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# MICROORGANISMS ASSOCIATED WITH TRADITIONAL PLANTAIN-BASED FOOD "DOCKOUNOU" DURING SPONTANEOUS FERMENTATION

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**Abstract:** *The objective of the present study was to investigate microorganisms associated with traditional plantain-based food Dockounou during spontaneous fermentation. Five samples including rice and maize flour, over ripe banana pulp, and over ripe banana pulp/rice or maize flour mixture were fermented separately during 4 hours. Microbial load was estimated by plate count using decimal dilution on selective medium depending of each microbe. The microorganisms isolated from all samples included yeast, lactic acid bacteria and Bacillus sp. The yeast was detected at levels of values ranged 2.54 $log_{10}$ to 3.50 $log_{10}$ at biginning of spontaneous fermentation and increased with value ranged from 4.03 $log_{10}$ to 4.56 $log_{10}$ at the end of the process. The bacterial load during this spontaneous fermentation decreased to 4.50 $log_{10}$ to 2.72 $log_{10}$ for some samples. Acetic acid bacteria were not detected in all samples during the spontaneous fermentation. The metabolic activities of yeast, Bacillus sp and lactic acid bacteria during fermentation could improve the sensory, nutritive and safety quality of Dockounou.*
***Key words**: Plantain, dockounou, fermentation, yeast, lactic acid bacteria, Bacillus sp*

## 1. Introduction

Bananas (Musa AAA, AA) is one of the major food resources in the world. With an average production of 106 million tones per year [1, 2], bananas occupy the fourth in the world after rice, wheat and milk [3, 4]. Sub-Saharan Africa accounts for over a third of global production of plantains, with nearly 7 million tones per year. In Côte d'Ivoire, plantain is the third food product after yam and cassava [5]. Interest in plantain is mainly related to its nutritional quality.

In fact, plantain constitutes an agriculture product with carbohydrates accounting for 22% of fruit weight and rich in vitamins A, B6, C, minerals and dietary fibre [6, 7].

This vegetable resource contributes significantly to food security and provides more than 25 and 10% of the daily intake of carbohydrates and calories, respectively, for more than 70 million people in Sub-Saharan Africa [8, 9, 10]. However, plantain is a highly perishable fruit and difficult to keep. What is causing huge post-harvest losses estimated at nearly 60% of the annual harvest. The difficulties of plantain preservation result from its easy ripening at ambient temperature, leading to a qualitative and quantitative degradation along the distribution chain [11]. According to **Palmer** [12], the ripening of banana fruit is associated with a massive breakdown of starch, pectin, cellulose and hemicellulosic substances concomitant with a rise of simple sugars. In Côte d'Ivoire, to reduce these post-havest losses, plantain fingers are rapidly consumed boiled, roasted and fried [7, 13, 14, 15, 16]. The over ripe fruits of plantain are transformed by farming women into dish called Dockounou in Côte d'Ivoire [14] and "Ofam" in Ghana [16] to reduce these losses. The Dockounou is a dish consists of over ripe banana supplemented with other starchy source mainly rice or maize flour [17]. Production process of this dish followed seven steps including fermentation which is still optional [18]. To date, importance of fermentation and microorganisms involved in this process during the Dockounou production are still unknown. However, **Akoa et al.** [19], in optimization of Dockounou manufacturing process, showed that the best sensory quality of this dish was obtained after 4 hours of fermentation of over ripe banana and rice or maize mixture. Therefore the aim of this study is a first time to investigate the microorganisms present in the dough of Dockounou during this fermentation.

## 2. Experimental

### *2.1. Samples Collection*

Senescent plantains fingers, maize and rice grains used in this study were purchased from Adjame market (Abidjan).

