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Biopreservation against Fungi Contamination of Traditional Food (*Iassa*) Produce by Fermentation of *Hibiscus sabdariffa* Seeds

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Abstract Uncontrolled fermentation is a method of transformation which can expose food to risks of contamination by moulds and mycotoxins. Food contaminations represent a potential threat to the health of consumers. In the far North of Cameroun, *Iassa* obtained by spontaneous fermentation of *Hibiscus sabdariffa* grains belongs to this food category. This study was aimed at preserving the safety quality of *Iassa* against fungal growth during fermentation by using lactic acid bacteria (LAB). To carry out this work, moulds and LAB were first isolated from *Iassa* samples collected in the locality of Gamboura (Cameroon). This was followed by the production of *Iassa* at the laboratory which was inoculated with moulds and LAB, and the entire batch was left to ferment for 120 hours at 25°C. The antifungal activity of LAB and titratable acidity were evaluated during fermentation. The results showed that, *Iassa* is a favourable food to the development of moulds. High contamination of this product by moulds of the genus *Aspergillus* and *Fusarium* were observed. Generally, this contamination ranged from 2.9Log_{10}CFU/g to 6.9Log_{10}CFU/g. Among the isolated LAB, *Lactobacillus brevis* and *Lactobacillus paracasei* were identified as the best LAB with good antifungal activity. *Lactobacillus brevis* inhibited completely the growth of *Aspergillus* in *Iassa* after 96 hours of fermentation. During this assay, a high growth of *Lactobacillus brevis* varying between 12.0 and 13.3 Log_{10}CFU/g was also observed. Unlike *Lactobacillus brevis*, *Lactobacillus paracasei* produced a high quantity of acidity evaluated at 0.9%. From this work, these bacteria can be used as a LAB starter to ensure safety of the *Iassa* against moulds after 96 hours of fermentation.

Keywords Fermentation, *Hibiscus sabdariffa*, Antifungal activity

1. Introduction

In many African countries, the seeds of *Hibuscus sabdariffa* are used as condiments during the preparation of traditional food. The name of these spices varies from one country to another. In Niger, Mali, Sudan and Cameroon this condiment is respectively called *Dawadawa botso, Datou, Furundu* and *Mbuja* [1]. In the Far North of Cameroon (Gamboura), these same seeds of *Hibuscus sabdariffa* are used for the production of a fermented food called *Iassa.* This product, little known beyond this particular area where it is produced [2], is a very popular food and widely consumed throughout the year by local populations. It is consumed as a sauce accompanied with red millet or corn *fufu* on special occasions.

According to local customs, *Iassa* must be automatically consumed by women who have just given birth in order to restore their lost strength during childbirth and for the maintenance of the baby during breastfeeding which can sometimes span over 2 years.

Thus, due to the high protein content (39.5g/100g) of *Hibiscus sabdariffa* seeds, *Iassa* contributes significantly to the dietary needs of consumers. Unfortunately, studies carried out on fermented products obtained from *Hibuscus sabdariffa* seeds showed the bad sanitary quality of these products.

Moreover, studies conducted on the hygienic quality of *Soumbala,* a condiment obtained by fermentation of the seeds of *Hibiscus sabdariffa* and *Parkia biglobosa* in Burkina Fasso have revealed the presence of the moulds in this food [4]. Likewise, the analysis of *Mbuja* consumed in the North region of Cameroon has shown the presence of spores of *Bacillus cereus* and *enterococci* [5]. In addition, traditionally fermented foods are generally contaminated with acid tolerant fungi species as *Penicillium*, *Fusarium* and *Aspergillus* [6]. Given the importance occupied by *Iassa* in the diet of these local populations and the potential dangers it represents, it would be wise to seek solutions to this problem of fungal contamination during spontaneous fermentation. However, the solutions to this problem should be part of the production system for this food. The use of lactic acid

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bacteria starters widely involved in traditional food fermentation has demonstrated their ability to inhibit the growth of moulds during fermentation [7,8] through the secretion of many antifungal compounds such as organic acids, diacetyl, bacteriocins, hydrogen peroxide, alcohols and reuterin [9]. In order to improve the safety of *Iassa*, the present study was aimed at evaluating the antifungal activity of lactic acid strains during fermentation of *Iassa.*

2. Material and Methods

2.1. Sample Collection

The *Iassa* samples were collected in Gamboura, located in the Department of Mayo-Tsanaga in Cameroon and Mokolo (Far North) as its main town. A total of 30 samples of *Iassa,* weighing 6000g, were collected and stored in sterile plastic bags for various analysis. The figure 1 shows what the *Iassa* samples look like.

Figure 1. Sample of *Iassa*

2.2. *Iassa* Production Investigation

An investigation carried out with the producers made it possible to reproduce the *Iassa* production process in the laboratory. Firstly, *Hibiscus sabdariffa* seeds were washed and cooked on fire to make them tender. Then, these seeds were left to ferment in a container for 5 days at room temperature. This allows the bacteria to use by fermentation, the sugars present in the product. After this period, the fermented seeds are finely ground on stone and mixed with basic leachate obtained from the ash of dry cotton seeds. This step directs microbial metabolism towards intense proteolytic activity, promoting the production of metabolites such as various amino acids and ammonia. These changes contribute to the improvement of its organoleptic characteristics appreciated by consumers. The figure 2 gives the production process of the *Iassa*.

