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Improvement of Sanitary Quality of the *Digue* by Use of Lactic Starters

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Abstract *Digue* is a traditional food obtained from the addition of the paste of roasted peanuts in pumpkin juice obtained by spontaneous fermentation. This spontaneous fermentation unfortunately contributes to its alteration by molds and certain pathogenic bacteria. The aim of this work is to use lactic starters to inhibit the growth of certain pathogenic germs during controlled fermentation. A total of 24 lactic acid bacteria were isolated from the samples of *Digue* collected in the locality of Gamboura in the Far North of Cameroon. The screening allowed to retaining 02 strains of lactic acid bacteria for their best antimicrobial activities. The molecular identification of these two strains showed that they belong to the species *Lactobacillus casei* and *Pediococcus acidilactici*. The use of these two 02 lactic acid bacteria during the controlled fermentation of the *Digue* contaminated by *Aspergillus* M2, *Aspergillus* M3, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A showed that all the pathogen germs were totally inhibited by *Lactobacillus casei* after 21 days of fermentation. The same result was obtained with *Pediococcus acidilactici* on all molds and *Escherichia coli* ATCC 25922. At the end of this study, it appears that, the lactic acid bacteria *Lactobacillus casei* can be used to ensure the hygienic quality of the *Digue* after 21 days of fermentation.

Keywords: *lactic acid bacteria, Aspergillus, Escherichia coli, Salmonella enteritidis, antimicrobial activity*

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1. Introduction

The countries of sub-Saharan Africa are characterized by high population growth within a population with low financial income and having agriculture as a means of subsistence. African agricultural production is unfortunately faced with enormous post-harvest losses due to the limited means of transport from rural to urban areas and especially the lack of technological means of conservation [1]. Faced with this situation, the transformation of agricultural products into other foods meeting needs of the populations and leading to new markets is exploited by the populations to conserve part of their production and thus fight against food insecurity. The *Digue* is one of the traditional foods, allowing the populations of the Far North Cameroon to manage the undernourishment they face during long periods of drought. This product is obtained from the addition of roasted peanut paste in the fermented melon juice [2]. Among the unit operations involved in the transformation of many African agricultural products, lactic fermentation occupies a prominent place. This is indeed known to ensure the preservation of food and also to improve the organoleptic and nutritional qualities of food [3]. Unfortunately, the lactic fermentation African agricultural products is carried out in uncontrolled tropical conditions and handicapped by the lack of economic and technological means at the origin of numerous microbial contaminations of food. Among the microorganisms most implicated in the alteration of traditional products are mainly molds responsible for the production of mycotoxins [4], and pathogenic bacteria including enterobacteriaceae [5]. In the case of *Digue*, studies conducted by Tchikoua *et al*. [2] on the effect of fermentation on the microbial and nutritional composition of *Digue* have shown that the food is contaminated by three main pathogen groups. These were primarily staphylococci, enterobacteriaceae and molds respectively at concentrations of 3.2, 3.8 and 3.28 Log_{10}CFU/ml. This observation justified the interest of this study based on the inhibition of the growth of pathogenic germs by the use of lactic starters during controlled fermentation.

2. Methodology

2.1. Biological Material

The antibacterial activities were carried out with the strains of *Eschirichia coli* ATCC 25922 and *Salmonella enteritidis* 155A which were kindly offered by the Microbiology laboratory of the University of Yaounde I.

2.2. Collecting Samples

In order to isolate the lactic acid bacteria, 30 dried *Digue* samples with a total weight of 7.5kg were collected in Gamboura, located in the Department of Mayo-Tsanaga (Cameroon) and transported under aseptic conditions to the Microbiology Laboratory of the University of Yaounde I for analysis.

2.3. Isolation and Purification of Lactic Acid Bacteria

Lactic acid bacteria were isolated from the *Digue* samples. 10 g of *Digue* was crushed and introduced into an Erlenmeyer containing 90 ml of physiological water, to make the mother dilution (10^{-1}). This dilution was then used to make a series of decimal dilutions. Then 0.1 ml of each dilution was spread on the surface in Petri dishes containing 15 ml of MRS agar supplemented with 0.5% Calcium carbonate (CaCO3). All the Petri dishes were incubated at 37°C for 48 hours under anaerobic conditions. The colonies of the isolated bacteria were purified by successive seeded on MRS agar and incubated at 37°C for 48 hours [6]. Pure colonies obtained were subjected to the catalase test and microscopic observation after Gram stain. Only Gram positive and catalase negative strains were retained as lactic acid bacteria.

2.4. Isolation and Purification of Molds

The molds were isolated on Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (0.5 g/l) to avoid bacterial contamination. For this, 0.1 ml of each dilution produced was inoculated by spreading into sterile Petri dishes containing 15 ml of PDA medium. These were then incubated at 25°C for 3 to 5 days. The molds exhibiting the general appearance of the different genera were successively seeded on PDA medium and the dishes were incubated at 25°C for 5 days [7]. Pure fungal strains were seeded on tilted PDA agar, incubated at 25°C for 5 days and stored at 4°C.

2.5. Characterization of Molds

The macroscopic and microscopic characterization of the molds was carried out according to the method described by Compaore *et al.* [8], based on the appearance of the colonies, the color of the mycelium, texture, the shape of the mycelial head, the presence or not of a septate mycelium.

2.6. Molecular Identification of Lactic Acid Bacteria

The lactic acid bacteria isolated from the *Digue* were first purified by making isolation by streaks on MRS medium. The pure strains of lactic acid bacteria obtained were multiplied in MRS broth at 37°C for 48 hours. The product obtained was centrifuged at 5000 rpm for 5 minutes and the pellet formed was collected for DNA extraction.

The PCR amplification was perform with the primers LacF (5'-AGCAGTAGGGGAATCTTCCA-3') and LacR (5'-ATTCCACCGCTACACACATG-3') of 20 base pairs. These primers have been amplified 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 PCR conditions were initial denaturation at 94°C for 2 minutes followed by 30 cycles denaturation of 94°C for 15 seconds, hybridization at 51°C for15 seconds. The elongation phase began at 72°C for 30 seconds, followed by a final elongation at 72°C for 7 minutes. 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.

2.7. Antifungal Activity of Lactic Acid Bacteria

2.7.1. Preparation of Fungal Inocula

The mold spores, which were grown on PDA agar for 7 days were collected and placed in 10ml of sterile physiological water. The stock solution obtained was mixed with tween 80 (0.1 g/100ml). Dilutions of the stock solution were made and a Malassez cell count was performed to determine the concentration of spores [9].