### *2.2. Samples preparation*

Samples preparation was performed according to Dockounou optimization method described by **Akoa et al.** [19]**.** The rice and maize grains were soaked in water respectively for 2 and 10 hours. After drying, the grains were grinded in a home wooden mortar and the flour was sieved (500 µm). After that, senescent plantain fingers were washed with potable water to eliminate all impurity and peeled manually using ordinary knife. Pulp crushing was made in traditional hygienic wood mortar and 450 g of this mushy pulp was mixed with 50 g of rice or maize flour**.** In all, five (5) samples were made including 500 g of rice flour, 500 g of maize flour, 500 g of over ripe banana dough, 500 g of banana pulp and rice flour mixture and 500 g of banana pulp and maize flour mixture.

### *2.3. Sample fermentation process*

Each sample was puted in a sterile container which hermetically closed with a top. All containers were placed at room temperature that was set at 25 $^{o}$ C- 28 °C and allowed to ferment for 4 hours [19]. Each hour (T0, T1, T2, T3 and T4) 10 g of each sample was tacked and analyzed for monitoring, isolation, and enumeration of fermenting flora according to AFNOR V 08-015 guidelines. Briefly, 10 g of each sample was added to 90 mL of peptone water buffer contained in a sterile Stomacher bag that was shaken for 2 min to obtain a uniform homogenate. Samples (1.0 ml) of the homogenate were serially diluted 10-fold in trypton salt buffer, from which aliquots (0.1 ml) were plated on different selective agar media that were incubated at 30 °C for 1 to 3 days in a standard incubator (Jouan, St. Herblain, France). Specific medium was used for each microorganism: MYGP (Malt yeast glucose, peptone) supplemented with chloramphenicol (1 %) for yeast, MRS (Man Rogossa and Sharpe) for lactic acid bacteria, **Duthathai et Pathom-Aree** [20] agar (0.5 % D-glucose, 1 % yeasts extract, 1 % peptone, 2 % glycerol, 1.5 % potato and 4 % ethanol (v/v) and 0.0016 % of bromocresol green) for acetic acid bacteria and nutrient agar (BioRad, France) for *Bacillus* sp isolation. Morphologically different colonies of acetic acid bacteria, lactic acid bacteria and *Bacillus* were picked up from each sample on specific medium and purified through subculturing and confirmed by using of both phenotypic (colony and cell morphology, mobility, Gram staining) and biochemical analyzes (catalase activity and oxidase activity). While yeast were identified by morphological characteristics (colony and cell morphology) after pick on MYGP agar.

### *2.4. Colonies counting*

After the incubation periods, colonies were counted by using a colony counter (JP Selecta, Spain) and results were expressed as colony forming unit per gram (cfu/g) of sample.

## 3. Results and discussion

The evolution of microbial cell counts in all samples during fermentation step can be seen in figure 1 and 2.