2.3. Isolation of Microorganisms

To isolated Lactic acid bacteria and mould, 10g of fermented *Iassa* sample was diluted in 90ml of sterile saline water (0.85% NaCl) to prepare the first dilution. 0.1 ml of each dilution was plated on the surface of Potato Dextrose Agar (PDA) to isolate mould and on De Mann, Rogosa and Sharpe Agar (MRS) supplemented with benzimidazole to isolate lactic acid bacteria. The MRS Petri dishes were incubated in a jar containing a candle for creation of anaerobic conditions, and all the dishes were incubated at 37°C for 24 at 48 hours. For the mould, PDA dishes were incubated at 25°C for 3 to 5 days [10,11].

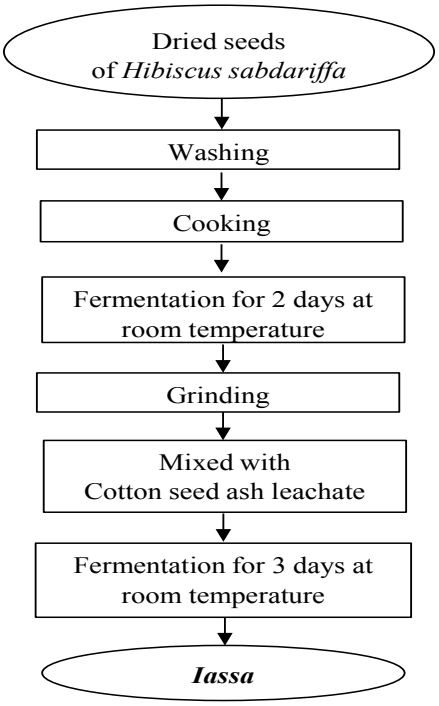

Figure 2. The process of *Iassa* preparation

2.4. Screening of Antifungal Lactic Acid Bacteria

Firstly, the isolated moulds of *Iassa*, were cultivated on the PDA agar for 7 days until sporulation. The spores were then collected using a sterile platinum loop and introduced into 10ml of sterile physiological water. The stock solution obtained was mixed with tween 80 (0.1g/100ml). Dilutions of the stock solution were made and a count using the Malassez cell was performed to assess the concentration of the spores. Suspensions of 10^5CFU/ml were prepared and stored at 4°C. The lactic acid bacteria were streaked in the middle of Petri dishes containing MRS agar. The dishes were incubated for 48 hours at 37°C. After this time, the preparation was covered with 10 ml of Mueller Hinton medium (MH) contaminated with 10^5spores/ml of mould. Then, the entire preparation was incubated at 25°C for 48hours. The presence of an inhibition zone made it possible to select lactic acid bacteria which have antifungal activity [12].

2.5. Quantitative Test of Antifungal Lactic Acid Bacteria

This test permitted to choose among the bacteria selected, those having the best diameter of inhibition. First, a well of 6mm was drilled in the centre of each Petri dish containing 20mL of MRS agar, using a sterile tip. Then, 20µl of a lactic acid bacteria inoculum of concentration 10^9CFU/ml

was mixed with 30µl of the MRS agar and introduced using a micropipette into each well. After solidification, the dishes were incubated at 37°C for 48 hours. After incubation, each preparation was covered with 10mL of contaminated MH medium with a spore concentration estimated at 10^5spores/ml. The Petri dish was incubated at 25°C for 48 hours. The zone of inhibition formed was measured using a calliper and expressed in mm [13].

2.6. Characterization of Mould

The isolated moulds were first cultured onto PDA-Chloramphenicol medium (250 mg/l) and incubated at 25°C for 7 days. The characterization was based on macroscopic studies on the appearance, colour and the texture of the mycelium. The Microscopic studies were also carried out on the shape of the vegetative thallus, conodiophore, and head morphology [10].

2.7. Molecular Identification of Active Lactic Acid Bacteria

The pure strains of lactic acid bacteria isolated from *Iassa* were multiplied in an MRS broth at 37°C for 48 hours. The culture obtained was then centrifuged at 5000 rpm for 5 minutes. The pellet formed was collected for DNA extraction, which was carried out with the Qiaamp cador pathogen kit (qiagen kit supplied by Biozyme, Romania) according to the manufacturer's instructions.

The PCR amplification was perform with the primers LacF (5'-AGCAGTAGGGGAATCTTCCA-3') and LacR (5'-ATTCCACCGCTACACACATG-3') of 20 base pairs. These primers are specific to the group of lactic acid bacteria and have been amplified by the program proposed by Ponnusamy *et al.* [14] in a multigene thermocycler (mycycler thermal cycle, BIO RAD, Hercules, USA). The PCR reaction product was made up to a final volume of 50 µl by mixing 5 µl of MgCl2, 1 µl of dNTPs, 2.5 µl of each primer, 0.25 µl of Taq polymerase (Promega corp., USA) and 10 µL of DNA. The program used was as follows: initial denaturation at 94°C / 2 minutes; 30 denaturation cycles at 94°C / 15 seconds, hybridization at 51°C /15 seconds, elongation at 72°C /30 seconds; final elongation at 72°C/7 minutes [15]. To separate and characterize the PCR products, 10 µL of this product were migrated by electrophoresis with 2% agarose containing ethidium bromide (7g/L). The amplified DNA was sequenced by Base Clear Netherlands and the sequences were submitted to the NCBI for their identification and recorded in the online database of the NCBI.