2.7.2. Preparation of the Bacterial Inoculum

The lactic acid bacteria strains were seeded by the streaks on MRS agar and incubated at 37°C for 48 hours in the anaerobic conditions. The colonies were then taken and introduced into test tubes containing sterile physiological water. The turbidities obtained were compared with the McFarland N°4 standard corresponding to a concentration of 10^9 CFU/ml [10].

2.7.3. Screening of Lactic Acid Bacteria with Antifungal Activity

A first screening was carried out in order to retain, among the lactic acid bacteria which were previously isolated, those which have an antifungal power. To carry out this test, the lactic acid bacteria were inoculated by streaks (3.5 mm) in the middle of Petri dishes containing the MRS agar. The dishes were incubated at 37°C for 48 hours. Then, this preparation covered with 10ml of Muller-Hilton agar containing 10^5spores/ml. The dishes were again incubated at 25°C for 48 hours. The presence of a zone of inhibition around the streak of lactic acid bacteria demonstrates antifungal activity of lactic acid bacteria [11].

2.7.4. Determination of the Inhibition Diameter

The strains showed good antifungal activity were selected in order to compare the antifungal power through the determination of the diameter of inhibition. The pure colonies of lactic acid bacteria were introduced into 5 ml of MRS broth contained in test tubes. These were incubated for 48 hours at 37°C. Then, in the Petri dishes

containing the MRS agar, wells of 5mm of diameter were dug using a sterile tip. In each well was introduced 20 µl of the bacteria (10^9 CFU/ml) preparation mixed with 30 µl of MRS agar and the dishes were incubated at 37°C for 48 hours. After incubation, each preparation was covered with 10 ml of Muller-Hilton medium containing 10^5spores/ml of molds and the dishes were again incubated at 25°C for 48 hours at 37°C. The diameter of the inhibition formed was measured using a caliper [12].

2.8. Production and Inoculation of *Digue*

The pumpkin was bought at the Mfoundi market (Yaoundé, Cameroon) and were washed with javel water (8 °chl) diluted at 2% (V/V) for 15 minutes. Then, they were rinsed three times with sterile water in order to eliminate the traces of javel still present on the pumpkin. The disinfected pumpkin was cut into pieces (3 cm × 3 cm) using a sterile knife and mixed with sterile distilled water (1/1.5; m/V). The resulting mixture was evenly distributed among 12 glass jars and each jar was inoculated with a group of microorganisms. Initially, all 04 jars were inoculated with *Lactobacillus casei* (10^6 CFU/g). 02 of these jars were each contaminated with *Aspergillus* M2 and *Aspergillus* M3. The remaining 02 jars were each contaminated with Escherichia coli ATCC 25922 (10^3 CFU/g) and *Salmonella enteritidis* 155A (10^3 CFU/g). Another 04 jars were inoculated with *Pediococcus acidilactici* (10^6 CFU/g) and similarly contaminated with the pathogenic germs mentioned above. The remaining 04 jars of the 12 jars were inoculated only with *Aspergillus* M2, *Aspergillus* M3, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A serving as a positive control. After inoculation, the jars were closed and fermented for 28 days at 25°C. After this period, the fermented preparation was sieved using a sterile sieve (20 microns) and the resulting liquid, called *Yam Digue* was mixed with sterile roasted peanut paste in 1/1.5 (m/v) proportions. The product obtained in paste form after mixing will be kept for 2 days at 25°C to obtain the *Digue*. Figure 1 summarizes the production and inoculation stages of the *Digue* in the laboratory. The tests were performed every 7 days of fermentation to assess bacterial growth, fungal growth and variation in titratable acidity during 28 days of fermentation and 30 days after introduction of peanut paste.

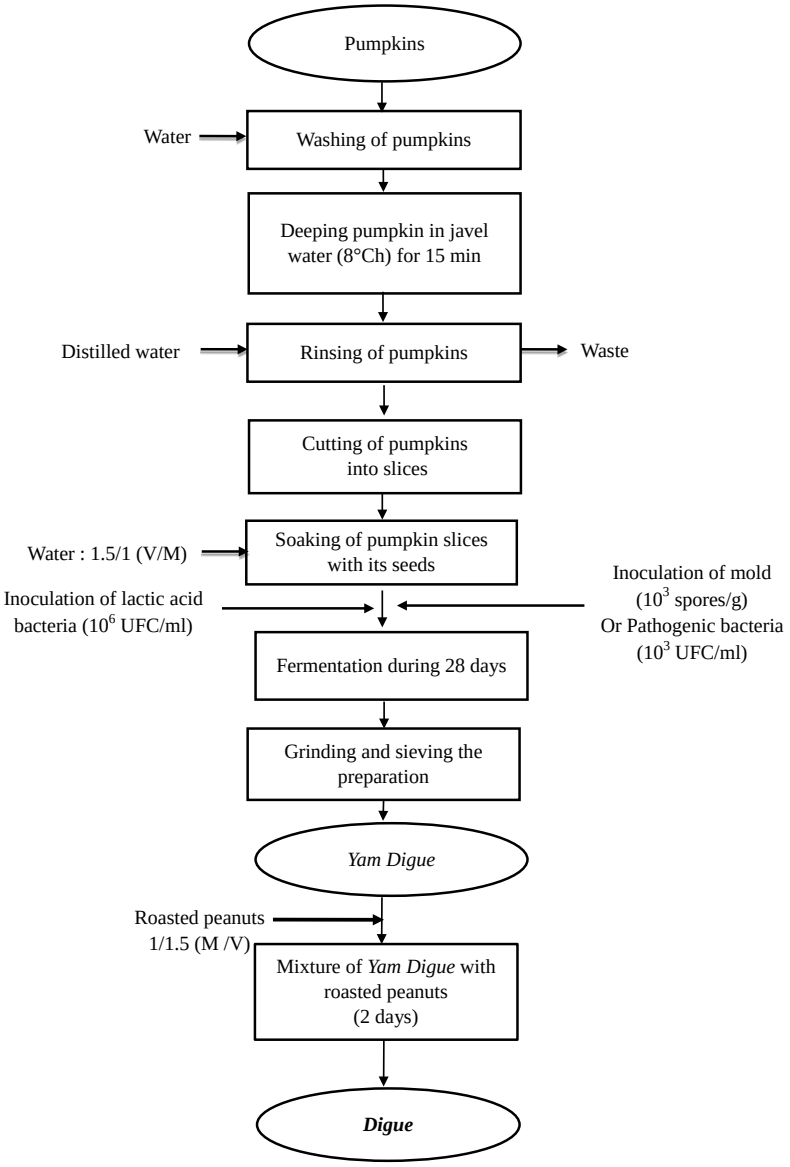


Figure 1. Diagram of production and inoculation of *Digue*

2.8.1. Microbiological Analyzes

After the decimal dilution carried out from 10g of the *Digue* sample, 0.1 ml of the stock solution of each decimal dilutions were inoculated on the MRS agar for the enumeration of lactic acid bacteria at 37°C for 48 hours under anaerobic conditions. The PDA agar was used for the enumeration of molds at 25°C for 3 to 5 days and EMB agar for the enumeration of pathogenic bacteria at 37°C for 24 hours [7].

