INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 20, No. 6, p. 32-44, 2022

RESEARCH PAPER OPEN ACCESS

Study of the Probiotic Potentialities of Lactic Acid Bacteria isolated from Maize (*Zea mays*) in Côte d'Ivoire

N'Guessan Yévi Delphine[1], Bonny Aya Carole[1*], Aké Moussan Désirée Francine[2], Assandi Kouamé Rivière[1], Koffi Djarys Michel[1]

[1]*Laboratory of Biotechnology, Agriculture and Valorisation of Biological Resources, Biosciences Training and Research Unit, Félix Houphouët-Boigny University, Côte d'Ivoire, 22 BP 582 Abidjan 22*

[2]*Laboratory of Biotechnology and Microbiology of Foods, Food Science and Technology Training and Research Unit, Nangui Abrogoua University, Côte d'Ivoire, 02 BP 801 Abidjan 02*

Key words: Maize (*Zea mays*), Lactic acid bacteria, probiotics, Ivory Coast.

http://dx.doi.org/10.12692/ijb/20.6.32-44 Article published on June 14, 2022

Abstract

The objective of this work is to evaluate the probiotic ability of lactic acid bacteria isolated from fermented maize (*Zea mays*) pulp in Côte d'Ivoire. To this end, selection criteria, namely antimicrobial activity, resistance to acidity and bile salts, self-aggregation, antibiogram and percentage of hydrophobicity, were carried out on fourteen lactic acid bacteria isolates (LAB). Seven isolates (T1.8; T1.9; T2.3; T2.7; T2.10; T3.1; T3.5) were able to inhibit the growth of *Salmonella enterica* O:8, *Escherichia Coli* and *Staphylococcus aureus*, with diameters ranging from 1.1 ± 0.14 to 31.75±2.3 mm. Two isolates (T1.9 and T2.7) showed growth at pH 2 (2.01% ± 0.6 and 3.24% ± 0.1). Isolates T3.4 and T3.5 showed growth (86.17± 0.05%) at 0.3% bile salts. Of all the antibiotics tested, Chloramphenicol was inactive on the isolates tested. However, isolates T3.5, T3.6, T1.4 and T0.5 showed sensitivity to Amoxicillin. As for surface hydrophobicity, a growth rate of 0.6% phenol was observed in isolate T1.9 (71.87 ± 0.06). The maximum rate of self-aggregation (77.35%) was observed in isolate T3.4. In sum, isolates T3.1, T3.7 and T3.4, selected as potential probiotics via the PHEAPMAP software, and revealed by the MalditoF test as belonging to the genera *Lactobacillus* (*Lactobactillus fermentum*), could be used to improve maize-based foods.

* **Corresponding Author:** Bonny aya carole ✉ bonnyayacarole@yahoo.fr

Introduction

Maize (*Zea mays*) is the most widely cultivated crop in the world and the most important cereal crop produced ahead of wheat (*Triticum aestivum* L. subsp. *aestivum*) (Boudouhi *et al.*, 2005). The dominant position of maize in Africa in general, and in West Africa in particular, has been favoured by its capacity to adapt to agro-ecological conditions and by its strategic roles, both as a cash crop and as a major consumer in many countries (Nago, 1997).

In Côte d'Ivoire, maize forms the basis of the diet of rural populations. It is used for both human and animal feed (poultry, pigs, cattle, etc.) and as a raw material in certain industries (brewery, soap factory and oil mill) (Boone *et al.*, 2008).

However, its nutritional quality remains relatively low in starch, fat, protein, crude fibre, ash and sugars. Potassium, magnesium and phosphorus are the main minerals in maize. Also, common maize varieties cannot quantitatively meet the protein and fat requirements of the populations that use them as a staple food (FAO/WHO, 1973), as they are generally low in lysine and tryptophan. Nevertheless, various processing methods would improve its nutritional value (Demont, 1997). Indeed, several maize-based products are obtained by fermentation with lactic bacteria. These bacteria, capable of bio-converting maize into new molecules, give it new organoleptic properties (Belarbi, 2011). In addition, they are tolerated by living beings, which has led to the recognition of their GRAS (Generally Recognized As Safe) status (Klaenhammer, 2005). Some strains of lactic acid bacteria are beneficial to humans and are called probiotics.

Indeed, they are used for the treatment of diarrhoea and other digestive disorders. Also, recent studies show properties of stimulating a specific immune response and some strains have the ability to reduce food allergy reactions, especially to maize proteins and fats (Cromwell *et al.*, 1999). Therefore, probiotics could be an alternative to improve the nutritional quality of maize-based foods, which are widely consumed in Côte d'Ivoire. The main objective would be to contribute to the formulation of maize-based foods, supplemented with these bacteria, in order to improve the nutritional quality of these foods and guarantee the health of the Ivorian population.

Materials and methods

The study material consisted of 14 isolates of lactic acid bacteria with antifungal potential on *Aspergillus niger*, *Aspergillus flavus*, *Fusarium sp*, *Penicillium sp*, characterised in the work of Yobouet (2020), and isolated from fermented yellow corn dough (Table 1). Also, three (03) strains of pathogenic bacteria, responsible for several infections in human food, namely *Escherichia coli*, *Salmonella enterica* O:8 and *Staphylococcus aureus*, targeted for bacterial antagonism tests, are of various origins (avian and human). The lactic acid bacteria isolates come from the strain library of the Unité Pédagogique et de Recherche de Biotechnologie (UPRB) of the Université Félix HOUPHOUËT-BOIGNY. The *E. coli* and *S. enterica* O:8 strains are from the collection of the Microbiology Unit of the Central Veterinary Laboratory of Bingerville (LCVB). The *S. aureus* strain was obtained from the Bio-bank of the Pasteur Institute of Côte d'Ivoire (IPCI). The reference strains ATCC 14028 and IPCI 8297 served as positive controls for the antibiogram.