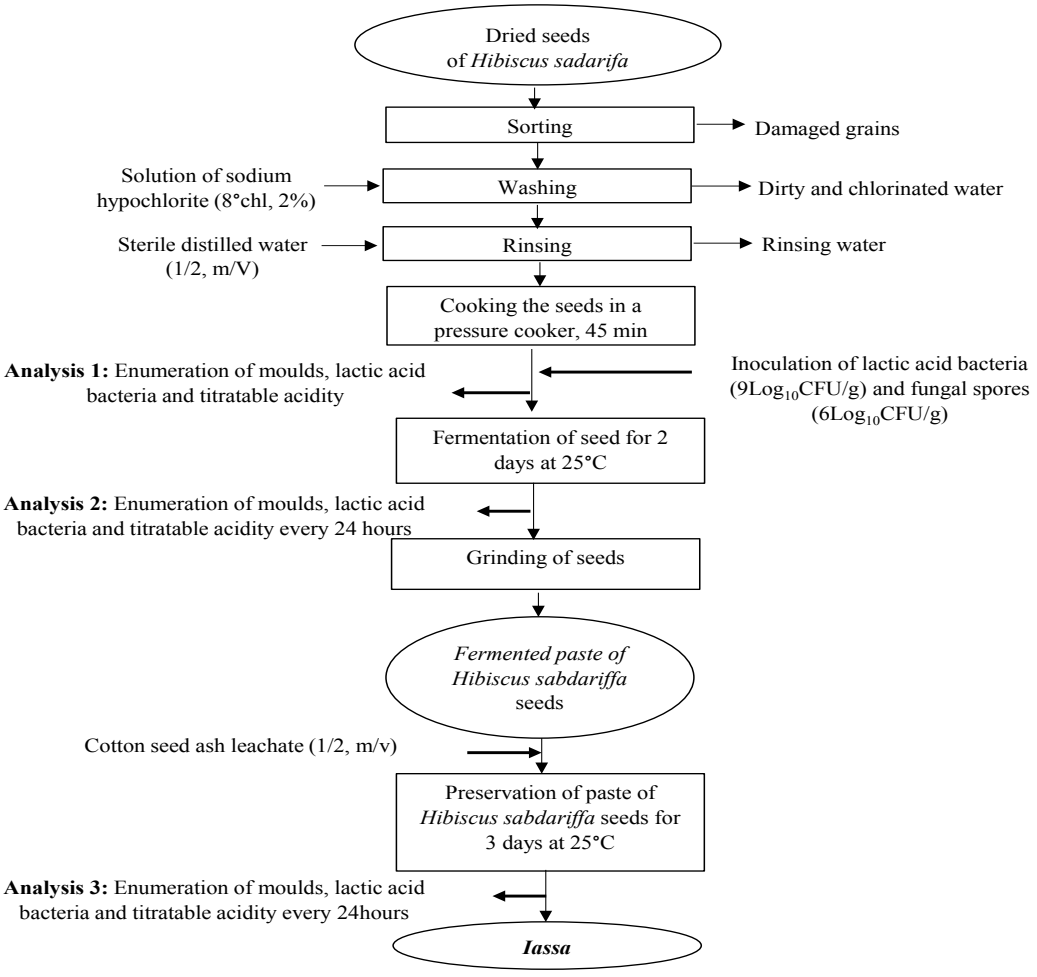

Figure 3. Process for the preparation and inoculation of *Iassa*

2.8. Antifungal Activity of Lactic Acid Bacteria during the Fermentation of *Iassa*

2.8.1. Production of *Iassa* and Inoculation

The seeds of *Hibiscus sabdariffa* which are used for the preparation of *Iassa* were first sorted, winnowed and disinfected in 2% aqueous solution of sodium hypochlorite (8°Chl) for one minute at room temperature in order to eliminate natural contaminants. The disinfected seeds of *Hibiscus sabdariffa* were then rinsed thoroughly with sterile distilled water (1/2, V/V) to remove traces of sodium hypochlorite. Subsequently, these seeds were cooked in a pressure cooker during 45 minutes. 200g of cooked *Hibiscus sabdariffa* seeds were inoculated with 2ml of inoculum of lactic acid bacteria and 2ml of mould spores with a concentration of 10^9 CFU/ml and 10^6 CFU/ml respectively. At the same time, control preparation was inoculated only with fungal spores. After this inoculation, 20g of the preparation were taken immediately for the enumeration of moulds, lactic acid bacteria and titratable acidity. The rest of the preparation was covered and incubated at 25°C for 2 days for the first fermentation. At the end of this fermentation, the seeds of *Hibiscus sabdariffa* were crushed aseptically and 20 g were again taken to carry out the same analysis mentioned above. The rest of the crushed seeds were added with cotton ash leachate (1/2, m/V). The wet paste obtained after the addition of leachate was fermented for 3 days at 25°C. Analyses were performed every 24 hours for the 3 days. The process for the preparation and inoculation of *Iassa* is shown in figure 3.

2.8.2. Enumeration of Microorganisms during the Fermentation of *Iassa*

Lactic acid bacteria and moulds were first isolated from the dilutions as described earlier in this study. The microorganism count was done in Petri dishes containing between 30 and 300 colonies. The microbial loads were expressed in CFU/g using the formula below:

$$\mathbf{C} = \frac{\sum \mathbf{N}}{\mathbf{1,1} * \mathbf{Fd} * \mathbf{V}}$$

C: Microbial concentration (CFU/g);

N: Total colonies counted on a Petri dish of two successive dilutions;

Fd: Dilution factor of the smallest dilution;

V: seed volume (ml).

