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Benchmarking Loads of Lactic Acid Bacteria from Traditional Ferment of Cassava Used for the Preparation of Cassava Meal in Abidjan

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Abstract

Lactic acid bacteria became very important in the food processing industries and farms because of theirs divers properties and characters. "Magnan" is among the variety of fermented food products where they would meet. "Magnan" is a traditional ferment used for the preparation of fermented cassava meal steamed called "attiéké". Studies on the magnan showed that flora consists of molds, yeast, bacillus, and lactic acid bacteria. However, in their diversity the lactic acid bacteria involved in the fermentation remain largely unknown. In fact some studies have shown the involvement of genus *Lactobacillus* in this process when other associated it to the genus *Leuconostoc*. Also among the lactic acid bacteria, the predominant type is not known. A study to identify the genus of lactic acid bacteria mainly involved in the fermentation process used to produce ''attiéké'' and the pH gave different results: *Lactobacillus* (7.2±0.7 log (UFC/g)), *Leuconostoc* (6.7±0.6 log (UFC/g)), *Streptococcus* (5.8±0.9 log(UFC/g)), *Lactococcus* (5.9±0.4 log(UFC/g)). It was noted that cassava peal was fermented by less loaded *Pediococcus* (5.0±1.7 log (UFC / g)). The pH of ferments ranged between 5.35±0.53 and 5.58±0.54.

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Magnan

Introduction

Lactic acid bacteria have an important role in food and feed. They are ubiquitous and commensal bacteria, which involved in spontaneous fermentation artisanal food. Starter's selection has made them auxiliaries manufacturing in the food industries. They are widely used as a probiotic. Today, probiotics are known as a potential stem to replace antibiotic growth promoters in livestock without creating new threats such as observed with antibiotics (Devie et al., 2005). In diversity of genus and species *Lactobacillus*, *Leuconostoc*, *Lactococcus Pediococcus* and *Streptococcus thermophilus* were recognized as those that best meet definition of lactic acid bacteria. Lactic acid bacteria are present in any fermented product meet in Côte d'Ivoire. Include the "dokounou" fermented sweet corn dough; "akassa" fermented sweet corn dough, "adjovan" fermented fish, "soumara" fermented grains of *Parkia biglobosamm* "attiéké" fermented cornmeal steamed cassava.

Attiéké is the main food accessible to all strata of the population. There are several types of attiéké depending on the region and the method of the preparation. Generally it is from a tapioca pulp of traditional ferment called "magnan". The magnan is generally obtained by bleaching of cassava tubers. Studies on the magnan showed that flora of magnan consists of mold, yeast, bacillus and lactic acid bacteria. The role of some of these microorganisms is well known. Bacillus and molds

intervened in softening pulp. Lactic acid bacteria are responsible for lactic fermentation that gives a sour taste appreciated by consumers, however, in their diversity; the genus lactic acid bacteria involved mainly in this process remain unknown. In fact some studies have shown the involvement of the genus of *Lactobacillus* while other studies have shown both genus *Lactobacillus* and *Leuconostoc*. This study have to identify the pH (potential hydrogen) and the genus of lactic acid bacteria mainly involved in the fermentation process used to produce attiéké on three sites artisanal production.

Materials and methods

Sampling

Seventy one (71) samples of traditional ferment each weighing 0.5 kg were collected from three production sites of professional producing cassava semolina " attiéké " in Abidjan, Ivory Coast. Table 1 shows the sites and the number of samples per site. The samples were transported to the laboratory where the determination of pH and lactic acid bacteria counts were made.

Table 1. Sampling sites and the number of samples per site

	Sampling 1	Sampling 2	Sampling 3	Sampling 4	Sampling 5	Total
Site 1	4	5	5	5	5	24
Site 2	5	5	3	5	5	23
Site 3	5	5	5	5	4	24
Total	14	15	13	15	14	71

Determination of pH

Each sample was crushed in a sterile mortar and pestle. After homogenization, ten grams (10g) was crushed and diluted in 90 ml of distilled water and then filtered on a filter paper (filter folded diam. 190 mm, qualitative analysi**s**). The pH was measured with an electronic pH meter (HANNA pH meter) by immersing the electrode in the filtrate. The analysis was performed in triplicate per sample.