Characterisation of the probiotic properties of lactic acid bacteria isolates isolated from fermented maize pulp

The characterisation of the probiotic potential of the isolates was done by applying the following selection criteria: antimicrobial activity, resistance to acidity, resistance to acid bile salts, self-aggregation, co-aggregation, and measurement of percentage (%) hydrophobicity and antibiogram (Cromwell *et al.*, 1999).

Antimicrobial activity

Antibacterial activity was carried out according to the method of Kim *et al.* (2007) with some modifications. A 24-hour culture of both pathogens and lactic acid bacteria strains on MRS agar (Condalab, Spain) was

performed. Then, a dense suspension estimated at 108 CFU/mL of each pathogen to be tested (*E. coli*, *Salmonella enterica* O:8 and *Staphylococcus aureus*) was made as follows: from a pure 24h culture of each of the pathogenic strains, well-isolated colonies were taken and splashed in 2 mL of sterile distilled water. The resulting bacterial suspension was homogenised and diluted 1:100 so that its opacity was equivalent to the McFarland control (108 CFU/mL).

The suspension obtained is diluted again to 1/10 in MRS agar (Condalab, Spain) maintained in supercooling (55°C). MRS agar (Condalab, Spain) inoculated with each. After homogenisation, the pathogen is poured into Petri dishes. After solidification of the agar at room temperature for 15 min, pure colonies of lactic acid bacteria isolates obtained after 24 h of incubation on another MRS agar (Condalab, Spain) were picked and then plated with sterile Pasteur pipettes, in spots, in order to promote direct contact with the target pathogens. Finally, the Petri dishes were incubated at 30°C for 24 h. The antimicrobial activity of the lactic acid bacterial isolates, marked by the appearance of zones of inhibition, was estimated by measuring two perpendicular diameters around the spots.

Antimicrobial susceptibility testing

An antibiogram was performed with the lactic acid bacteria isolates in order to test their sensitivity to certain antibiotic molecules. The susceptibility test was performed according to the recommendations of the Comité d'Antibiogramme de la Société Française de Microbiologie (CASFM, 2019), on MRS agar (CondaLab, Spain). The following antibiotic discs were tested: Amoxicillin (AMX, 10µg), nalidixic acid (Nal, 10 µg), Chloramphenicol (C, 10µg), cotrimoxazole (SXT, 25µg) and cefoxitin (30 µg). Salmonella reference strains ATCC 14028 and IPCI 8297, were used to validate the antibiogram test. Inhibition diameter values are interpreted and categorised as susceptible (S), intermediate (I) or resistant (R). Lactic acid bacterial isolates, which would show intermediate resistance traits, were categorised as resistant for further study.

Resistance to acidity

Resistance to gastric conditions was performed according to the modified method described by Kim *et al.*(2007). The MRS broth is prepared at different acidic pH ranging from 1 to 5. Also, suspensions of each lactic acid bacterial isolate were made from 24h cultures on MRS agar (CondaLab). The optical density of each LAB suspension was read with a spectrophotometer (BK-UV1000) at 600 nm and set at an OD of 0.1; this allowed the determination of the volume to be taken for each suspension, for the inoculation of the lactic bacteria, in the different broths prepared beforehand. The corresponding volume for each suspension of lactic acid bacteria isolates was added to test tubes containing 4 mL of MRS broth (CondaLab), at different pH levels (pH=1, pH=2, pH=3, pH=4, pH=5). The optical density (OD) is read immediately after plating (OD at T0: OD.I) and the tubes are incubated at 37°C for 24 hours. After this incubation time, the OD is read (OD. F). The resistance of the lactic acid bacteria isolates is assessed by calculating their percentage growth in the broths at different pH levels. Growth at pH=6 (pH of SRM agar) was taken as the maximum growth reference (100% growth). The percentage growth was calculated according to the following expression:

$$\text{Growth rate of LAB (\%)} = \frac{\text{OD. F - OD. I}}{\text{OD pH6}} \times 100$$

OD. F : Optical density after 24h incubation;
OD. I : Optical density before incubation;
OD pH6 : Optical reference density

Hydrophobicity of the cell surface: Adhesion to epithelial cells is assessed by cell surface hydrophobicity according to the method described by Zuo *et al.* (2015). Isolates of 18 h young lactic acid bacteria, obtained on MRS agar (CondaLab, Spain), are washed twice with phosphate buffer (PBS) at pH 7.4 and suspended in physiological saline (0.5M). The optical density of the physiological solution is adjusted to 0.5 and 0.7 at a wavelength of 600 nm. Subsequently, 1 mL of phenol (100 %) is added to test tubes containing 3 ml of the previous physiological solution. The tubes were shaken for 90 s and left to

stand for 15 min to separate the two phases. The optical density of the aqueous phase is then measured at 600 nm. The hydrophobicity is calculated as the percentage decrease in absorbance of the lactic acid bacteria suspension, according to the following formula:

Percentage hydrophobicity = [(absorbance before homogenisation - absorbance after homogenisation) / absorbance before homogenisation] × 100

Bile salt tolerance

Gut conditions are simulated by tolerance to bile salts at different concentrations (P /V) : 0.1 %, 0.2 %, 0.3 % and 0.4 %. For this purpose, MRS broth is prepared with the addition of bromocresol purple and then adjusted to the different concentrations of bile salts (0.1 %, 0.2 %, 0.3 % and 0.4 %). These different broths are used to inoculate the lactic acid bacteria isolates. The optical density is read immediately after inoculation (OD. I) of the lactic isolates, and the tubes are then incubated at 37°C for 24 hours. After incubation, the optical density is read (OD. F) at 600 nm. Growth at pH=6 is taken as the maximum growth reference (100 % growth). The percentage growth as a function of bile salt concentrations is calculated according to the expression described in the acid resistance protocol (Behira, 2012).