2.8.2. Determination of Titratable Acidity

The titratable acidity was determined by titration with sodium hydroxide solution (0.1 N) mixed with 3 drops of phenolphthalein (1%) used as a color indicator. The titratable acidity was expressed in g of lactic acid equivalent (MW = 90.08 g/Mol) per 10 g of sample. The final concentration of acid in the stock solution was given in g/100g of *Digue* [13].

2.9. Data Analysis

The data obtained were analyzed using Statgraphics 5.0 software for analysis of variances, calculation of means and standard deviations. The Sigma plot 11.0 software allowed the graphical representation of the data.

3. Results

3.1. Characteristics of Isolated Lactic Acid Bacteria

24 lactic acid bacteria were isolated from the *Digue* samples. Microscopic observation of the selected isolates made it possible to distinguish the cocci which were more representatives (54%), followed by the coccobacilli represented (29%). The bacilli were weakly represented by a percentage of 17%.

3.2. Characteristics of Isolated Molds

20 molds were isolated from the *Digue*. After macroscopic and microscopic observations, these molds were grouped into 4 isolates of *Aspergillus* namely *Aspergillus* M1, *Aspergillus* M2, *Aspergillus* M3 and *Aspergillus* M4. Among these isolates, *Aspergillus* M3 (40%) was the most represented, followed by *Aspergillus* M2 (35%) and *Aspergillus* M4 (20%). The representativeness of *Aspergillus* M2 and *Aspergillus* M3 in the samples from *Digue* guided their choice for the evaluation of the antifungal activity of lactic acid bacteria during *Digue* fermentation

3.3. Selection of Antifungal Lactic Acid Strains

Among the 24 lactic acid bacteria isolated, 16 showed antifungal activity on at least one mold. Depending on the intensity of the inhibition, 04 of them (BL8, BL36, BL46 and BL54) induced large areas of inhibition. Table 1 shows the results of antifungal activity of the 24 lactic acid bacteria.

Table 1. Selection of antifungal lactic acid bacteria

Isolated lactic acid bacteria	Molds		
	***Aspergillus* sp M2**	***Aspergillus* sp M3**	***Aspergillus* sp M4**
BL3	-	+	+
BL5	-	+	-
BL6	-	-	+
BL35	++	-	+
BL36	+++	+++	+++
BL8	+++	+++	+++
BL9	-	+	+
BL46	+++	+++	+++
BL51	+	-	+
BL17	+	-	+
BL18	+	+	++
BL19	-	++	+
BL20	+	+	+
BL34	+	-	+
BL66	+	+	++
BL54	+++	+++	+++
BL70	-	-	-
BL52	-	-	-
BL63	-	-	-
BL62	-	-	-
BL71	-	-	-
BL72	-	-	-
BL50	-	-	-
BL13	-	-	-

-: No visible inhibition; +: Zone of inhibition between 0.1-3% of the area of the Petri dish, ++: Zone of inhibition between 3-8% of the area of the Petri dish and +++: Zone inhibition > 8% of the area of the Petri dish.

3.4. Evaluation of the Inhibition Diameter

The results show that all 04 lactic acid bacteria inhibited 03 molds. However, the inhibition diameters vary depending on the bacteria and the mold tested. The bacteria BL8 and BL36 showed their ability to inhibit all the molds tested with inhibition diameters greater than those induced by BL46 and BL54 (Table 2). This result made it possible to retain the lactic acid bacteria BL8 and BL36 for antifungal activity in *Digue.*

Table 2. Determination of the diameter of inhibition induced by lactic acid bacteria

Mold growth inhibition diameter (mm ± standard deviation)			
Lactic acid bacteria codes	***Aspergillus* sp M2**	***Aspergillus* sp M3**	***Aspergillus* sp M4**
BL8	40.0 ± 0.0	40.0 ± 0.0	40.7 ± 1.1
BL36	42.0 ± 2.8	39.0 ± 3.5	39.0 ± 2.3
BL46	40.0 ± 0.0	37.0 ± 4.2	36.3 ± 3.5
BL54	22.5 ± 3.4	32.0 ± 2.8	26.0 ± 1.7

3.5. Antibacterial Activity of Lactic Acid Bacteria

Table 3 shows the results of the antibacterial activity of the lactic acid bacteria isolated against the pathogenic bacteria. Similar to the inhibition tests with molds, the lactic acid bacteria BL8 and BL36 were recorded the best antibacterial activity with inhibition diameters of 17; 15mm and 19; 17mm when tested with *Salmonella enteritidis* 155A and *Escherichia coli* ATCC 25922, respectively.

Table 3. Inhibition of pathogenic bacteria by lactic acid bacteria

Lactic acid bacteria codes	*Salmonella enteritidis* 155A	*Escherichia coli* ATCC 25922
BL3	11.0±1.1	11.0±2.1
BL5	10.0±0.0	10.0±0.0
BL6	0.0±0.0	0.0±0.0
BL35	10.0±0.5	10.0±0.7
BL36	17.0±0.7	15.0±1.4
BL8	19.0±1.4	17.0±4.2
BL9	0.0±0.0	0.0±0.0
BL46	15.5±3.5	14.5±2.1
BL51	11.0±0.6	0.0±0.0
BL17	0.0±0.0	0.0±0.0
BL18	0.0±0.0	0.0±0.0
BL19	11.0±0.6	0.0±0.0
BL20	0.0±0.0	0.0±0.0
BL34	0.0±0.0	0.0±0.0
BL66	16.5 ± 3.5	13.0±2.8
BL54	17.0±2.3	14.5±2.1
BL70	5.0±0.0	9.0±0.4
BL52	7.0±0.1	0.0±0.0
BL63	0.0±0.0	0.0±0.0
BL62	0.0±0.0	0.0±0.0
BL71	0.0±0.0	0.0±0.0
BL72	0.0±0.0	0.0±0.0
BL50	0.0±0.0	0.0±0.0
BL13	0.0±0.0	0.0±0.0

3.6. Molecular Identification of Lactic Acid Bacteria BL8 and BL36

Throughout the inhibition tests, it was clear that BL8 and BL36 stood out from all other lactic acid bacteria in their ability to inhibit the growth of the Enterobacteriaceae and molds tested. They were therefore retained to be identified by PCR. BL8 and BL36 were identified respectively as the *Lactobacillus casei* with percent identification of 100% and as *Pediococcus acidilactici* with percent identity of 99.7%.

