillus cereus* group is occasionally involved in food poisoning (Guinebretière et al., 2013) and the particular presence of this enterotoxin producing *Bacillus* species in the soumbara sample from the region of Tchologo might be of high risk for consumers (Guinebretière et al., 2013). This is the first time that *B. cytotoxicus* is isolated from soumbara together with many species such as *B. velezensis*, *B. drentensis*, *B. endophyticus*, *B. oleronius*, *Paenibacillus xylanilyticus*, *Rummeliibacillus stabekissi, Lysinibacillus macrolides*, *Weissella cibaria*, *Enterococcus faecium, Streptococcus oralis*, *Staphylococcus pasteuri, Staphylococcus sciuri*, *Acinetobacter variabilis, Micrococcus yunnanensis*, *Paracoccus siganidrum, Blastobotry sproliferans, Meyerozyma caribbica, Penicillium sclerotiorum*, *Penicillium chrysogenum*, *Absidia corymbifera*, *Cladosporium uredinicola, Fusarium oxysporum* and *Aspergillus flavus* that have never been isolated in other countries and seems to be specific to soumbara ecosystem in Cote d'Ivoire.

Among the microorganisms isolated from soumbara sample, fungal strains were also found representing a few proportion (4.61 %) of total isolates. The fungal microbiota consisted in six mold species and two yeasts species. Molds are more generally associated with food spoilage mainly altering the organoleptic quality through enzymes production and sometime producing mycotoxin (Pitt, 2000; Bennett and Klich, 2003). The presence of molds in soumbara may results from the fact that this is a non-packaged and non-protected food against humidity, allowing an easy mold contamination from air. Additionally, *Aspergillus flavus* was assumed to be the major producer of aflatoxin (Klich, 2007), and its occurrence in soumbara analyzed confirms that this fermented food can potentially pose a health problem for consumers. Formerly, Campbell-Platt (1980) have reported the incidence of few fungi in "dawadawa" (eg. soumbara in Nigeria) fermentation as contaminants, but further investigations were not carried out to identify the exact species. The processing of this traditional fermented food should be controlled to be completely safe.

Conclusion

This study showed that soumbara in Cote d'Ivoire is associated with a diversity of microorganisms consisting in major and autochthonous microbiota consistently present in sample from all regions and minor microbiota variable through the regions. The core microbiota composed of the species *B. subtilis* and *B. velezensis* may serve as valuable potential starters for improvement of this fermented food, whereas the variable microbiota including potential pathogen may be indicative of contamination from unacceptable hygienic conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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