Auto-aggregation test

The self-aggregation test is performed according to the method described by Zuo *et al.* (2015). To do so, cells from a 24h culture of lactic acid bacteria isolates are recovered by centrifugation at 6000 g for 10 min. Subsequently, the bacterial pellet is washed twice with PBS buffer (pH 7.4) and resuspended again in PBS buffer. Finally, the bacterial loads are adjusted to an optical density of 0.5 at 600 nm by dilution with MRS broth (CondaLab, Spain). Incubation is done for 2 h at 37°C. A volume of 0.1 ml of the upper part of the suspension (PBS) is transferred to another tube containing 1.9 mL of PBS and the optical density is read at 600 nm. The percentage of self-aggregation is calculated according to the following formula:

% Self-aggregation = (1-(A2h -A / A oh)) × 100

A oh: initial optical density; A2h: optical density after 2h; A: reference growth of lactic acid bacteria (growth in MRS at Ph6).

Selection of probiotics by the pheatmap software method

A pheatmap was used to group the different isolates according to the different characteristics assessed during the study, in order to show the significant difference between the probiotic properties of the isolates, using a colour key with a red and blue gradient. A comparison of the bands along the axis of the dendrogram allowed the identification of the lactic acid bacteria isolates with probiotic potential. The pheatmap processing was carried out with the R software version 3.1.3.

Identification of lactic acid bacteria isolates with probiotic potential by MALDITOF mass spectrometry

Subcultures on MRS agar (CondaLab, Spain) of lactic acid bacteria colonies with probiotic potential are used for MALDI-TOF MS analysis. Most colonies were grown the day before. Approximately 50 µg of fresh cells are taken from a single colony, without agar residue through the use of inoculation loops and transferred to stainless steel wells. Immediately, the bacterial material is extracted with 0.3 mL of matrix solution (10 mg of 2,5-dihydroxybenzoic acid in 1 mL of water: acetonitrile [1:1], acidified with 1% trifluoroacetic acid). Positive ion mass spectra were recorded for each strain using a MALDI-TOF mass spectrometer (AXIMA CFRplus, Schimadzu, Germany). For desorption of the components, a nitrogen laser beam (λ = 337 nm) is focused on the model. The accelerating voltage is set to 20 kV and the delay time to 950 ns. All measurements are performed in delayed extraction mode, allowing the determination of high resolved mass values (m/z; mass-to-charge ratio). Analyses are performed in the positive ion mode site, yielding mainly molecular ions ([M + H] +). All spectra are processed by the instrument software with baseline correction, peak

filtering and smoothing. The resulting site peak lists are exported to SARAMIS software (AnagnosTec). The peak lists of individual samples are compared to the superspectra database, generating a ranked list of matching spectra.

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's test with R software version 3.1 is used to compare the means of the optical densities. Differences are considered significant for values of $P < 0.05$.

Results

Antibacterial activities of lactic acid bacteria isolates

The study of the interaction between the lactic acid bacteria isolates and the targeted pathogenic strains, namely *Salmonella enterica* O:8, *Escherichia coli* and *Staphylococcus aureus*, shows that the 14 lactic acid bacteria isolates tested have an antagonistic effect on the growth of these pathogens (Fig. 1). The largest inhibition diameter was observed with isolate T1.6 on *Salmonella enterica* serogroup O:8 (31.75 ± 2.3 mm), while the smallest diameter was observed with isolate T2.7 on *Staphylococcus aureus* (1.1 ± 0.14 mm). All the lactic acid bacteria isolates were active on two pathogens (*E. coli* and *Salmonella enterica* O:8). As for the *Staphylococcus aureus* strain, only seven lactic acid bacteria isolates (T3.7, T3.1, T2.7, T3.5, T2.3, T2.10, T1.8 and T1.9) showed antagonistic activity (Table 2).

Table 1. Characteristics of lactic acid bacteria isolates.

LAB isolates	Characteristics of the isolates	Fungus germs
T0.5- T1.2- T1.4- T1.9- T2.3-T2.7- T2.10-	Cocci, Gram+, homofermentative	*Aspergillus niger, Aspergillus flavus, Pénicillium sp, Fusarium sp*
T1.6- T1.8-	Bacilles Gram+ homofermentative	
T3.1- T3.4- T3.5- T3.7	Bacilles Gram+ heterofermentative	

LAB: Lactic acid bacteria.

Resistance profile of lactic acid bacteria isolates

The results of this test showed that all isolates tested were susceptible to Chloramphenicol (C) (Table 3). Apart from isolates T3.5, T3.6, T1.4 and T0.5, resistance was observed to Amoxicillin, Cotrimoxazole, Cefoxitin and Nalidixic acid.

Table 2. Diameters of the inhibition zones (in mm) of lactic acid bacteria isolates (LAB) on the target pathogens.

	T0.5	T3.7	T3.1	T3.6	T2.7	T3.4	T3.5	T1.2	T1.4	T2.3	T2.10	T1.6	T1.8	T1.9
E. coli	8.5 ± 0.71^{e}	0	24± 0.71^{bc}	10.5± 0.70^{de}	31.0 ± 1.41^{a}	0	22.5 ± 0.71^{c}	13.5 ± 0.71^{d}	30.5 ± 2.12^{a}	13.5 ± 0.71^{d}	30 ± 0.71^{a}	0	22.5±0.71^{c}	26.5± 0.71^{b}
Staph	0	14.0± 1.41^{a}	13.0 ± 1.41^{a}	0	1.1 ± 0.14^{b}	0	13.0± 1.41^{a}	0	0	15.5± 2.12^{a}	16.5± 0.71^{a}	0	14.5±0.71^{a}	15.0±1. 41^{a}
Sal	30.01 ±0.01^{a}	21.50± 12.02^{a}	22.00 ±11.31^{a}	28.75 ±1.77^{a}	18.50 ± 12.02^{a}	29.55 ±0.64^{a}	21.5 ±10.61^{a}	31.0 ±1.41^{a}	31.0 ±1.41^{a}	29.5 ±0.71^{a}	21.0 ±5.66^{a}	31.75 ±2.47^{a}	18.5± 6.36^{a}	22.00± 11.31^{a}

Staph: Staphylococcus aureus; E. coli: Escherichia coli; Sal: Salmonella enterica serogroup O:8

There is a significant difference between the isolates in relation to the same pathogen and also a significant difference between the isolates in relation to different pathogens. This difference is seen in the different letters that accompany the numbers.