3.7. Antifungal Activity Lactic Acid Bacteria and Variation of Acidity during Fermentation of the Digue

Figure 2 shows the results of the antifungal activity of *Lactobacillus casei* and *Pediococcus acidilactici* and the change in titratable acidity during fermentation of *Digue.* After the first 7 days of fermentation, an increase of concentration of *Aspergillus* M2 and *Aspergillus* M3 of 3.6 Log10UFC/g each in the *Digue* fermented with *Lactobacillus casei* is noted. The same observation was made for *Aspergillus* M2 with an increase in the concentration of 4 Log_{10}UFC/g in the *Digue* fermented with *Pediococcus acidilactici*. However, after the 14th day of fermentation, we observed a gradual decrease in fungal growth at non-detectable concentrations in the *Digue* until the 21st day of fermentation.

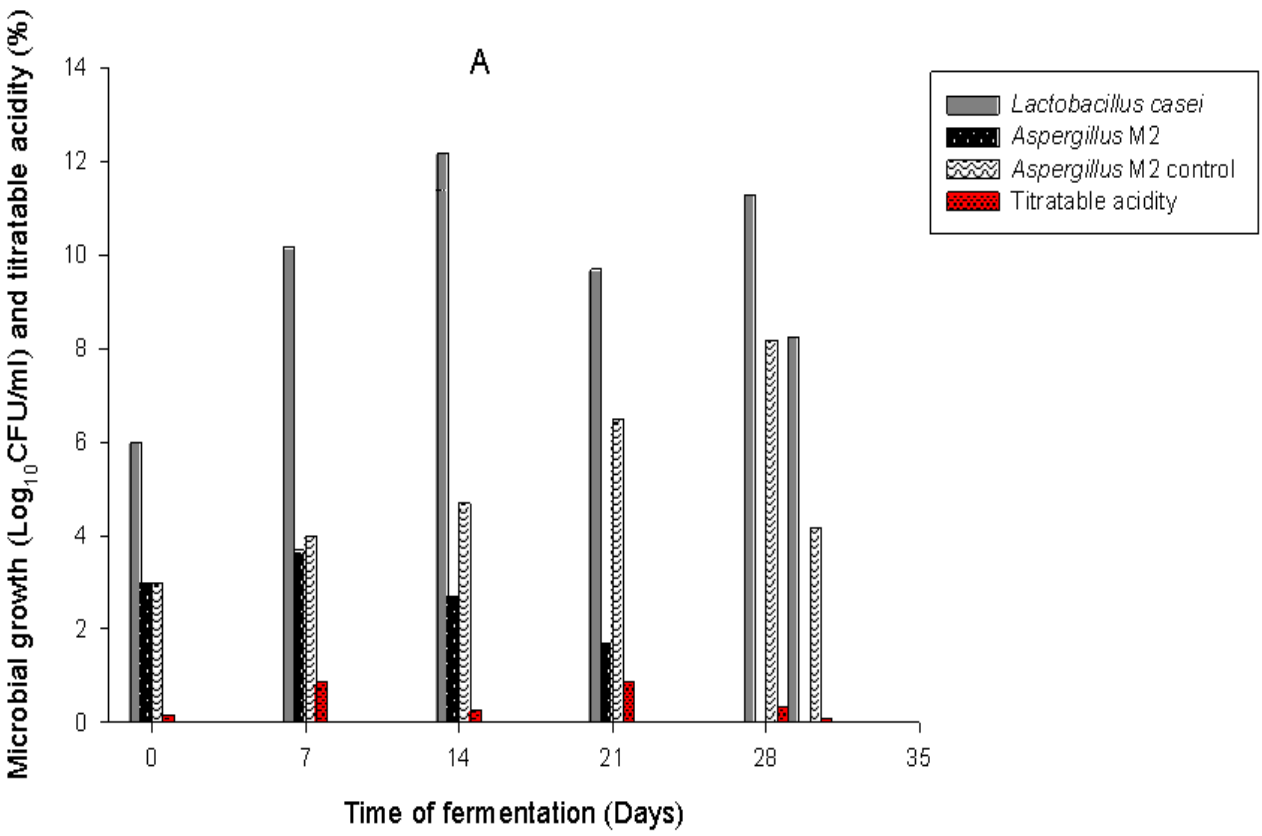
During the fermentation, the titratable acidity was also determined in the *Digue*. The result obtained show that the titratable acidity increase during the first 7 days of fermentation, period above which a reduction in mold growth was observed in the *Digue* treated with lactic acid bacteria. The level of titratable acidity varies from one lactic acid bacteria to another and depending on the mold tested. In the *Digue* fermented with *Lactobacillus casei* and contaminated with *Aspergillus* M2 and *Aspergillus* M3, the titratable acidity rate recorded is 0.85% and 1% respectively. The *Digue* fermented with *Pediococcus acidilactici* and contaminated by *Aspergillus* M2 and *Aspergillus* M3, the titratable acidity rate is respectively 0.49% and 0.87%.This titratable acidity continued to increase until the 21st day in the *Digue* fermented with *Lactobacillus casei* and contaminated by *Aspergillus* M2 and *Aspergillus* M3. The titratable acidity rate recorded was 0.86% and 0.93% respectively.

In general, the growth of lactic acid bacteria in the presence of these molds was noted during the 28 days of fermentation of the *Digue.* However, a decrease in the concentration of these lactic bacteria was observed on the 30th day of fermentation, after *Yam Digue* was added to the sterile roasted peanut paste on the 28th day.