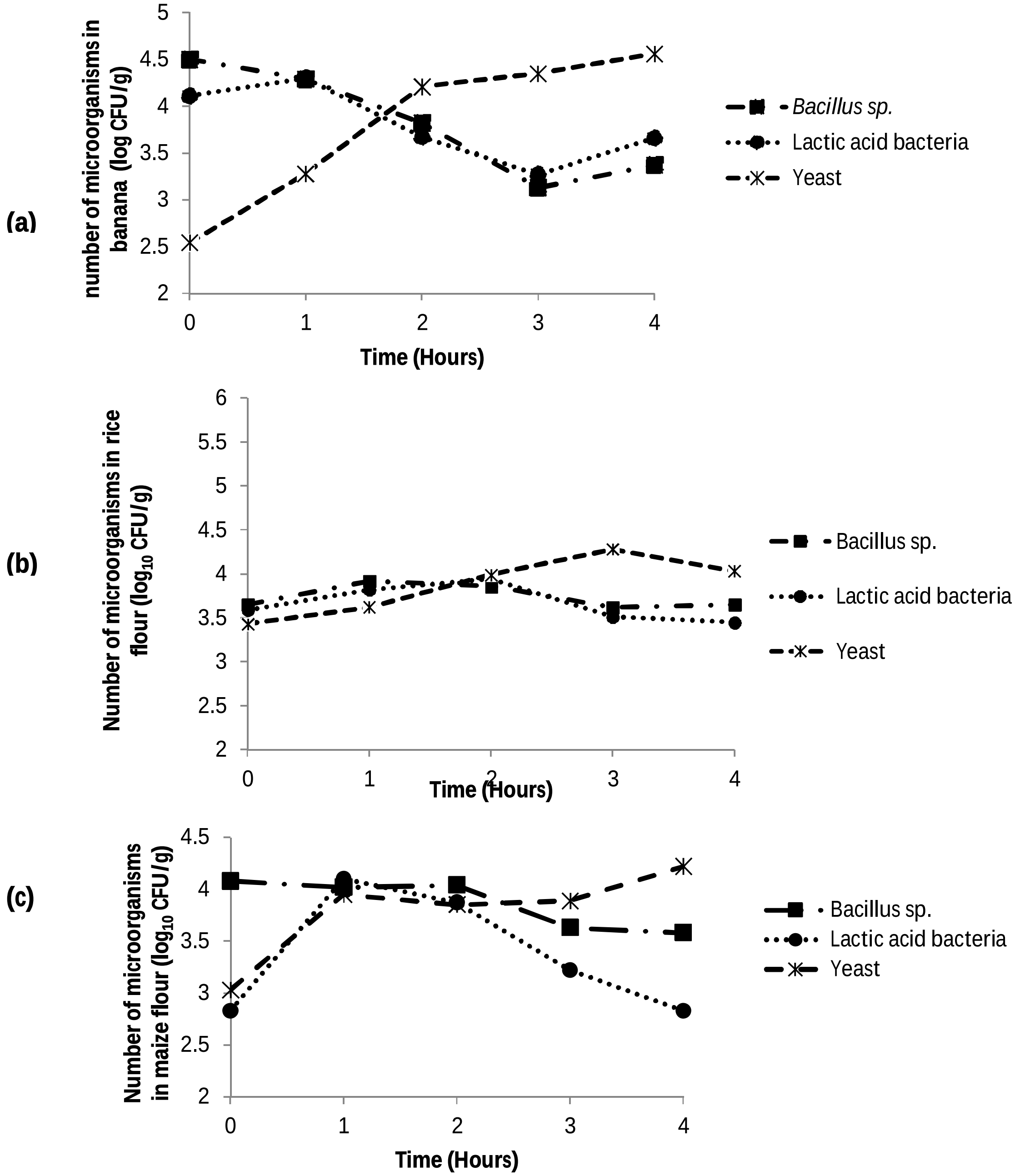
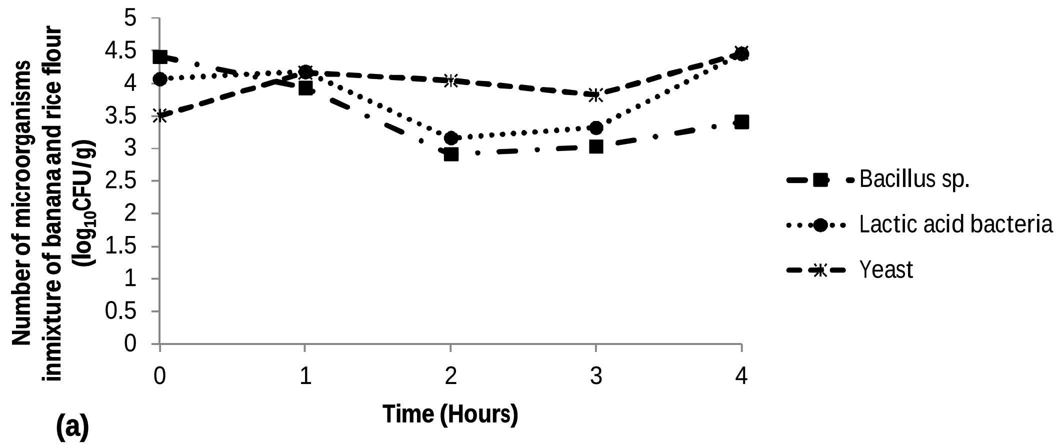