Growth of isolates at acidic pH

The growth of bacteria as a function of pH was carried out on all lactic acid bacterial isolates and made it possible to determine the number of lactic acid bacterial isolates resistant to different pH values (1, 2, 3, 4, 5 and 6) after 24 hours of culture. At pH values between (1 and 6), the growth rate varied from 0 to 99.72%. At pH 1 and 2, growth is almost zero for some strains and very low for others. From pH 3 onwards, isolates T3.1, T3.5 and T1.8 show more than 50% resistance. At pH 4, isolate T2.10 has a percentage resistance of 58.43. Isolate T2.3 shows a percentage resistance of 38.31 and 73.38 at pH 3 and pH 4, respectively, after 24 hours of incubation. The pH6 is

considered as a controlled pH to evaluate the growth percentage of the other pH values. In sum, the lactic acid bacteria isolates with better resistance to acidic pH are T2.3, T3.1, T3.5, T1.8, T2.7 and T2.10 (Table 4).

Surface hydrophobicity

All the isolates tested show a growth percentage higher than 50 %, at concentrations ranging from 0 to 0.2 % phenol (Table 5). At 0.3 % phenol, four lactic acid bacteria isolates (T3.5, T1.4, T1.8 and T2.10) show less than 50 % resistance, with growth rates of 45.76 ± 0.12, 37.78 ± 0.06, 33.18 ± 0.11, 46.48 ± 0.13 and 46.48 ± 0.13 respectively. At the 0.4 concentration, isolates T3.4, T1.9, T3.1, T2.7, T3.7, T3.6 and T0.5 showed a percentage growth of more than 50 %, with respective rates of 91.14 ± 0.11, 77.09 ± 0.06, 72.19 ± 0.11, 50.19 ± 0.10, 60.57 ± 0.14 and 52.89 ± 0.06. However, at the 0.5 % phenol concentration, only isolates T3.1, T1.9 and T3.4 had growth percentages above 50 %.

However, isolate T3.6 shows a growth percentage of 50 at 0.6 % phenol concentration. Growth at 0 % phenol concentration is the reference for the other concentrations.

Table 3. Inhibition diameters (mm) of lactic acid bacteria isolates (LAB) tested in the presence of antibiotics.

ATB	Lactic acid bacteria isolates													
	T1.8	T2.3	T1.9	T2.10	T3.5	T2.7	T3.1	T3.6	T3.4	T1.2	T1.4	T0.5	T1.6	T3.7
C	29.5±0.07^{a}	29.5±0.07^{a}	29.5±0.21^{a}	29.5±0.1^{a}	30.25±0.07^{a}	30±0.07^{a}	30±0.01^{a}	28.5±0.1^{a}	27.5±0.1	29.5±0.07^{a}	28,5±0,07^{a}	30.5±0.02^{a}	27.5±0.21^{ab}	23.5±0.1^{b}
AMX	14.5±0.07^{c}	14.5±0.21^{c}	14.5±0.35^{c}	14.5±0.7^{c}	20.2±0.03^{a}	14.5±0.02^{c}	13.5±0.07^{c}	15.5±0.7^{bc}	13.5±0.7^{c}	13.5±0.07^{c}	18.2±0.01^{a}	16.5±0.07^{b}	11.5±0.3^{d}	13.5±0.07^{c}
SXT	0	0	0	0	0	0	0	0	0	0	0	0	0	0
FOX	0	0	0	0	12.5±0.7^{c}	0	0	0	11.5±0.7^{c}	0	0	0	0	0
NAL	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ATB: Antibiotics; C: Chloramphenicole; AMX: Amoxicillin; SXT: Cotrimoxazole; FOX: Cefoxitine; NAL: Nalidixic acidHorizontally, mean values bearing the same letter are not statistically different at the 5% level.

Resistance of isolates to different concentrations of bile salts

The lactic acid bacteria isolates showed good growth ability in the presence of different concentrations of bile salts (0 to 0.4 %) (Fig. 2). At salt concentrations of 0.1% and 0.2 %, all isolates had higher growth percentages (above 50%), except isolate T2.10 which had a growth percentage of 46.43 ± 0.06 % at 0.1% concentration and 39.86 ± 0.07%, at 0.2 % salt concentration. At the 0.3 % concentration, isolates T3.5 and T3.1 had growth percentages of 86.17 ± 0.05 and 62.19 ± 0.13, respectively. At the 0.4 % concentration, no isolate reached 50 % growth.

Auto-aggregation test

The different isolates showed self-aggregation capacity with values ranging from 34.07±0.08 % to 77.280.09 %, after 2 hours of incubation (Table 6). Most isolates showed more than 50 % self-aggregation after 2 hours of incubation, with a significant difference observed between the different isolates. The T3.1 lactic acid bacterium isolate showed the highest rate of auto-aggregation (77.28±0.09 %), while the lowest rate (34.07±0.08 %) was observed in the T3.1 lactic acid bacterium isolate after 2 hours of incubation.