Enumeration of lactic acid bacteria

Twenty-five grams (25 g) each ground sample are put into a stomacher bag containing 225 ml of EPT and homogenized by grinding in a Stomacher (AES Laboratoire, France). Decimal dilutions were performed to the dilution 10^{-8} in nine milliliters (9 mL) tryptone salt. The seedings were performed in duplicate for each dilution, spreading 0.1 mL of the inoculum. Genus of *Lactobacillus* and *Streptococcus* were counted respectively on MRS agar and M17 agar supplemented with cycloheximide 0.01%.

The genus *Leuconostoc* and the genus *Pediococcus* were enumerated on MRS agar supplemented with cycloheximide (0.01%) and vancomycin (0.01%). *Lactococcus* were counted on agar Chalmers added cycloheximide 0.1%. All inoculated media were placed in anaerobic jars. Petri dishes for the enumeration of the genus *Lactobacillus* were incubated at 37°C, those of the genus *Streptococcus* to 44°C and those used for *Lactococcus*, *Leuconostoc* and *Pediococcus* were incubated at 30°C. The incubation period is 48 hrs.

Identification of different bacterial genera

Identification of the different genera is made by performing the main biochemical and physiological tests used for lactic acid bacteria (Pillet, 1998). Among characteristics colonies presumed counted, five were used each time. These tests include Gram stain, research catalase, cytochrome oxidase, the homo fermentation character or hetero fermentation, degradation of arginine, growth at 10°C, 15°C and 45°C.

Expression of results

After confirming the method of calculating the number of each genus was carried out in accordance with paragraph 9.3.5.3 of ISO 7218: 1996 / Amd.1: 2001 (E) Microbiology of General Rules - food microbiological examination.

Storage of stem

Identified strains were kept in stock at -24 C in MRS broth at 30% glycerol.

Statistical analyses

The XLSTAT 2015 software was used for statistical analysis. Statistical analysis of medium loads was done according to the Kruskal -Wallis test. Multiple

pair wise comparisons the procedure of Steel- Dwass - Cristchow - Fligner has identified the differences between charges.

Results

Hydrogen potential (pH) values of cassava - ferments of the three production sites attiéké

The mean pH of all 71 samples is 5.46 ± 0.57. The minimum and the maximum value are respectively 3.91 ± 0.57 and 6.91 ± 0.57. The average pH of the various sites are 5.54 ± 0.63 for site 1, 5.50 ± 0.54 for the site 2 and 5.34 ± 0.53 for the site 3 (Fig. 1). The linear trend line shows that the pH of the cassava - ferments vary between 5.35 ± 0.53 and 5.58 ± 0.54.

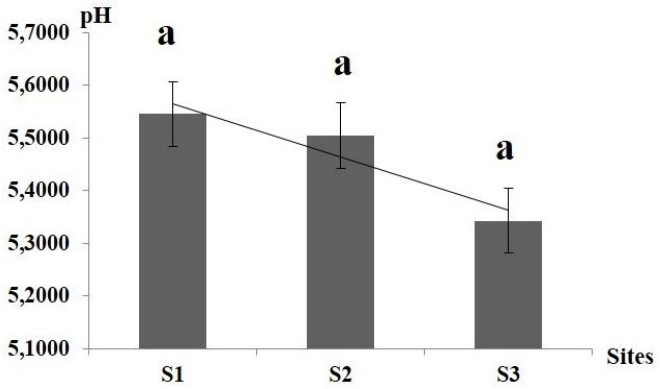

Fig. 1: Average pH of ferment per site.

Load of lactic acid bacteria of technological interest on the three sites

Analyzes reveal the presence of the five genus of lactic acid bacteria of technological interest. The isolation frequency is 1 for the genus *Lactobacillus, Leuconostoc, Streptococcus, Lactococcus* it is 0.9 for the genus *Pediococcus* (Table 2). The average load of lactic acid bacteria genus of cassava - ferments are shown in (Table 3). The different average charges for all samples for the genus *Lactobacillus* is 7.2 ± 0.7 log (CFU / g), which is more numerous. The genus *Leuconostoc* is second with 6.7 ± 0.6 log (UFC/g). The genus Streptococcus and *Lactococcus* statistically occupy the same position with respectively 5.8±0.9 log (UFC/g) and 5.9 ± 0.4 log (UFC/g). The charge of *Pediococcus* genus is lowest with 5.0 ± 1.7 log (UFC/g). Fig. 2 shows the average distribution of different bacterial loads on all samples analyzed. Fig. 3 presents loads of genus studied on the site 1. The order of distribution of the different loads on the site 1 (Fig. 3) is identical to that observed for all 71 samples. Loads of *Lactobacillus* and *Leuconostoc* are respectively 7.4 ± 0.7 log (CFU / g) and 6.7 ± 0.6 log (CFU / g). Those of *Streptococcus* and *Lactococcus* are respectively 6.0 ± 0.4 log (CFU / g) and 5.9 ± 0.3 log (CFU / g). The charge of the genus *Pediococcus* is 5.2 ± 1.6 log (CFU / g). Statistical analysis showed that there is no significant difference between the loads of *Lactococcus* and *Streptococcus*. Loads of other genus are different from each other (p<0.05).

