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Simultaneous Use of Two Strains of Lactic Acid Bacteria *Lactobacillus plantarum* and *Pediococcusacidilactici* to Extend the Shelf Life of Attiéké

(Côte d'Ivoire traditional food)

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ABSTRACT: Lactic acid bacteria (LAB) have been used for centuries in the fermentation of a variety of foods. The preservative ability of LAB in foods is attributed to the production of anti-microbial metabolites including organic acids and bacteriocins. Avoiding the growth of spoilage bacteria in the Attiéké « food made in Côte d'Ivoire » is one of the major challenge for food industry in Côte d'Ivoire. In this work, two very strong bacteriocinogenic strain of (LAB) namely *Lactobacillus plantarum*, producing the bacteriocinplantaricin*and Pediococcusacidilactici* producing the bactoriocinpediocin was used as protective culture against growth of Attiéké's foodborne spoilage bacteria. These bacteria have been isolated from the attiéké, characterized amplified and produced as starters. Several tests were carried out on different samples of Attiéké for each strain taken differently or in combination to update their effectiveness in protecting of Attiéké. During storage 2.5% of each inoculum were added in powder form, the spoilage bacteria cfu count was initially reduced by approximately 3 log, but a growth rebound occurred after two or three weeks, coinciding with loss of 70% of the bacteriocin activity (result non shown). However a combination of the two strains has a better result in the conservation of the Attiéke. The observed activity levels are discussed in relation to the degree of the microbial food spoilage protectionconferred.

KEYWORDS: Attiéké, *Lactobacillus plantarum, P.acidilactici,* bacteriocin, foodborne spoilage bacteria

I.INTRODUCTION

Product resulting from the processing of cassava, Attiéké is a semolina obtained after several methods including fermentation step [8]. Even today in Côte d'Ivoire, the activity of manufacturing Attiéké offers at least seven (7) main job and at least nine (9) subsidiary jobs in the informal sector. These jobs once exclusively female seduced male skills through invigorating and promising perspectives offered by this sector[3]. Given the importance of this product in the economic sector, improve the safety of it is a major challenge for the Ivorian authorities.

To cope with the problems of contamination of foodstuffs, the development of chemistry has allowed the emergence and application of new chemicals substances as synthetic food preservatives [22]. They have been widely used to prevent food spoilage[23]. Subsequently, several synthetic preservatives have been limited in several countries because of their long-term adverse toxicological effects, including carcinogenicity[15; 5]. Similarly, the current trend of consumers to seek a more natural diet has prompted research, development and application of new natural products with antimicrobial and antioxidant activities in order to use them as alternatives to synthetic preservatives in the field of food industries.

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So taking into account the requirements of consumers and also food security standards, innovative technologies are increasingly exploited including those involving the use of lactic acid bacteria and or their bacteriocin to control spoilage bacteria responsible of the deterioration of the commercial and sanitary quality of food.
Lactic acid bacteria (LAB) are important industrially, mainly in food fermentation processes[1; 12]. It is well known that such bacteria have the potential to produce numerous antimicrobial compounds, such as acetic acid, ethanol, aroma compounds, exopolysaccharides, enzymes and bacteriocins, that increase the shelf-life and microbial safety of the end product, improve its texture, or contribute to a pleasant sensory profile[27]. It should be noted that, among the metabolites synthesized by LAB, bacteriocins are important for their strong antibacterial action. These ribosomally synthesized proteinaceous compounds typically inhibit the growth of strains closely related to the producer strain[27], competing microflora[20] and can also affect more distantly related species such as *Listeria monocytogenes*, a foodborne pathogen that has received considerable attention[20; 31; 7]. Bacteriocin, however, is sensitive to several factors limiting their effectiveness. We can mention limited bioavailability due to poor solubility (as a result of an unfavourable pH), limited diffusion, localised accumulation (as a result of binding to food particles and surfaces), the presence of inhibitors and ⁄ or proteases, and hydrophobic interactions with fats and proteins[21; 18; 25]. Evidence suggests that these numerous factors affecting negatively the effectiveness of the bacteriocin may contribute to the spoilage and pathogenic bacteria'growth rebound' observed in foods after their initial inhibition. But also to make ready-to-use starters available to manufacturers. The present work was an attempt to limit this problem.

II.MATERIAL AND METHODS

A.Bacterial strains and growth media

Lactobacillus plantarum, a producer of bactericidal, fungicidal compounds and *Pediococcoccusacidilactici*, was previously isolated from attiéké [26]. Total flora, sensitive to the bacteriocin produced by *L. plantarum and P.acidilactici* and isolated from attiéké, was used as indicator strain to bacteriocin activity measurement in Attiekes samples. *L. plantarum* were grown on de Man, Rogosa and Sharp medium (MRS broth) (Biokar, Beauvais, France), rendered selective for plasmid-containing cells by addition of streptomycin (50 mgmL-1)[9]. The same media were used to the growth of *P. acidilactici*rendered selective with streptomycin[13; 14; 24]. However, since the two strains are streptomycin-tolerant and they grow on MRS medium, it is necessary to produce a minimum medium by adding or not arginine according to the strain to be highlighted. *Total flora* was regularly spread over Plate Count Agar (Oxoid, Beauvais, France). All strains were stored at -80°C in their respective media with added glycerol (40%).