3. Statistical Analysis

The results obtained were analysed using Statgraphics 5.0 software for analysis of variances, calculation of means and standard deviations. Sigma Plot 11.0 software use for graphical representation of data.

4. Results and Discussion

4.1. Evaluation of the Fungal and Lactic Profile of *Iassa*

After analysis, the results revealed the presence of LAB in the *Iassa*. The contamination of *Iassa* by moulds was also noted. 71 moulds were isolated from samples analysed, thus contaminating 80% of samples collected. The evaluation of the degree of contamination shows a strong contamination of certain samples of the *Iassa* with the concentrations of the fungal flora ranging between 2.9Log_{10}CFU/g and 6.9Log_{10}CFU/g. However, samples S7, S8, S9, S10, S11, S13, S16, S19, S20, S23 and S28 were the most contaminated with concentrations between 5.5 and 6.9 Log_{10}CFU/g. The moderately contaminated samples made of S12, S17, S18, S21, S22, S25, S26, S27, S14, S24 and S29 with concentrations between 2.9 and 4Log10CFU/ml. While the samples ranging from S1 to S6 were not contaminated with mould. Table 1 shows the contamination rate of *Iassa* by moulds.

Table 1. Contamination of *Iassa* by mould

Concentration of mould in *Iassa*					
Samples	**Concentration (Log_{10}CFU/g) and number of moulds**	**Samples**	**Concentration (Log_{10}CFU/g) and number of moulds**	**Samples**	**Concentration (Log_{10}CFU/g) and number of moulds**
S 1	*Abs*	S 11	6.43 (2)	S 21	4.95 (3)
S 2	*Abs*	S 12	4.43 (3)	S 22	4.95 (4)
S 3	*Abs*	S 13	5.56 (3)	S 23	6.65 (3)
S 4	*Abs*	S 14	2.95 (3)	S 24	2.95 (4)
S 5	*Abs*	S 15	2.65 (2)	S 25	4.95 (1)
S 6	*Abs*	S 16	5.26 (5)	S 26	4.95 (4)
S 7	6.95 (3)	S 17	3.95 (2)	S 27	3.40 (3)
S 8	5.43 (1)	S 18	4.11 (4)	S 28	5.43 (4)
S 9	6.80 (2)	S 19	6.26 (2)	S 29	2.95 (2)
S 10	6.08 (3)	S 20	6.95 (5)	S 30	5.26 (3)

Abs : Absent ; S : Sample

Table 2. Macroscopic and microscopic characteristics of isolated moulds from *Iassa*

Macroscopic and microscopic characteristics of isolated moulds from *Iassa*								
		Macroscopic observations			Microscopic observations			
Moulds	Codes	Recto	Verso	Aspect	Type of hypha	Conidiophore	Head morphology	Genus
Group 1	**M0,** M01, M02, M03, M04, M05, M06, M07, M08, M09, M010, M011, M012, M013, M014, M015, M016, M017	Green	yellow	Cottony	septate hypha	Short and not septate	Aspergillary head	*Aspergillus sp*
Group 2	**M1,** M11, M12, M13, M14, M15	Brown	Brown	Powdery	septate hypha	Short and not septate	Aspergillary head	*Aspergillus sp*
Group 3	M2, M21, M23, M24, M25, M26 M27, M28, M29, M211, M212, M213, M214, M215	Brown	Beige	Cottony	septate hypha	Long and not septate	Aspergillary head	*Aspergillus sp*
Group 4	M3, M31, M32, M33	Black	Black	Glabrous	septate hypha	Long and not septate	Aspergillary head	*Aspergillus sp*
Group 5	M4, M41, M43, M44, M45, M46, M47, M48, M49, M411	White	Yellow-orange	Cottony	septate hypha	Long and not septate	Aspergillary head	*Aspergillus sp*
Group 6	M5, M51, M53, M54, M55, M56	Greenish	Orange	cottony	septate hypha	Long and not septate	Aspergillary head	*Aspergillus sp*
Group 7	M6, M61, M63	Green	Milky white	Cottony	septate hypha	Short and not septate	Aspergillary head	*Aspergillus sp*
Group 8	**M7,** M71, M72, M73, M74, M75, M76, M77, M78, M79	Green	Orange	Powdery	septate hypha	Short and not septate	Aspergillary head	*Aspergillus sp*