3.8. Antibacterial Activity of Lactic Acid Bacteria and Evolution of Acidity during Fermentation

The Figure 3 shows inhibition of *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A by *Lactobacillus casei*, *Pediococcus acidilactici* and variation of acidity during fermentation of the *Digue*. Despite the inoculation of lactic starters in the *Digue*, during the first 7 days of fermentation, growth of *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A was observed, respectively of 7.4 Log_{10}UFC/g and 7.3 Log_{10}UFC/g in the *Digue* fermented by *Lactobacillus casei.* While the *Digue* fermented with *Pediococcus acidilactici* recorded a growth rate of 7.4 Log_{10}UFC/g and 8.0 Log_{10}UFC/g, respectively in *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A. However, after these 21 days of fermentation, it was noted that *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A were reduced below the non-detectable threshold by *Lactobacillus casei*. The same observations were made with *Pediococcus acidilactici* which reduced below the non-detectable threshold *Escherichia coli* ATCC 25922. Beyond the inhibition of the pathogenic germs observed, there is also a change in titratable acidity during the controlled fermentation of the *Digue*. *Digue* fermented with *Lactobacillus casei* and contaminated with *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A recorded a titratable acidity level of 0.72% and 0.86%, respectively. While the *Digue* fermented with *Pediococcus acidilactici* and contaminated with the same pathogenic germs recorded an acidity rate of 0.63% and 0.37%.

Although this acidity drops on the 14th day in the *Digue* fermented with *Lactobacillus casei* and *Pediococcus acidilactici*, it nevertheless remains high on the 21st day. It is 0.63% and 0.74% in the *Digue* fermented with *Lactobacillus casei* in the presence of *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A respectively. In the *Digue* fermented with *Pediococcus acidilactici* and contaminated with the same germs, an acidity level of 0.63% and 0.41% was recorded.


A
Microbial growth (Log_{10}CFU/ml) and titratable acidity (%)
Time of fermentation (Days)
Lactobacillus casei
Aspergillus M2
Aspergillus M2 control
Titratable acidity
0
2
4
6
8
10
12
14
0
7
14
21
28
35

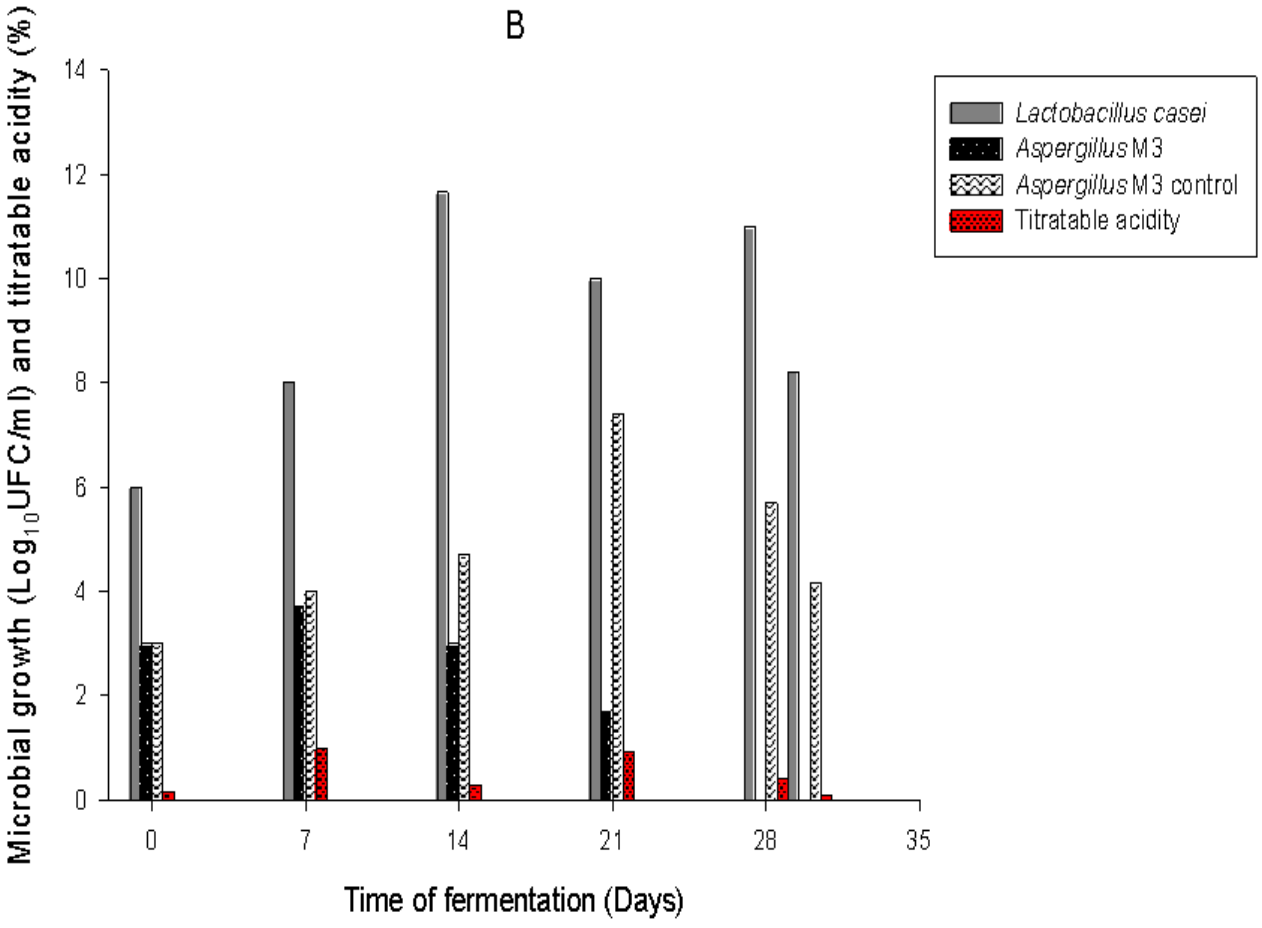
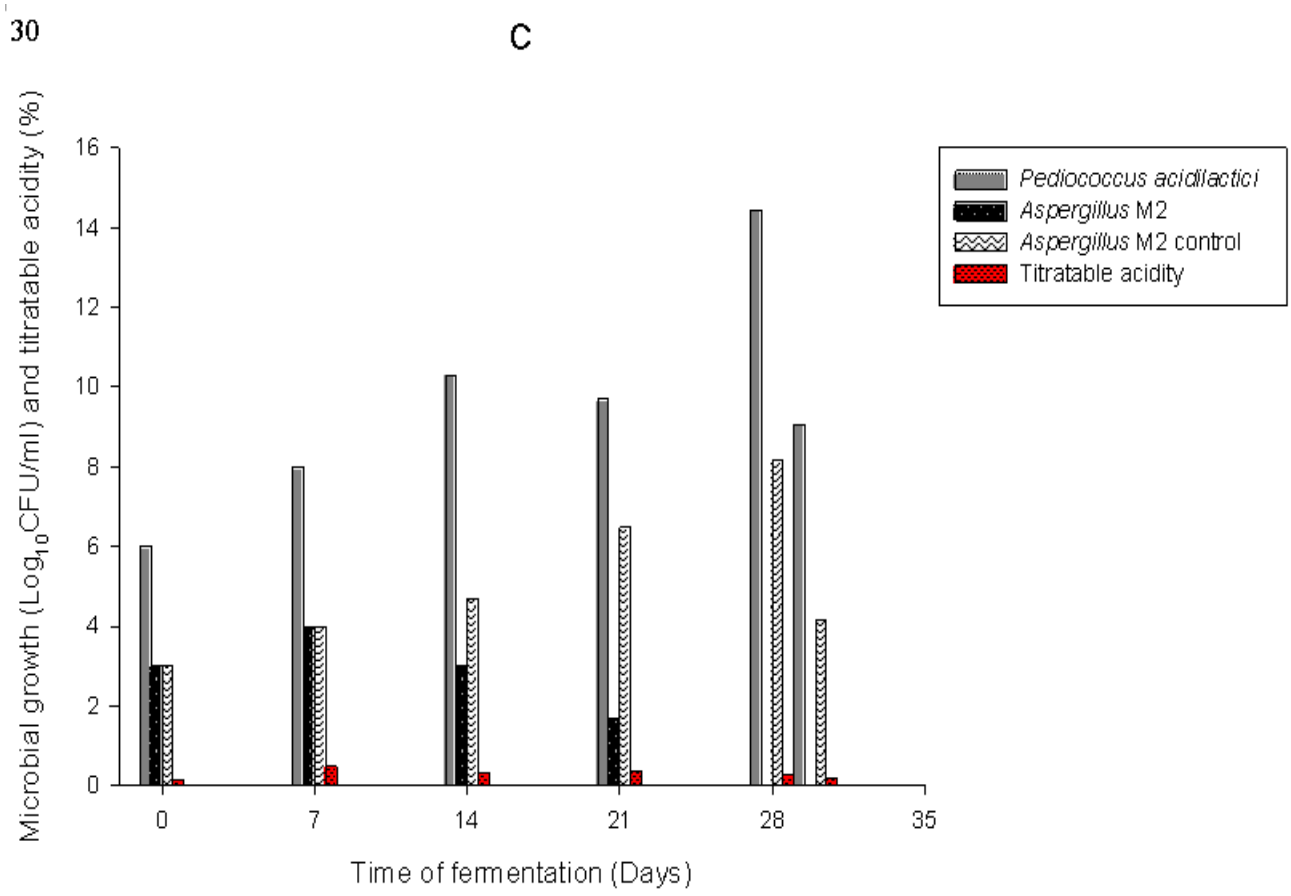