B.Using *L. plantarum*and *P. acidilacticit*o control Attieké'sspoilage bacteria growth

B.1.Attieké preparation and inoculation

Attieké's blocks were obtained from various Ivorian commercial producers. Four blocks of 200 g were aseptically transferred separately to four sterile large beaker then *P. acidilactici* (2.5% per g of attiéké) for the first beaker and (2.5% per g of attiéké) of *L. plantarum*for the second were added. In the third beaker a combination of *P. acidilacti* and *L. plantarum* were added at the rate of 2.5% per g of attiéké for each bacteria. A control without *L.plantarum and P. acidilactici* added for the fourth beaker was included at the same time under a laminar flow hood (Clean Air,VWR, Belgium). The contents of each beaker is mixed for 2 minutes using a sterile spatula before vaccum packaged to sterile Stomacher bags and storage at 4°C for six weeks.
Each experiment was performed twice and each determination was done in triplicate. Data are presented as means of two independent experiments with SD.

B.2.Attieké sampling

The Attieké were sampled at regular intervals (1, 2, 3, 4, 5 weeks) of incubation. At each sampling, 20-g samples were taken aseptically from the Stomacher bags, diluted with 10 mL sterile saline solution (0.85% sodium chloride), and pressed manually in another Stomacher bag to extract as much liquid as possible. This liquid (called the 'Attieké juice' hereafter) was then used for microbiological analyses.

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B.3.Microbiological analysis

Growth of the inoculated *L. plantarum*, *P.acidilactici* strains and Attiéké's spoilage bacteria were determined on the basis of cfu counts after homogenisation of 1 mL attiéké's juice in 9 mL peptone water, as described by[19]. A decimal dilution series was prepared and 1-mL aliquots were plated. *L. plantarum*strains were plated on streptomycin-MRS selective agar media. The same method were done with *P. acidilactici by adding* streptomycin in MRS, while Attiéké's spoilage bacteria were plated on natural MRS agar and enumerated after incubation for 48–72 h at 37°C. The enumeration of Attiéké's spoilage bacteria was done by subtracting L. plantarum strains and *P.acidilactici* view of the fact that both grow on natural MRS agar.

B.4.Statistical analysis

Each trial was repeated twice and each determination was done in triplicate. Statistical analysis (analysis of variance α = 0.05% and Student's t-test) of was done with Excel software.

III.RESULTS

Table 1 show the results of the growth of *L. plantarum*, *P.acidilactici* and the spoilage bacteria naturally present in Attiéké during storage.

As shown in the table, starting from week 1, the number of colonies of spoilage bacteria present in the attiéké samples supplemented with *P. acidilactici* reached $1{,}4.10^{.6}$cfu g^{-1} while the one added with *L. plantarum* was at $1{,}4.10^{6}$cfug^{-1}. At the same time, the number of cells of spoilage bacteria present in the samples containing the association *L.plantarum* and *P.acidilactici* is 2.10^{5}cfu/g. This value of spoilage bacteria decreases to a value of $1{,}3.10^{5}$cfu/g for P.acidilactici, lowest value at week 3. It is the same profile as with *L.plantarum* but with a value slightly higher that to say $1{,}1.10^{6}$cfu/g.

This dynamics of decay is also observed in the combination *L.plantarum* - *P.acidilactici*. Thus a drastic decrease in the population of spoilage bacteria at week 2 to a value of $1{,}1.10^{3}$cfu/g. Meanwhile the spoilage bacteria cfu count in the control kept increasing throughout the duration of the experiment reaching $1{,}9.10^{7}$cfu/ g for about 1 week and a maximum of $1{,}4.10^{9}$cfu/g before decreasing at the end of the experiment.

	Log CFU g-1 ± SD			
Time (weeks)	**Control**	***P.acidilatici* (2.5% per g of attiéké)**	***L.plantarum* (2.5% per g of attiéké)**	***L.plantarum+P.acidilatici* (2.5% per g of attiéké)**
0	6.23 ± 0.3	6.26 ± 0.01	6.23 ± 0.12	6.20 ± 0.02
1	7.28 ± 0.1	6.15 ± 0.3	6.15 ± 0.15	5.30 ± 0.12
2	9.26 ± 0.02	5.11 ± 0.16	6.04 ±0.3	3.04 ± 0.3
3	9.28 ± 0.3	5.04 ± 0.22	7.26 ± 0.21	3.65 ± 0.15
4	10.15 ± 0.21	6.70 ± 0.12	7.74 ± 0.02	4.08 ± 0.2
5	9.20 ± 0.16	7.04 ± 0.15	8.08 ± 0.03	4.70 ± 0.12

Table 1. Cell counts as a function of time: Attiéké's spoilage bacteria; *P.acidilactici* (2.5% per g of attiéké); *L.plantarum* (2.5% per g of attiéké) and *L.plantarum*+ *P.acidilactici* (2.5% per g of attiéké).