Table 1. Frequency of occurrence of different genera.

Total number of positive samples	Total number of samples	Total number of positive samples	Frequency of positivity
Lactobacillus	71	71	1
Leuconostoc	71	71	1
Streptococcus	71	71	1
Lactococcus	71	71	1
Pediococcus	71	64	0.9

Table 2. Bacterial load expressed in log (CFU / g) by site and all sites.

	Bacterial load log (UFC/g)				
	Lactobacillus	***Leuconostoc***	***Lactococcus***	***Pediococcus***	***Streptococcus***
Site 1	7.4 ± 0.7	6.7 ± 0.6	5.9 ± 0.3	5.2 ± 1.6	6.0 ± 0.3
Site 2	6.8 ± 0.6	6.5 ± 0.5	5.8 ± 0.3	5.5 ± 0.5	5.8 ± 0.6
Site 3	7.2 ± 0.6	6.7 ± 0.6	6.2 ± 0.5	4.3 ± 1.3	5.7 ± 1.3
All three sites	7.2 ± 0.7	6.7 ± 0.6	5.9 ± 0.4	5.0 ± 1.7	5.8 ± 0.9

On sites 1 and 2 loads in bacteria belonging to the genus *Leuconostoc* and *Lactobacillus* are not statistically different. These charges are higher than those of the genera *Lactococcus*, *Streptococcus* and *Pediococcus* (Figs. 3 and 4). *Pediococcus* gender is the least abundant (Table 3).

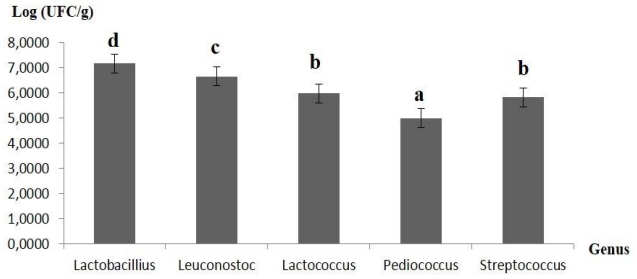

Fig. 2: Average distribution of bacterial loads in log transformed UFC per gram on all three sites.

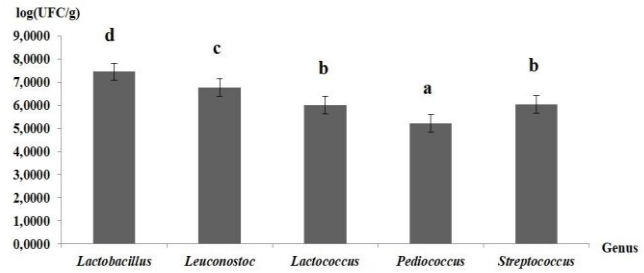

Fig. 3: Average distribution of bacterial loads in transformed UFC per gram from site 1.

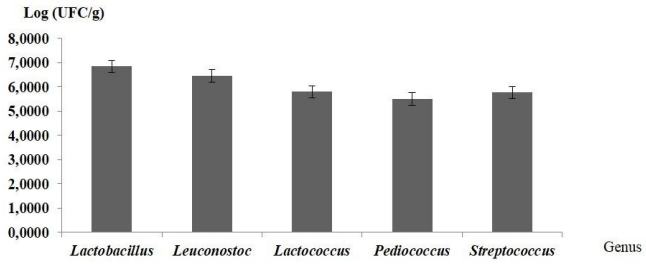

Fig. 4: Average distribution of bacterial loads in transformed UFC per gram from site 2.