B
Microbial growth (Log_{10}UFC/ml) and titratable acidity (%)
Time of fermentation (Days)
Lactobacillus casei
Aspergillus M3
Aspergillus M3 control
Titratable acidity
0
2
4
6
8
10
12
14
0
7
14
21
28
35

Figure 2. A-B



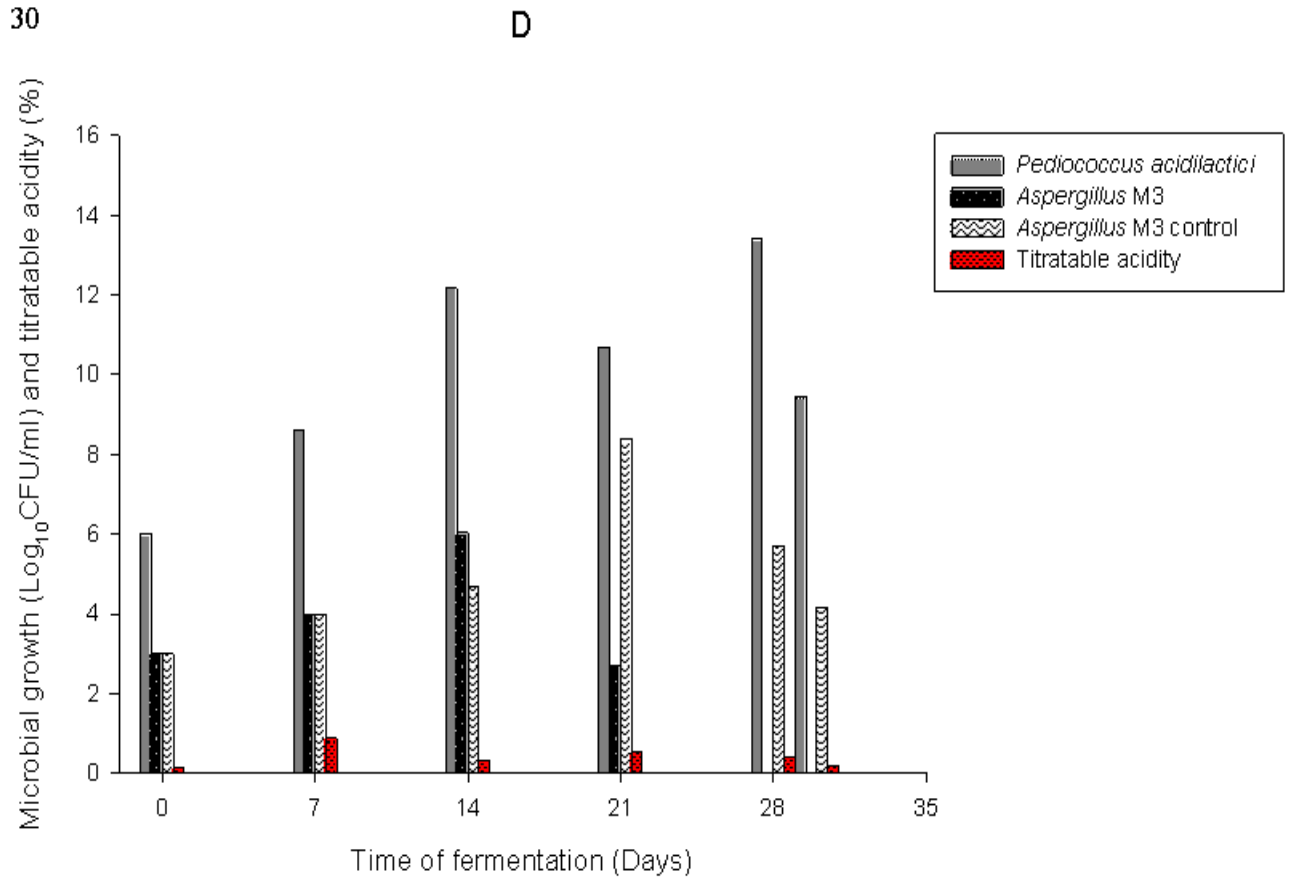


Figure 2. Inhibition of *Aspergillus* M2 (A, C), *Aspergillus* M3 (B, D) by *Lactobacillus casei* and *Pediococcus acidilactici* and variation of acidity during fermentation of the *Digue*