The value of spoilage bacteria is obtained by the following formula: $\boldsymbol{F_T - (F_{Lp+}F_{Pa}) = F_b}$

$\boldsymbol{F_T}$: Total flora + *L.plantarum*+ *P.acidilactici*;

$\boldsymbol{F_{Lp}}$: Total flora unless *L.plantrum*;

$\boldsymbol{F_{Pa}}$: Total flora unless *P.acidilactici*;

$\boldsymbol{F_b}$: Total flora grew.

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IV.DISCUSSION

These results demonstrate that the addition of lactic acid bacteria that are *L.plantarum* and *P.acidilactici* in the samples attiéké contributes to the elimination of Attiéké's spoilage bacteria. Taken individually lactic strains have a limiting effect on the growth of stem alterations as shown the result. Indeed, in the table, attiéké samples supplemented with strains of *L.plantarum* (2.5% per g of attiéké) exert an inhibitory action on the deterioration bacteria naturally present in the attiéké [11]**.**It is the same for the second strain lactic namely *P.acidilactici*[4]. And at week 3, the spoilage bacteria present in the control reached a rate of $1,9.10^{9}$cfu/gwhile this value declines to $1,1.10^{5}$cfu / g for samples supplemented with *P.acidilactici*against $1.8.10^{7}$cfu / g for*L.plantarum*. Considering the beginning of the experiment with *P.acidilactici*, with a value of $1,8.10^{6}$cfu /g of spoilage bacteria removal rate of 93.88% until week 3 whichcorresponds to the lowest value of spoilage bacteria and consequently the highest value of *P.acidilactici* in the attiéké sample, which is statistically significant.On the same basis of the lowest value with *L.plantarum*, an elimination rate of 64.70%.

This action of *L.plantarum* and *P.acidilactici* on the development of the spoilage bacteria means that the production of metabolites during its growth prevents the development of this flora present in the Attiéké. Indeed as pointed out many works,[32; 17; 28; 6], that produced by *L. plantarum* and *P.acidilacitici* is secreted during the exponential and early stationary growth phases of fermentation. Throughout the exponential phase the bacteriocin activity increases with the cfu count, suggesting that bacteriocin production follows primary metabolite kinetics. A drastic decrease is observed upon further incubation.[10] observed a similar decrease during the stationary phase for *Carnobacteriumdivergens* and *Carnobacteriumpiscicola* cultures in a simulated cold smoked fish system and suggested that it might be due to degradation by endogenous proteases [10; 34]induced during the growth phase and/or to adsorption of bacteriocin on the surface of producer cells [34].

The fact that the microbial population of alteration of the attiéké decreases strongly with *P.acidilactici* that *L.plantarum* shows that this strain produces a much more effective compound on these strains than does *L.plantarum. I*ndeed, *P.acidilactici* besides being an acidifying strain produces the bacteriocin in the growth that has a strong bactericidal potential[33].

In all test samples, an increase in spoilage bacteria counts was noted after they have reached their lowest values. Such an increase (i.e. rebound phenomenon) was observed from week 4 with *P.acidilactici*. This phenomenon takes place a week rather with the samples treated by *L.plantarum*. The steady decrease in the bacteriocin activity during storage may explain the spoilage bacteria growth recovery (Table 1). Inactivation of bacteriocins in food has been attributed to indigenous or microbial proteases[21]. But this can also be attributed to an adaptation of the spoilage bacteria to sublethal injury[21; 35] spontaneous development of resistant mutants [35; 30] interactions with food constituents[2; 16]. However, the combination of both lactic acid bacteria present better results in the development of the spoilage bacteria of Attiéké seen the results in the table. Indeed, the early experience with $1,6.10^{6}$cfu / g counted at week 2 which gives lower values of alteration or stem $1,1.10^{3}$cfu / g, the discount rate is 99.99% . Seeking appropriate combination of probiotic strains capable of ensuring the harmlessness of food and keep them longer was the subject of numerous studies in recent years. Indeed the work of[29] showed the action of its two combined strains on the development of *Escherichia coli*. As well as the work of [11]showed that the use of the starter composed *Lactobacillus plantarum*, *Rhyzopusoryzae* spores, reduced the duration of retting by five and to improve the microbiological quality of cassava by eliminating the development of pathogens.

Although comparing the results of the combined action of the two strains with those taken individually shows that the synergistic action leads to a better action on the development of the alteration flora present in the attiéké.

V.CONCLUSION

The combination of *Pediococcusacidilactici* and *Lactobacillus plantarum* has improved the control of the spoilage bacteria in our traditional Attiéké food as compared with their utilization separately. However, the synergistic action of the two LCAB provided more efficient and a longer protection of Attiéké food from the growth of the spoilage bacteria. Therefore the use of lactic strains is a path to explore to keep our traditionalAttiéké food healthy but also to extend its shelf life.

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