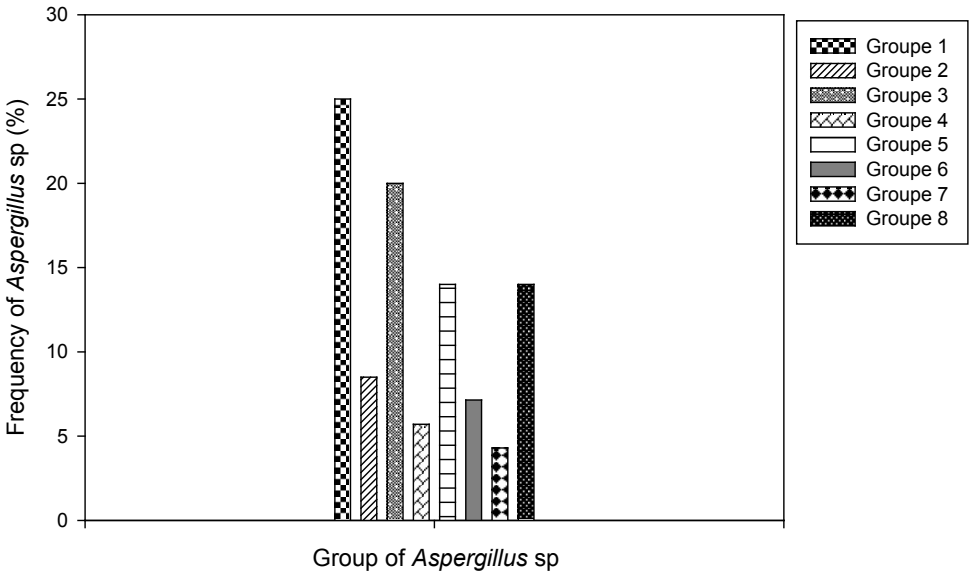

Figure 4. Frequency of appearance of *Aspergillus* sp

Microscopic and macroscopic observations of these fungal contaminants made it possible to group them into genus based on their resemblance. From this classification, two genera have been identified. *Aspergillus* and *Fusarium* with frequencies of 99% and 1% respectively. These genera are a major concern for the safety of cereals and fruits [6]. The dominance of *Aspergillus* in foodstuffs stored in regions with a warm climate is well known [16]. *Aspergillus* is an ubiquitous fungus able of colonizing various substrates and has a great capacity for sporulation [17]. This strong contamination of *Aspergillus* in the *Iassa* could be explained by temperature (28.5°C) of the locality which corresponds to the optimal growth temperatures of *Aspergillus* (25-30°C). From macroscopic observations, the genus *Aspergillus* was divided into 8 groups according to the colour and appearance of the mycelia on PDA medium (Figure 4). The great heterogeneity observed among *Aspergillus* could be influenced by many factors such as temperature, humidity, water activity, chemical composition [18]. Among the groups of *Aspergillus* identified, certain

groups of *Aspergillus* are strongly represented more than others. This includes group 1 which is the most represented with 25% (18) and is followed by group 3 with 20% (14) as well as groups 5 and 8 with 14% (10). A mould was chosen from each of these groups to evaluate the antifungal activity of LAB against the selected mould. Table 2 shows the macroscopic and microscopic characteristics of the isolated moulds of *Iassa.*

4.2. Screening of Active Lactic Acid Bacteria

Table 3. Antifungal activity of lactic acid bacteria

	Moulds			
Lactic acid bacteria	***Aspergillus* M0**	***Aspergillus* M1**	***Aspergillus* M3**	***Aspergillus* M7**
Lab 1	+	+	+	+
Lab 2	-	++	+	+
Lab 3	+++	+++	+++	++
Lab 4	+	+	-	-
Lab 5	+++	++	++	++
Lab 6	+	-	-	+
Lab 7	++	-	+	+
Lab 8	-	++	-	-
Lab 9	+++	+++	+++	+++
Lab 10	+	-	-	+
Lab 11	+	++	+	+
Lab 12	-	-	+	+
Lab 13, Lab 14	-	-	-	-
Lab 15	+++	+++	++	+
Lab 16	-	+	+	++
Lab 17	+	++	+	+
Lab 18	-	++	+	+
Lab 19	+	-	-	-
Lab 20	-	-	+	-
Lab 21	+	-	+	-
Lab 22	+++	++	++	+++

- : Absence of inhibition; + : Zone of inhibition between 0,1-3% of Petri dish area; ++ : Zone of inhibition between 3-8% of Petri dish area; +++ : Zone of inhibition greater than 8% of Petri dish area

After analysis, 53 LAB were isolated from the *Iassa*. The catalase tests, macroscopic and microscopic criteria, permitted to select and regroup these bacteria into 22 LAB. The presence of LAB in *Iassa* confirms the results of several authors who isolated LAB in foods obtained from the fermentation of *Hibiscus sabdariffa* seeds [4,5]. The confrontation of these LAB with *Aspergillus* revealed that out of the 22 lactic acid bacteria isolated, 20 LAB showed antifungal activity on at least one mould. The intensity of this antifungal activity varies from one LAB to another and from one mould to another. Among the 22 LAB tested, 05 showed an inhibition greater than 8% on the surface of the Petri dish. These were Lab3, Lab5, Lab9, Lab15 and Lab22. The LAB Lab2, Lab11, Lab17 and Lab18 had an inhibition zone between 3 and 8% on the surface of the Petri dish. In the rest of this study, the 05 LAB which exhibited the best antifungal activities were selected for the determination of their inhibition diameter. Table 3 shows the antifungal power of the 22 lactic acid bacteria retained after characterisation.

4.3. Determination of the Inhibition Diameter

In general, the LAB selected had antifungal activity on all of the moulds tested. However, this antifungal activity depended on the strain of LAB and the type of mould. It appears that Lab9 presented the best inhibitions on the moulds tested. It is followed by Lab3. However, Lab15 registered a greater inhibition diameter when it was tested on *Aspergillus* M1 (36 ± 0.7mm). The smallest inhibition was observed with Lab5 when it was tested on *Aspergillus* M7 (8 ± 0.0 mm). Given the efficiency of Lab3 and Lab9, they were chosen for the rest of the work. Table 4 shows the diameters of inhibitions induced by the different LAB selected.