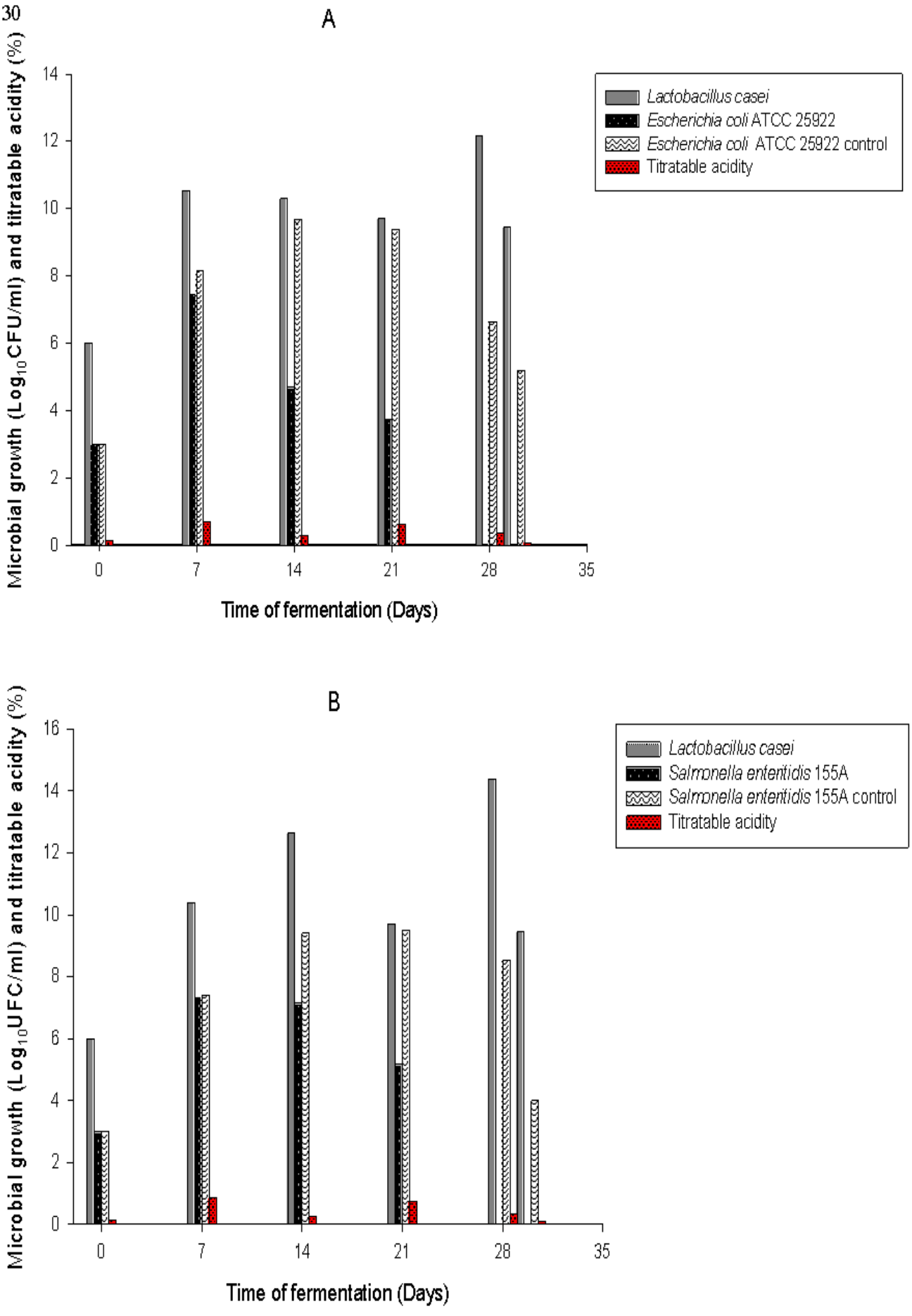

A
30
Microbial growth (Log10CFU/ml) and titratable acidity (%)
14
12
10
8
6
4
2
0
0
7
14
21
28
35
Time of fermentation (Days)
Lactobacillus casei
Escherichia coli ATCC 25922
Escherichia coli ATCC 25922 control
Titratable acidity
B
Microbial growth (Log10UFC/ml) and titratable acidity (%)
16
14
12
10
8
6
4
2
0
0
7
14
21
28
35
Time of fermentation (Days)
Lactobacillus casei
Salmonella enteritidis 155A
Salmonella enteritidis 155A control
Titratable acidity