Discussion

The pH (potential hydrogen) cassava-ferments analyzed are acidic and have averaged between 5.35 ± 0.53 and 5.58 ± 0.54. These results are consistent with those of the study by Louembé et al (2002) which have found an acidic pH at the end of the fermentation process. It is the same for Djouldé et al. (2003) which showed that the pH of cassava ferments were acid although the original tubers have a neutral pH. On the three sampling sites, the pH of ferments was not statistically different. This could be related to the fact that producers use the same manufacturing process of the ferment. Microorganisms causing pH lowering have a common range of growth pH. pH values for optimal growth of lactic acid bacteria are generally between 5.5 and 6.5. However there may be differences between genera and even between strains of the same species (Corrieu et al., 2008).

The 5 types of lactic acid bacteria that are *Lactococcus, Streptococcus, Pediococcus, Leuconostoc* and *Lactobacillus* were isolated in close-cassava used for the preparation of manioc "attiéké" analyzed in this study. This number is higher than the study of Assanvo et al. (2002) which had demonstrated the presence of *Lactobacillus plantarum*. The work of Okafor (1977), Ngaba Lee (1979) and Lei et al. (1999) revealed in turn, the presence of *Leuconostoc* in the ferment of cassava. This qualitative difference of lactic acid bacteria may be due to the diversity of cassava variety used for the production or the difference process used by each producer.

To our knowledge, this is the first time that the presence of the five genera of lactic acid bacteria is revealed simultaneously in a ferment-cassava, better than *Lactococcus* and *Pediococcus* had never been made. These were isolated rather other substrates (Pilet et al., 1998; Leveau et al., 1991) including corn meal.

Loads of different genera remain variable with a continuous predominance of the genus *Lactobacillus* and the genus *Leuconostoc* (Fig. 2, 3 and 4). Note also that even if the presence of *Leuconostoc* and *Lactobacillus* has been reported, no study to our knowledge has highlighted the predominance of one genus over the other four genera. This dominance can be attributed to varying pH in the fermentation process. Indeed, cassava has a pH close to neutrality 6.5 to 7.0. During the fermentation process cassava - ferments, development and the action of certain microbial groups would create pH conditions for the development of another bacterial group. Bacteria of the genus *Lactobacillus* and *Leuconostoc* have an optimum development of acidic pH between 5.5 and 6 (Corrieu et al., 2008). According Carolle Lapointe (2002), some species of *Lactococcus* carry out acidification during the first hours of fermentation thereafter because of their sensitivity to acidic pH, they would be destroyed allowing resistant bacteria to grow. Magni (1999) argued that only bacteria capable of fermenting citrate have a resistance to the inhibitory effect lactate. That would justify the predominance of *Lactobacillus* and *Leuconostoc* in this study. The changes observed could be consequences of conditions productions. Although composed of the same steps and processes, they could be heavily impacted by human and environmental conditions. Most fermented products are obtained after spontaneous fermentation or by inoculation of the raw material with a portion of a previous fermentation, as is the case in the preparation of cassava-close (Assanvo et al., 2002; Yao et al., 2009).

Also microbial succession related to the pH, the temperature of incubation of ferment used by each producer could contribute to this selection. The species of *Pediococcus* genus have optimum development at temperatures between 25°C and 40°C, the genus *Leuconostoc* between 18°C-30°C, the *Lactococcus* between 25°C and 32°C , *Lactobacillus* mesophilic 30 or 35°C, *Lactobacillus* thermophilic between 40°C and 45°C and *Streptococcus thermophilus* 42°C-43°C (Corrieu et al., 2008). It has been widely demonstrated that lactic acid bacteria come from the raw material or the environment (Steinkraus; 1983; 1997; Caplice and Fitzgerald, 1999). Environmental conditions, adaptation to the substrate and the repeated use of the same utensils contribute to their selection (Assanvo et al., 2002).

Conclusion

In conclusion, this study shows that the pH of the "Magnan" is acidic and is located between the values 5.35 and 5.58. Moreover the bacterial genera *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Pediococcus* and *Lactococcus* have been highlighted. *Lactobacillus* is the dominant genus followed by *Leuconostoc* and the least being the genus *Pediococcus*. Although these five genres are known for their interesting technological properties, extensive studies should be performed on isolates including their antibacterial power, their enzyme profile. Allowing them use as starter in the food process or as probiotic.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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