Table 4. Determination of the inhibition diameter (mm)

	Lactic acid bacteria				
Moulds	Lab22	Lab3	Lab9	Lab5	Lab15
***Aspergillus* sp M0**	22±1.0	34±0.5	34±1.0	32±1.0	24±0.5
***Aspergillus* sp M1**	18±0.5	30± 1.0	32±1.0	22±0.5	36±0.7
***Aspergillus* sp M3**	17±0.7	20±0.7	20±0.7	10±1.0	16±1.0
***Aspergillus* sp M7**	22±0.5	10±1.0	25±1.0	8±0.0	12±1.5

4.4. Molecular Identification of Lactic Acid Bacteria

At the end of agarose gel electrophoresis, the DNA of Lab3 and Lab9 had the same molecular weight (340 base pairs). After sequencing, Lab3 was identified as *Lactobacillus paracasei* with 97.73% and Lab9 lactic bacteria as *Lactobacillus brevis* with 97.54%. The studies of Swain *et al.* [19] have demonstrated the presence of *Lactobacillus brevis* in fruits, vegetables and plants. In addition, *Lactobacillus brevis* has been found in *Mbuja,* a condiment obtained from the seeds of *Hibiscus sabdariffa* [5] and in many non-alcoholic starch products based on cereals [20]. *Lactobacillus paracasei* has also been identified in *Kefir*, a food traditionally fermented in Brazil [21] and in cocoa beans fermented in Cameroon [22].

4.5. Antifungal Activity of Lactic Acid Bacteria during the Fermentation of *Iassa*

The results of the antifungal activity of *Lactobacillus paracasei* and *Lactobacillus brevis* in the *Iassa* contaminated by *Aspergillus* M0 and *Aspergillus* M3 as well as the level of titratable acidity are shown in figure 5.

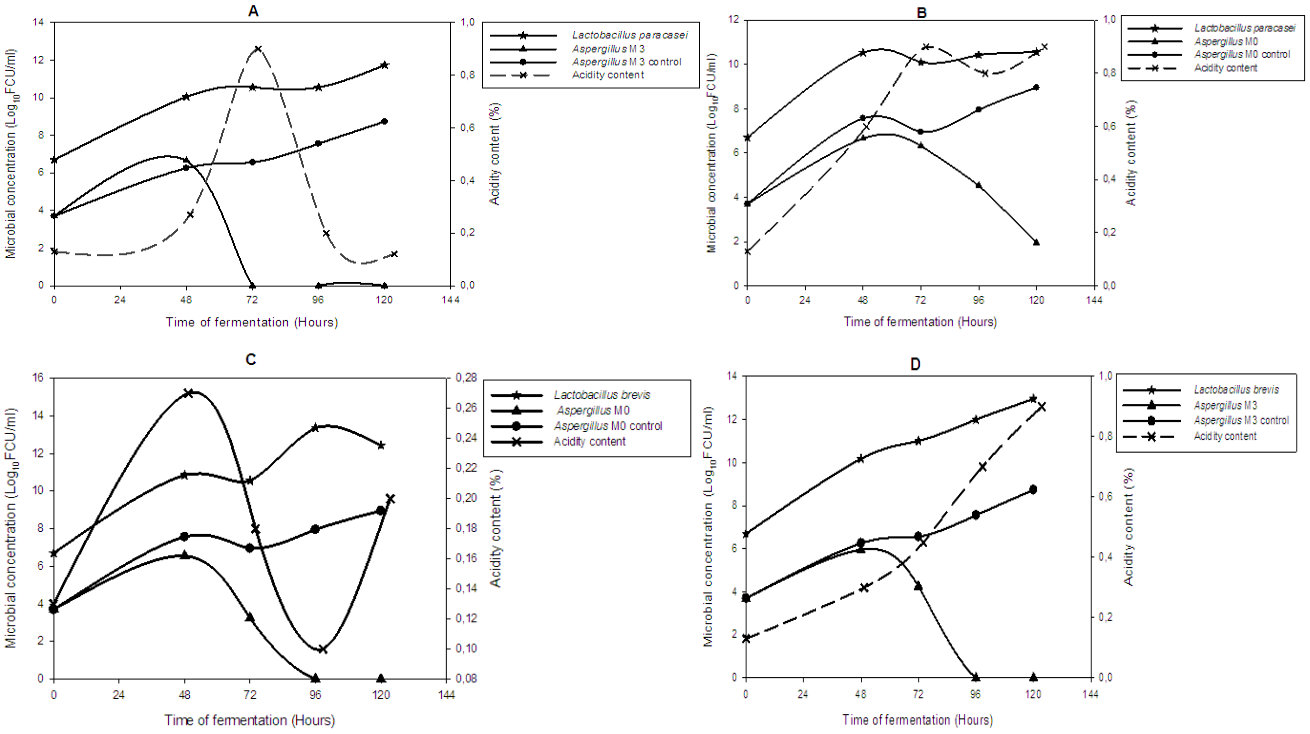

Figure 5. Inhibition of the growth of *Aspergillus* M3 and *Aspergillus* M0 by *Lactobacillus paracasei* (A and B) and *Lactobacillus brevis* (C and D) and evolution of the acidity content in the *Iassa* during 05 days