Figure 3. A-B

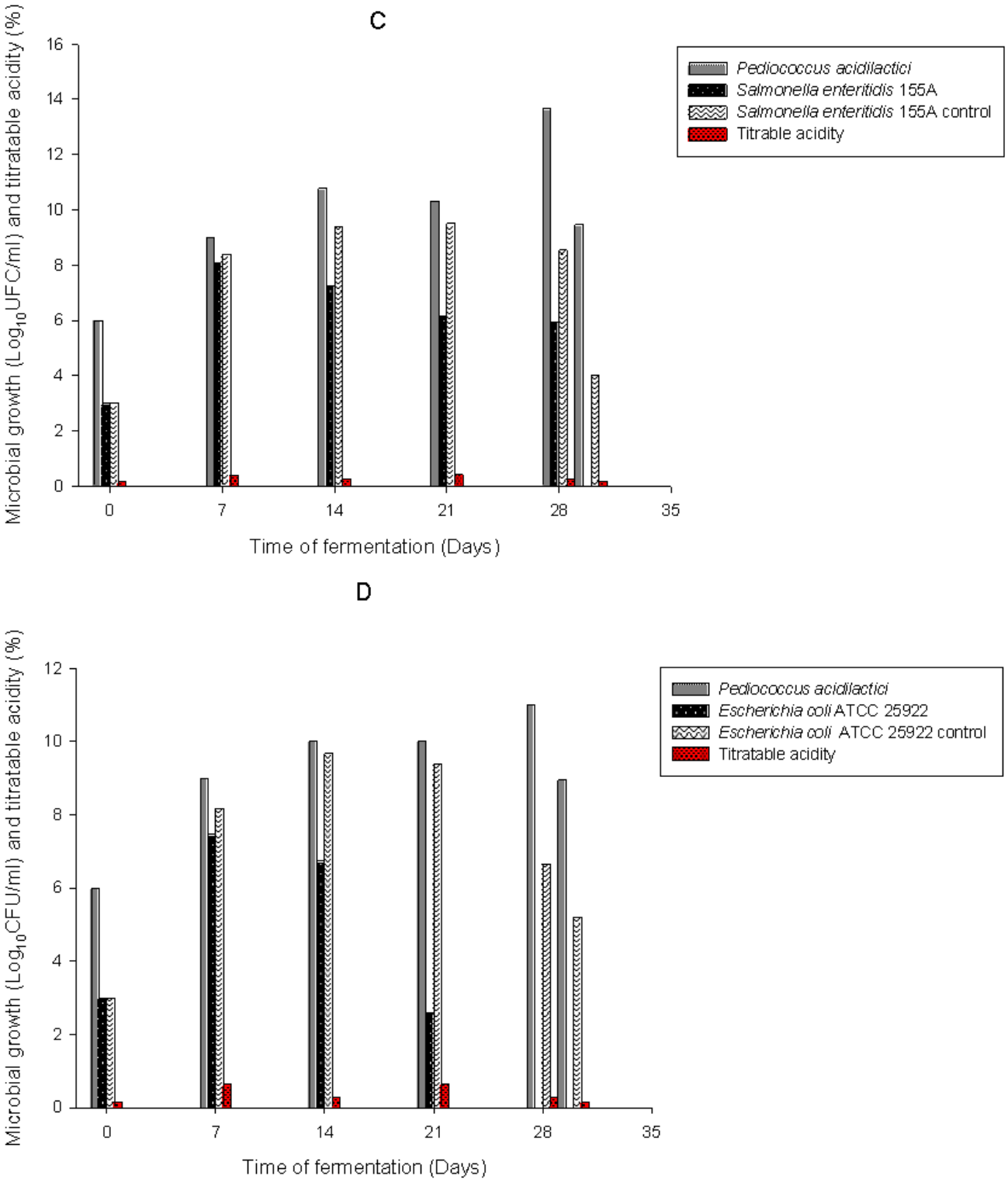


Figure 3. Inhibition of *Escherichia coli* ATCC 25922 (A, D), *Salmonella enteritidis* 155A (B, C) by *Lactobacillus casei* and *Pediococcus acidilactici* and variation of acidity during fermentation of the *Digue*

4. Discussion

The results obtained during the analyzes of the *Digue* samples collected in the locality of Gamboura showed the presence of lactic acid bacteria and molds. The presence of lactic acid bacteria may be explained by the fact that they are the main microorganisms associated with food fermentation [14]. For molds, their presence could be explained by the disrespect of hygiene rules during production of *Digue* [15]. In addition, climatic conditions such as temperature and humidity could also promote the growth of these molds in the *Digue*. Gamboura is a locality with an average temperature of 24.6°C that is close to the optimal growth temperature of the genus *Aspergillus* (25°C), a species mostly found in the *Digue*. In comparison to other mold species, the genus *Aspergillus* is frequently found in local foods [16].

The results of the antimicrobial activity showed that lactic acid bacteria inhibited the growth of molds and pathogenic germs during *in vitro* inhibition tests. The work by other authors has also shown that lactic acid bacteria inhibit the growth of pathogenic germs during food fermentation. David and Esther [17] showed that the genera *Lactobacillus* and *Pediococcus* inhibit the growth of several pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* sp. and *Pseudomonas aeruginosa* during the fermentation of *Ogiri*. Adebayo and Aderiye [18] also showed that *Lactobacillus casei* and *Pediococcus acidilactici* inhibit the growth of *Escherichia coli*, *Salmonella* and *Shigella flexneri* during the fermentation of cereals (*ogi*) and cassava (*fufu*).

The molecular identification of the two lactic bacteria selected for their best antimicrobial activity revealed they were *Lactobacillus casei* (BL8) and *Pediococcus acidilactici* (BL36). Several studies have revealed the presence of these species in fermented foods. Some work has identified *Lactobacillus casei* and *Pediococcus acidilactici* in fermented products [18].

The antifungal and antibacterial activity of *Lactobacillus casei* and *Pediococcus acidilactici* during the production of *Digue* showed a reduction in microbial growth as observed in *in vitro* tests. This antimicrobial activity would be linked to the production of organic acids by these lactic bacteria that grow exponentially during fermentation. Indeed, organic acids have been considered until now as the main metabolites of lactic bacteria that inhibit the growth of pathogenic bacteria and significantly affect the growth of molds by inhibiting the growth of mycelium. Indeed, organic acids have the ability to passively cross the cell membrane of these pathogenic germs and acidify the intracellular cytoplasm by proton release, thus affecting metabolism by inhibiting certain cell functions [19].

Although both lactic acid bacteria showed antimicrobial activity against the molds and pathogenic bacteria tested, it is noted that the inhibition of contaminants by *Lactobacillus case*i is faster than *Pediococcus acidilactici*. This difference in activity would be related to the amount of organic acids produced by these two lactic bacteria. *Lactobacillus casei* is a heterofermentary strain characterized by the high acid production estimated at 1% during fermentation. While *Pediococcus acidilactici* is a homofermentary strain characterized by an acid production estimated at 0.87% during fermentation. This production of organic acids by these lactic bacteria during fermentation would also be facilitated by the presence of sugars in the *Digue*.

5. Conclusion

At the end of this work, 02 lactic acid bacteria out of the 24 isolated from *Digue* were selected for their antibacterial efficacy. They have been identified as *Lactobacillus casei* and *Pediococcus acidilactici.* However, only *Lactobacillus casei* reduced below the non-detectable threshold *Aspergillus* M2 and *Aspergillus* M3, *Escherichia coli* ATCC 25922 and *Salmonella enteritidis* 155A after 21 days of controlled fermentation. These results show that *Lactobacillus casei* can be used to ensure the safety of *Digue* by eliminating the presence of carcinogenic molds and enterobacteriaceae after 21 days of fermentation..

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Conflict of Interest

None of the authors have conflict of interest.

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