**Figure 1: Microbial growth dynamic during fermentation of banana (a), rice flour (b) and maize flour (c)**

Yeast, lactic acid bacteria and *Bacillus* sp were detected in all samples throughout the fermentation period. Additionnally, result show that yeast population increase during this process while the bacterial load decrease. Indeed, microbial load of yeast increased to a value of 2.54 to 4.56 $\log_{10}$ (CFU/g) in banana pulp (Figure 1 a), 3.43 to 4.03 $\log_{10}$ (CFU/g) in rice flour (Figure 1 b) and 3.03 to 4.22 $\log_{10}$ (CFU/g) in maize flour (Figure 1 c).

In mixture samples corresponding to Dockounou dough, the yeast population increased also during fermentation step with microbial ranged from 4.06 to 4.56 $\log_{10}$ (CFU/g) for over ripe banana pulp and maize flour mixture and 3.50 to 4.46 $\log_{10}$ (CFU/g) for over ripe banana and rice flour mixture (Figure 2).



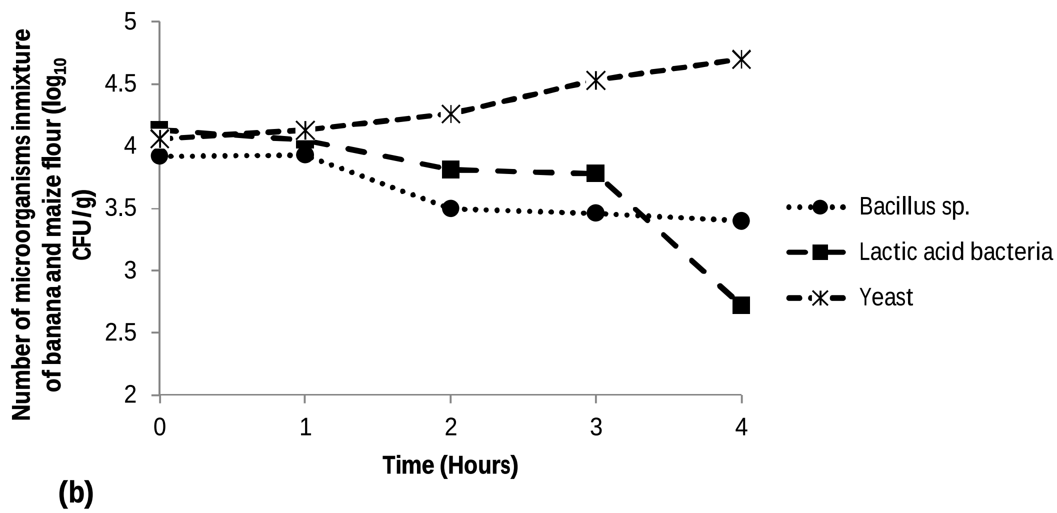




**Figure 2: Microbial growth dynamic during banana/rice flour mixture (a) and banana/maize flour mixture fermentation**

**Soro-Yao et al.** [21] was also reported that yeast, lactic acid bacteria and *Bacillus* sp are the major microorganisms implicated in production of many Ivoirian fermented food. In additionally **Kim et al.** [22] indicated an increase of yeast throughout the fermentation process of Korean traditional rice wine supplemented with banana during fermentation.

The increase of yeast population in this study could be related to the fact that banana, maize and rice contain favorable substrates (such as glucose) for the growth of yeast. Indeed during ripening of banana, starch is converted in glucose through α-amylase activity [12] while rice and maize contain starch essentially [23] which will be converted in glucose eventually [24].

According to **Amoa-Awua and Jakobsen** [25], an environment containing high sugar constituted a favorable medium for yeast. Concerning the *Bacillus* sp population, evolution of microbial load in banana, maize and rice presented globally a own phase. Indeed, the bacterial population decreased slightly during fermentation with microbial load ranged 4.50 -3.66 $\log_{10}$ (CFU/g) for the banana pulp (Figure 1 a), 3.65 -3.45 $\log_{10}$ (CFU/g) for the rice flour (Figure 1 b) and 4.08-3.58 $\log_{10}$ (CFU/g) for the maize flour (Figure 1 c).