During the first 48 hours of the fermentation of *Iassa, Aspergillus* M0 and *Aspergillus* M3 continued to multiply, despite inoculation with *Lactobacillus paracasei* and *Lactobacillus brevis*. In the course of these 48 hours of fermentation, *Aspergillus* M0 recorded a concentration of 6.6Log10CFU/g and 6.5Log10CFU/g respectively in the *Iassa* treated with *Lactobacillus paracasei* and *Lactobacillus brevis.* Whereas *Aspergillus* M3 registered a concentration of 6.6Log_{10}CFU/g and 5.9 Log_{10}CFU/g respectively in the *Iassa* treated with *Lactobacillus paracasei* and *Lactobacillus brevis*. However, beyond the 48 hours of fermentation, we observed the onset of the reduction in growth of *Aspergillus* M0 and *Aspergillus* M3 in the *Iassa* treated with *Lactobacillus paracasei* and *Lactobacillus brevis*. It should also be noted that this percentage of reduction observed varied according to the mould and the LAB tested. Among the two moulds studied, *Aspergillus* M3 was the most sensitive in the presence of *Lactobacillus paracasei and* it was completely inhibited by *Lactobacillus paracasei* after 72 hours of fermentation. However, it remained less efficient against *Aspergillus* M0 since it wasn't completely inhibited at the end of fermentation period (78%). Indeed, certain microorganisms have the capacity to develop natural resistance or to acquire resistance by mutation or genetic exchange in order to survive and continue their development [23]. Unlike *Lactobacillus paracasei*, *Lactobacillus brevis* totally inhibited the growth of *Aspergillus* M0 and *Aspergillus* M3 in *Iassa* after 96 hours of fermentation. The work carried out by Tchikoua *et al.* [8] on fermented corn pasta has demonstrated the ability of *Lactobacillus brevis* to completely inhibit *Aspergillus flavus* after 120 hours of fermentation. A similar study carried out on the antifungal activity of 4 strains of *Lactobacillus brevis* in *Katak*, milk traditionally fermented in Bulgari, showed the total elimination of *Penicillium claviforme*, *Aspergillus awamori* and *Aspergillus niger* after 24 hours of fermentation [24]. The antifungal activity of *Lactobacillus paracasei* and *Lactobacillus brevis* observed could be linked to the production of organic acids in *Iassa* during fermentation. Organic acids are considered to be the main metabolites of LAB which considerably affect the growth of fungi by inhibiting mycelia growth. This statement is in conformity with results of this study which show a correlation between the acid produced by microorganisms and the reduction of mould growth. We note for example, when the acidity rate in the *Iassa* treated with *Lactobacillus paracasei* increases to 0.9% after 72h, *Aspergillus* M3 was inhibited completely. It is also at this time, that we observed the degradation of *Aspergillus* M0. Unlike the *Iassa* treated with *Lactobacillus paracasei* where there is a large production of acidity after 72 hours of fermentation (0.9%), *Lactobacillus brevis* recorded a low rate of acidity despite its high growth. The growth of this bacteria was evaluated to 12 and 13.3Log10CFU/g respectively in the *Iassa* contaminated with *Aspergillus* M3 and *Aspergillus* M0. However, we noted that *Lactobacillus brevis* is the only bacteria to have inhibited two moulds after 96h of fermentation. This difference is associated to the metabolism of the two microorganisms. *Lactobacillus brevis* is described as a strict

heterofermentative bacterium while *Lactobacillus paracasei* is considered as a heterofermentative lactic acid bacterium [25]. The quantity and quality of lactic acid produced would, therefore, be different. In fact, in homofermentative, the metabolism only leads to the formation of lactic acid, while in heterofermentative, it leads to the formation of lactic acid and other products such as acetic acid, formic acid, ethanol and carbon dioxide [26].

5. Conclusions

The *Iassa* produced from the fermentation of the grains of *Hibiscus sabdariffa* presents strong contamination by moulds which are mainly composed of the genus *Aspergillus*. Nevertheless, one of the lactic acid bacteria isolated from *Iassa*, *Lactobacillus brevis*, completely eliminates contaminants such as *Aspergillus* M0 and *Aspergillus* M3 after 96 hours of fermentation. The use of this bacteria as a starter during fermentation makes it possible to control and eliminate the fungal contamination before the 120 hours of fermentation of *Iassa*. Thus, this controlled fermentation guarantees the safety quality of *Iassa*.

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REFERENCES

[1] Charles, P., Bréhima, D. and Labia, I.I.O. 2008, Technology and physico-chemical characteristics of Bikalga, alkaline fermented seeds of *Hibiscus sabdariffa.*, African Journal of Biotechnology, 7 (7): 916-922.

[2] Mohamadou, B.A., Mbofung, C.M.F. and Thouvenot, D. 2007, Functional Potentials of a product from Traditional Biotechnology: Antioxidant and Probiotic Potential of *Mbuja* produced by fermentation of *Hibiscus sabdariffa seeds* in Cameroon., Journal of food technology, 5(2): 164-168.

[3] Yagoub, A.E.G.A., Mohamed, B.E., Ahmed, A.H.R. and El Tinay, A.H. 2004, Study on *furundu*, a traditional Sudanese fermented Roselle (*Hibiscus sabdariffa* L.) seed: Effect on in vitro protein digestibility, chemical composition, and functional properties of the total proteins., Journal of agricultural and food chemistry, 52:6143-6150.

[4] Marius, K.S., Aly, S., François, T., Nicolas, O., Cheikna, Z. and Alfred, S.T. 2014, Impact of traditional process on hygienic quality of *Soumbala* a fermented Cooked Condiment in Burkina Faso., Journal of Food Security, 2 (2): 59-64.