Selection of isolates with probiotic potential using the pheatmap software

The creation of a heatmap made it possible to group the different isolates of lactic acid bacteria on the basis of the different characteristics evaluated. This study on the probiotic properties of lactic acid bacteria isolated from fermented maize pulp shows that the LAB isolates (T3.1, T3.7 and T3.4) have probiotic strain capacities. These strains are identified by comparing the bands along the axis of the dendrogram (Fig. 3).

Table 4. Growth rate (%) of lactic acid bacteria isolates (LAB) as a function of pH.

LAB isolates	Growth rate (%) of lactic acid bacteria					
	pH1	pH2	pH3	pH4	pH5	pH6
T3.4	0	0	75.25 ± 0.09^{c}	81.13 ± 0.54^{b}	88.32 ± 0.24^{a}	100.000
T3.5	0.09 ± 0.04^{d}	0.22 ±0.01^{d}	74.98 ± 0.22^{c}	79.19 ± 0.35^{b}	91.01 ± 0.32^{a}	100.000
T1.2	0.11 ± 0.002^{c}	0.21 ± 0.05^{c}	44.96 ± 0.08^{b}	61.95 ± 0.24^{a}	71.11 ±0.001^{c}	100.000
T1.4	0	1.34 ± 0.10^{c}	13.25 ± 0.15^{b}	82.01 ± 0.34^{a}	82.01 ± 0.34^{a}	100.000
T1.6	0	1.04 ± 0.56^{d}	3.25 ± 0.003^{c}	45.22 ±0.001^{b}	85.84 ± 0.39^{a}	100.000
T1.8	0	1.32 ± 0.01^{d}	59.12 ± 0.17^{b}	62.33 ± 0.02^{a}	56.85 ± 0.51^{c}	100.000
T1.9	0.52 ± 0.03^{d}	2.01 ± 0.60^{c}	24.04 ± 0.43^{b}	38.31 ± 0.001^{a}	88.31 ± 0.002^{a}	100.000
T2.3	0	0.34 ± 0.002^{d}	74.19 ± 0.36^{b}	38.31 ± 0.001^{c}	87.03 ± 0.01^{a}	100.000
T2.10	0.02 ± 0.001^{d}	0.35 ± 0.10^{d}	21.34 ± 0.001^{c}	44.99 ±4.56^{a}	58.44 ± 0.1^{b}	100.000
T3.1	0.32 ± 0.01^{d}	0.34 ± 0.09^{d}	22.34 ± 0.001^{c}	74.99 ± 4.51^{b}	97.11 ± 0.16^{a}	100.000
T2.7	3.10 ± 0.03^{d}	3.24 ± 0.10^{d}	13.06 ± 0.21^{c}	37.01 ± 0.19^{b}	99.58 ± 0.20^{a}	100.000
T3.7	0	0	25.81 ± 0.29^{c}	72.04 ± 0.56^{b}	82.35 ± 0.04^{a}	100.000
T3.6	0	0	4.96 ± 0.035^{c}	47.50 ± 0.002^{b}	73.50 ± 0.08^{a}	100.000
To.5	1.02 ± 0.54^{c}	1.28 ± 0.04^{c}	1.32 ± 0.001^{bc}	2.10 ± 0.16^{b}	96.32 ± 0.00^{a}	100.000

Identification of lactic acid bacteria isolates with probiotic potential by MALDITOF mass spectrometry

The identification of the three isolates of lactic acid bacteria with probiotic potential reveals that the LAB isolates T3.1, T3.4 and T3.7 belong to the genus *Lactobacillus* (*Lactobacillus fermentum*).

Discussion

Characterisation of the probiotic properties of lactic acid bacteria isolates isolated from fermented maize pulp

The characterisation of probiotic potentialities allowed the identification of strains with interesting antibacterial activities. These lactic acid bacterial isolates have different antimicrobial activities against the tested indicator strains. Some LAB isolates showed significant activity against the target pathogens, with inhibition diameters ranging from 8.5 ± 0.07 to 31.5±2.3 mm. Out of a total of 14 isolates tested, 7 showed an effect simultaneously on *E. coli*, *S. enterica* serogroup O:8 and *S. aureus*. The antibacterial activities obtained with *Salmonella*, *Staphylococcus aureus* and *E. coli* appear to be an important factor in the use of probiotics for human consumption (Gusils *et al.*, 2003). These results corroborate those of Hyung *et al.* (2006), who showed that among the strains isolated from Jeotgal (Korean fermented food), Leuconostoc mesenteroides strains showed inhibitions against *S. aureus* with a diameter of 22 mm. This is because lactic acid bacteria metabolise lactose into lactic acid, thereby lowering the pH and creating an unfavourable environment for the development of pathogenic bacteria and spoilage microorganisms (Ehrmann *et al.*, 2002). This antagonistic effect of LAB against pathogens could be explained by the fact that LAB produces antimicrobial substances of a protein nature called bacteriocins, such as nisin produced by lactococci directed against Bacillus and Clostridium. Plantaricin and sakacin, both produced by lactobacilli active against E. coli, Listeria and some yeasts (Ogunbanwo *et al.*, 2003), contribute to the preservation of the microbial and organoleptic balance of cheese (Georgalaki *et al.*, 2002). This characteristic is used in industry for the destruction of undesirable bacteria and pathogens in food manufacturing. Another important characteristic when selecting probiotics is antibiotic resistance.