Lactic acid bacteria population presented similar variation in the three samples. In mixture samples, the bacterial load increase within 3-4 hours for banana pulp and rice flour mixture (Figure 2 a) and remain stable during fermentation period in banana pulp and maize flour mixture (Figure 2 b).

So our results indicated that lactic acid bacteria and *Bacillus* sp are predominant at beginning of this spontaneous fermentation. This phenomen was also reported by other researchers during fermentation of variable food [21, 26, 27, 28, 29]. The slightly decrease of bacterial load during all samples fermentation is probably due to yeast activity during fermentation mainly alcohol production [30]. On the over end, variable acid (Malic, succinic, lactic and acetic acids…) which rate increase during fermentation of banana [22] contributed also to this decrease of bacterial population. However, the increase of bacterial load observed within 3-4 hours during mixture samples fermentation imply that these microorganisms could be competitive and capable to survive under certain stress conditions and eventually to express their fermentative potential during the fermentation process. Lactic acid bacteria, *Bacillus* sp and yeast are involved in many fermentation to a varying extent [31, 32, 33]. **Kim et al.** [22] and **Rhee et al.** [30] for example demonstrated the contribution of these microorganisms in improvement of Asian fermented product quality. According to **Soro-Yao et al.** [21], these microorganisms allowed improvement of food quality during fermentation step by affecting sensory properties (aroma, taste, colour, texture), enhancing food safety by inhibition of pathogens bacteria and removal undesired compounds such as mycotoxins, improving the nutritive value, and reducing energy required for cooking. Yeast are characterized by their pectinase activity and capacity to ferment glucose or other sugar into alcohol and $CO_2$.

These technological properties contributed probably in the first time to improve the texture of Dockounou by hydrolysis of pectin contain in banana and in second time to inhibit the growth pathogen bacteria in this dish during fermentation. Moreover, **Bouatenin et al.** [24], in their studies on cassava dough fermentation, reported that yeast, lactic acid bacteria and *Bacillus* are able to produce some enzymes mainly amylases.

Consequently, these microorganisms contributed probably of improvement of Dockounou quality by hydrolysis of starch contained in over ripe banana and in cereals by production of α-amylases. That allowed improving the digestibility of Dockounou and availability of fermentable sugar for ulterior acidification.

**Amoa-Awua et al.** [34] showed also the ability of some *Bacillus* sp to produce organics acid mainly lactic acid from starch and fermentable sugar. In additionally, it well-know that lactic acid bacteria produced from fermentable sugar the amount varies acid depends of the product type during fermentation [35]**.**

This food acidification during fermentation could have many advantages notably renders food resistant to microbial spoilage and the development of pathogenic microorganisms in food, produce desirable flavors and often improves nutritional value [30, 36]**.**

Therefore, this activity of lactic acid bacteria and *Bacillus* during Dokounou dough fermentation contribute certainly to improving the taste, aroma and nutritional and health qualities of the product at the end of cooking process. What could explain that Dockounou baked from fermented dough were the best sensory qualities according testers in **Akoa et al.** [19] studies. In this study, no colony corresponding to acetic acid bacteria was identified on the plate at this time during fermentation.

Absence of characteristic colony of acetic acid bacteria at the beginning (0-24 hours) of fermentation as also reported by some researchers [37, 38]. It well knows that acetic acid bacteria growth in aerobic atmosphere so the low level of oxygen in our fermenting medium is probably responsible of this growth inhibition.

## 4. Conclusion

This study allowed to known that yeast, lactic acid bacteria and *Bacillus* sp implicated in Dockounou fermentation. However, further studies are needed to characterize these microorganisms and to investigate the technological properties of each microbial group to understand their role during Dockounou dough fermentation.

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