[5] Mohamadou, B.A., Mbofung, C.M.F. and Thouvenot, D. 2009, Microbiological and organoleptic profiles of *Mbuja*: A traditional condiment produced by fermentation of *Hibiscus sabdariffa* seeds in Cameroon., Journal of food technology, 7(3): 84-91.

[6] Salas, M., Mounier, J., Valence, F., Coton, M. and Coton, A. 2017, Antifungal Microbial Agents for Food Biopreservation., Microorganisms., 5-37.

[7] Etem, S. and Ignatova-Ivanova, T. 2015, Antifungal Activity of Lactic Acid Bacteria, Isolated from Bulgarian Wheat and Rye Flour., Journal of Life Sciences, 9: 1-6.

[8] Tchikoua, R., Tatsadjieu, N.L., Mbofung, C.M.F. 2015, Effect of Selected Lactic Acid Bacteria on Growth of *Aspergillus flavus* and Aflatoxin B1 Production in Kutukutu., Journal of Microbiology Research, 5(3): 84-94.

[9] Crowley, S., Mahony, J. and Van, S.D. 2013, Current perspectives on antifungal lactic acid bacteria as natural bio-preservatives., Trends in Food Science & Technology, 33(2): 93-109.

[10] Compaore, H., Sawadogo-Lingani, H., Savagado, A., Dianou, D. and Traore, S.A. 2016, Isolement et caractérisation morphologique des moisissures productrices des substances antibactériennes à partir d'aliments locaux au Burkina Fasso., International Journal of Biological and Chemical Sciences, 10 (1): 198-210.

[11] Lacanin, I., Mounier, J., Pawtowski, A., Duskova, M., Kamenik, J. and Karpiskova, R. 2007, Assessment of the antifungal activity of *Lactobacillus* and *Pediococcus* spp. for use as bioprotective cultures in dairy products., World Journal of Microbiology and Biotechnology, 33 (10): 188.

[12] Lind, H., Jonsson, H. and Schnürer, J. 2005, Antifungal effect of dairy propionibacteria-contribution of organic acids., International journal of food Microbiology, 98: 157-165.

[13] Voulgari, K., Hatzikamari, M., Delepoglou, A., Georgakopoulos, P., Litopoulou-Tzanetak, E. and Tzanetakis, N. 2010, Antifungal activity of non-starter lactic acid bacteria isolates from dairy products., Food control, 21: 136-142.

[14] Ponnusamy, K., Choi, J.N., Kim, J., Lee, S. and Lee, C.H. 2011, Microbial community and metabolomic comparison of irritable bowel syndrome faeces., Journal of Medical Microbiology, 60: 817–827.

[15] Matei, B., Salzat, J., Diguță, C.., Cornea, C.P., Gabriela, L., Utoiu, E.R. and Matei, F. 2018, Lactic bacteria strains isolated from Kombucha with potential probiotic effect. Romanian Biotechnological Letters, 20 (10): 1-7.

[16] Pitt, J.I. and Hocking, A.D. 1997, Fungi and food spoilage, Blackie Academic and Professionnal., London.

[17] Gourama, H. and Bullerman, L.B 1995, *Aspergillus flavus* and *Aspergillus parasiticus*, aflatoxigenic fungi of concern in foods and feed- A review., Journal of food protection, 58: 1395-1404.

[18] Magan, N. and Alferd, D. 2005, Conditions of fermentation of ochratoxine A in drying, transport and in different commodities. Food additives and contaminants, 1: 10-16.

[19] Swain, M.R., Anandharaj, M., Ray, R.C. and Rani, R.P. 2014, Fermented fruits and vegetables of Asia: A Potential

Source of Pobiotics., Biotchnology Research International, 19p.

[20] Yao, A.A., M. Egounlety, L.P. Kouame, P. and Thonard, 2009. Les bactéries lactiques dans les aliments ou les boissons amylacées de l'Afrique de l'Ouest: leur utilisation actuelle., Annales de médecine vétérinaire, 153, 54-65.

[21] Leite, O.M.A., Miguel, L.M.A., Peixoto, S.R., Ruas-Madieto, P., Paschoalin, V.M., Mayo, B. and Delgado, S. 2014, Probiotic potential of selected lactic acid bacteria strains isolated from Brazilian *Kefir* grains., American Dairy Science Association, 98: 3622-3632.

[22] Fossi, T.B., Bonsgsiysi, G.N., Nchanji, T.G., Wandji, S. and Ndjounkeu, R. 2016, Lactobacilli cultures against Ochratoxine A moulds isolated from cocoa in the South West Region of Cameroun., Britisch Microbiology Research Journal, 15 (1): 1-16.

[23] Bower, C.K. and Daeschel, M.A. 1999, Resistance Responses of Microorganisms in Food Environments., International Journal of Food Microbiology, 50, 33-44.

[24] Tropcheva, R., Nikolova, D., evstatieva, D. and Danova, D. 2014, Antifungal activity and identification of Lactobacilli, isolated from traditional dairy product katak., Anaerobe, 20: 1-7.

[25] Faizan, A.S., Bowen, Y., Fengwei, T., Jianxin, Z., Hao, Z. and Wei, C. 2019, Lactic acid bacteria as antifungal and anti-mycotoxigenic agents., Food Science and food safety, 12481: 1541-4337.

[26] Liu, S. 2003, Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations., International journal of food microbiology, 83 (2): 115-131.