In the treatment of gastro-intestinal disorders, the constant use of antibiotics confers a certain resistance of pathogens to antibiotics and therefore, a probiotic bacterium must be resistant to these antibiotics. Lactic acid bacteria are inherently resistant to the quinolone family by an unknown mechanism (Bruns and Abbas, 2005). According to Zhou *et al.* (2005), several strains of lactic acid bacteria are resistant to antibiotics (fusidic acid, nalixidic acid) and aminoglycosidoses (gentamicin, kanamycin, streptomycin). These bacteria have a very high natural resistance to several antibiotics, but this

resistance in many cases is not transmissible (Ashraf and Shah, 2011). It should be noted that the majority of lactic acid bacteria isolates tested in this study are resistant to the majority of antibiotics tested (80-100 %). This is an important feature as the most common bacterial diseases are caused by *Staphylococcus aureus*, *Escherichia coli* and *Salmonella,* which are reportedly resistant to many antibiotics. Thus, the addition of probiotics would reduce the use of antibiotics in the curative setting, given the antagonistic effect of these bacteria against pathogenic germs.

Table 5. Percentage (%) of hydrophobicity of lactic acid bacteria isolates.

LAB isolates	Phenol concentrations						
	0	0,1 %	0,2 %	0,3 %	0,4 %	0,5 %	0,6 %
T3.4	100	97.18 ± 0.05^{a}	96.50 ± 0.12^{b}	94.08 ± 0.09^{c}	91.14 ± 0.11^{d}	76.12 ± 0.06^{e}	43.77 ± 0.07^{f}
T3.5	100	86.34 ± 0.09^{a}	55.54 ± 0.21^{b}	45.76 ± 0.12^{c}	38.62 ± 0.08^{d}	31.70 ± 0.08^{e}	24.21 ± 0.09^{f}
T1.2	100	80.17 ± 0.11^{a}	77.22 ±0.11^{b}	50.31 ± 0.11^{c}	42.80 ± 0.08^{d}	42.19 ± 0.13^{e}	40.78 ± 0.15^{f}
T1.4	100	56.16 ± 0.08^{a}	54.28 ± 0.09^{b}	37.78 ± 0.06^{c}	35.18 ± 0.08^{d}	28.22 ± 0.13^{e}	26.84 ± 0.13^{f}
T1.6	100	83.31 ± 0.09^{a}	76.87 ± 0.04^{b}	59.46 ± 0.08^{c}	48.26 ± 0.07^{d}	47.61 ± 0.11^{e}	42.68 ± 0.14^{f}
T1.8	100	95.31 ± 0.9^{a}	70.25 ± 0.06^{b}	43.18 ± 0.11^{c}	33.18 ± 0.11^{c}	31.17±0.11^{c}	18.32 ±14.30^{c}
T1.9	100	91.19 ± 0.09^{a}	86.46 ± 0.13^{b}	80.46 ± 0.13^{b}	77.09 ± 0.06^{d}	75.20 ± 0.05^{e}	71.87 ± 0.06^{f}
T2.3	100	69.86 ± 0.13^{a}	68.12 ± 0.04^{b}	50.70 ± 0.13^{c}	40.60 ± 0.08^{d}	30.23 ± 0.04^{e}	27.11 ± 0.07^{f}
T2.10	100	67.50 ± 0.13^{b}	59.21 ± 0.13^{a}	46.48 ± 0.13^{c}	43.87 ± 0.06^{d}	41.29 ± 0.11^{e}	33.38 ± 0.06^{f}
T3.1	100	94.08 ± 0.11^{a}	90.90 ± 0,06^{b}	87.74 ± 0.09^{c}	72.19 ± 0.11^{d}	62.16 ± 0.08^{e}	33.12 ± 0.11^{f}
T2.7	100	92.26 ± 0.15^{a}	78.20 ± 0.04^{b}	65.60 ± 0.11^{c}	52.36 ± 0.12^{d}	48.89 ± 0.03^{e}	39 ± 0.13^{f}
T3.7	100	85.65 ± 0.08^{a}	73.50 ± 0.08^{b}	61.90 ± 0.08^{c}	50.19 ± 0.10^{d}	49.11 ± 0.15^{e}	38.57 ± 0.14^{f}
T3.6	100	94.71 ± 0.08^{a}	90.44 ± 0.09^{b}	84.48 ± 0.09^{c}	60.57 ± 0.14^{d}	52.18 ± 0.12^{e}	50.00 ± 0.00^{f}
T0.5	100	85.21 ± 0.13^{a}	78.93 ± 0.08^{b}	62.91 ± 0.06^{c}	51.89 ± 0.06^{d}	42.21 ± 0.12^{e}	26.99 ± 0.01^{f}

The pH is one of the parameters that influence the growth of probiotics in the gastro-intestinal tract. Thus, the isolates were tested at different acidic pH and the growth rate of the strains observed in an acidic environment was higher than 50 % after 24 hours. All the isolates tested were resistant to acidity after 24 hours of incubation.

Table 6. Percentage (%) of auto-aggregation of lactic acid bacteria isolates.

LAB isolates	Auto-aggregation % after 2 hours
T3.1	77.28 ± 0.09^{a}
T3.5	62.89 ± 0.11^{f}
T1.2	65.55 ± 0,08^{d}
T1.4	60.78 ± 0.15^{g}
T1.6	71.27 ± 0.05^{b}
T1.8	54.55 ± 0.10^{j}
T1.9	53.67 ± 0,15^{k}
T2.3	69.41 ± 0.17^{c}
T2.10	59.21 ± 0.15^{i}
T3.4	54.25 ± 0.09^{j}
T2.7	58.09 ± 0.06^{i}
T3.7	34.07 ± 0.08^{l}
T3.6	63.46 ± 0.11^{e}
T0.5	61.08 ± 0.78^{g}

This growth in an acid environment could allow them to pass the gastro-intestinal barrier. These results are similar to those of Bruno (2012), who showed that all the strains of lactic acid bacteria tested in his work were able to resist pH 3, after 3h of incubation and some isolates showed growth after 1h of incubation at pH 2. The ability of LAB to grow in an acidic environment could be explained by the fact that they are widespread in nature, and are found in different ecological niches such as milk and milk products, corn, meat, fish, human and animal mucosa and in the digestive tract of humans and animals (Bielecka *et al.*, 1993), and constitute the majority flora of many fermented products. The tolerance of lactic acid bacteria to different concentrations of bile salts is a criterion for the in vitro selection of probiotics. It is generally considered necessary to assess their ability to resist the effects of bile salts that condition their ability to survive the conditions of the Gastro-Intestinal Tract (GIT), and to colonise the intestinal environment (Boudouhi *et al.*, 2005). Indeed, the optimal concentration of bile in the human intestinal environment varies from 0.3% to 0.6% (Psomas *et al.*, 2001).

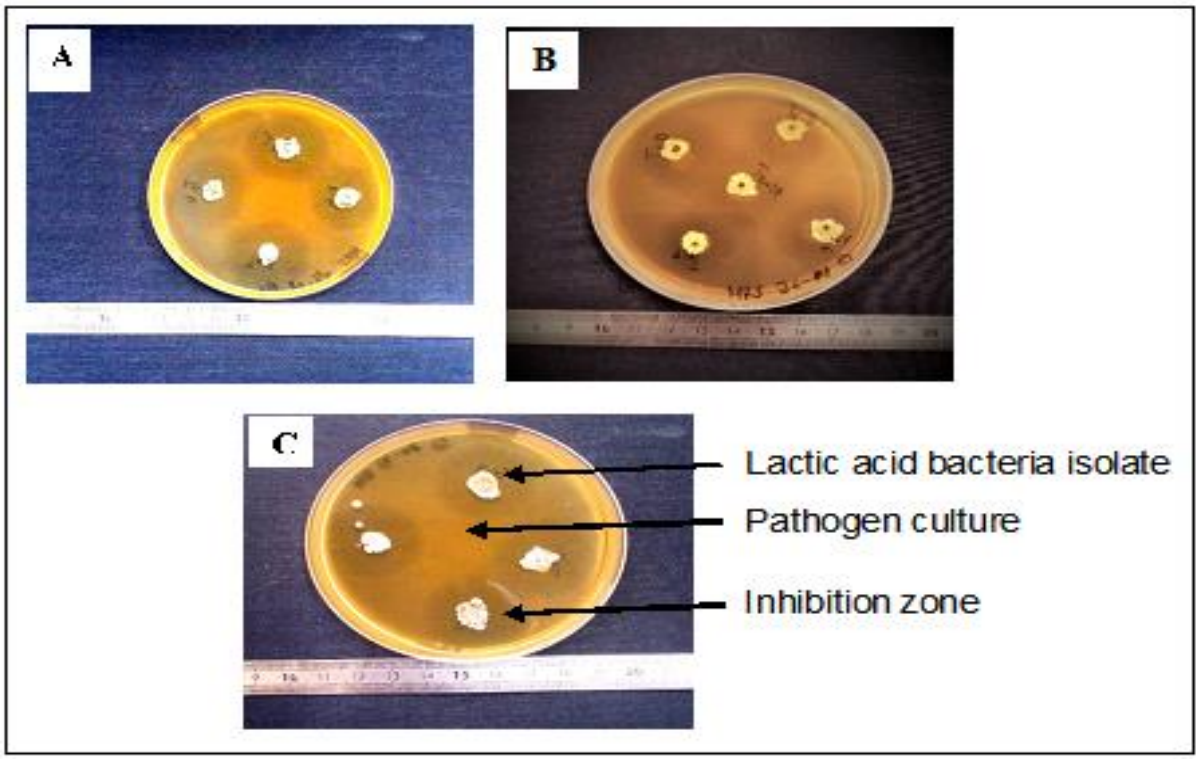

Fig. 1. Interaction of lactic acid bacteria isolates with target pathogens. A: *Escherichia coli*; B: *Salmonella enterica* O:8; C: *Staphylococcus aureus*.

All lactic acid bacteria isolates tested in this study showed growth at 0.1 %, 0.2 % and 0.3 % bile salts for 24 hours. However, no strain showed a growth rate of more than 50 % at the 0.4 % salt concentration. These results are similar to those of Klaenhammer (2000), who showed that the resistance of bacteria to bile salts comes from transporter proteins belonging to the ABC (ATP-Binding-Cassette) family. According to Moser and Savage (2001), two hypotheses could explain the resistance of lactic bacteria to bile salts. One hypothesis claims that there are bacterial species that can de-conjugate bile salts, in order to exploit taurine (an amino acid in the composition of bile salts) as an electron acceptor. The second hypothesis assumes that Bile Salt Hydrolase (BSH) enzymes protect the bacteria from bile salt toxicity. These types of transporters contribute to the mechanism of cellular defense through resistance to antibiotics, bile salts and peptides. The concentration of bile salts in humans is on average 0.3 % during digestion (Bakari *et al.*, 2011). Therefore, the tested lactic acid bacteria isolates that showed growth in the presence of bile salts up to 0.4 % can survive the different concentrations of salts produced in the digestive tract for pathogen inhibition. In an approach to assess a relationship between lactic acid bacteria and surface cells in the TGI, hydrophobicity and self-aggregation tests were performed. The criterion of adhesion to intestinal mucus is frequently studied when selecting probiotic strains (Van Tassell and Miller, 2011).

The results obtained showed that 90 % of the isolates had a high percentage of hydrophobicity. The results of the auto-aggregation test showed that all the lactic acid bacteria isolates tested had a self-aggregation capacity of more than 50 %.

This result is in agreement with those of Erhmann *et al.* (2002) who found that out of 112 isolates of lactobacilli isolated from duck crop, 31 isolates have a high self-aggregation capacity. These results are also similar to those of Boukhalfi (2020), who showed that the presence of a protein monolayer in most lactobacillus species confers probiotic traits (aggregation, adhesion to eukaryotic cells). Strains with a high self-aggregation capacity also have a high hydrophobicity and, thus, a high adhesion to intestinal mucus (Taheri *et al.*, 2009).

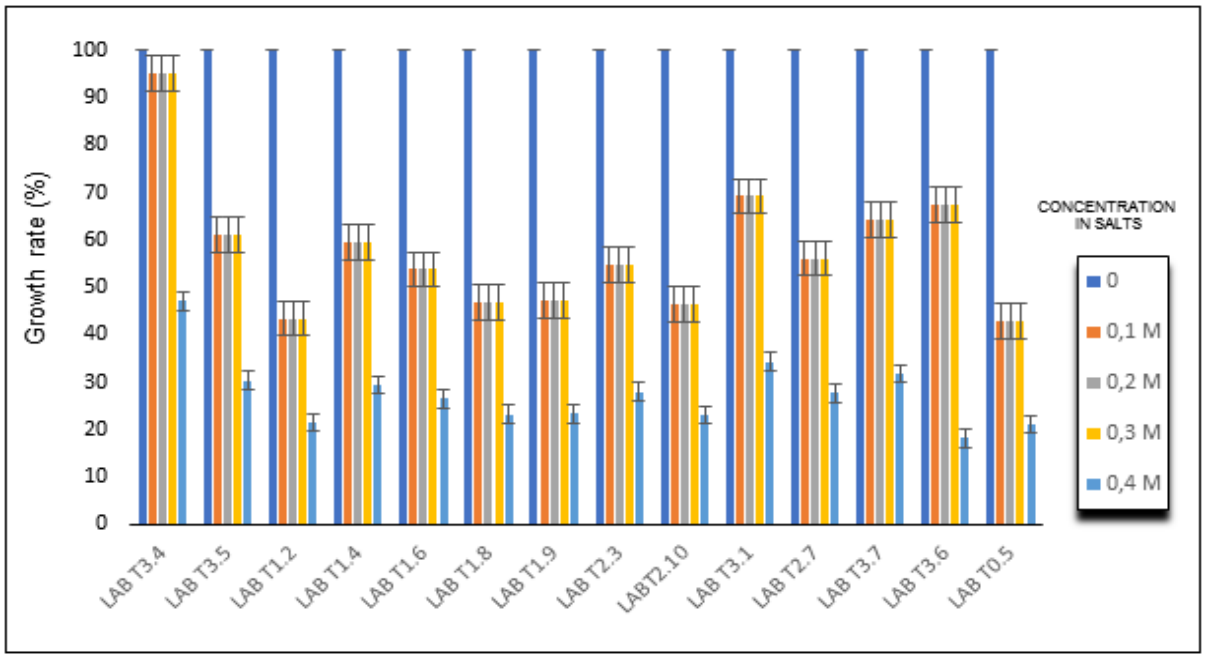

Fig. 2. Growth rate of lactic acid bacteria isolates as a function of bile salt concentrations.

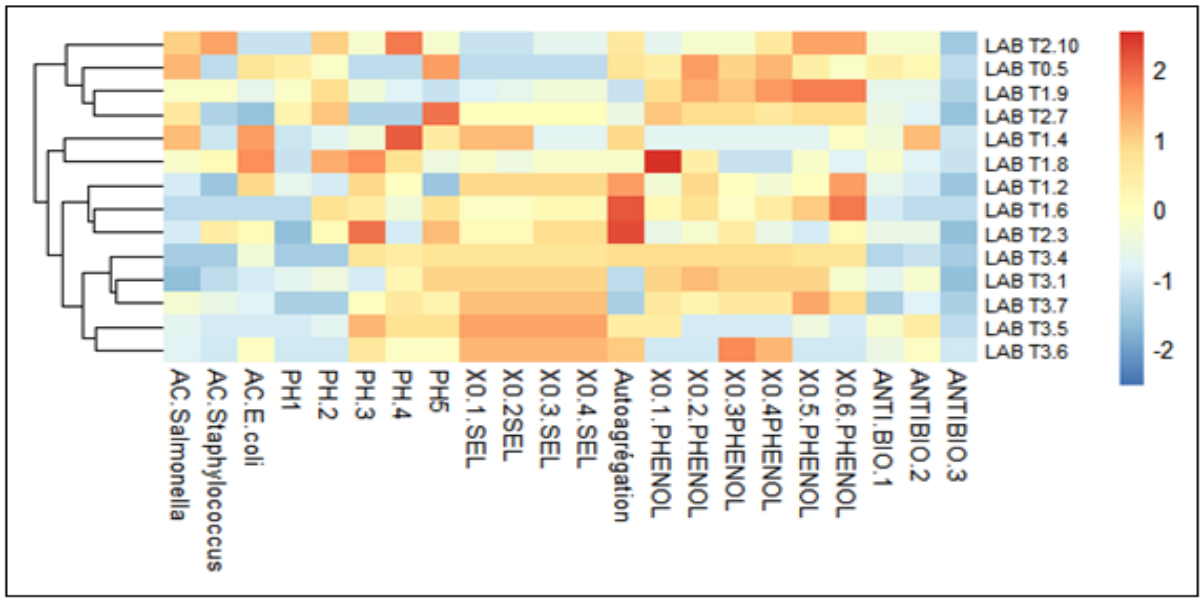

Fig. 3. Pheatmap of the studied lactic acid bacteria isolates.

Antibio: antibiogram; x phenol: phenol concentration; x salt: bile salt concentration; A.C: antibacterial activity. The hierarchical heat map shows the significant difference between some probiotic properties of the grouped isolates. The difference in property is represented by a colour key with a red and blue gradient.

Selection of probiotics by the pheatmap software method and identification of lactic acid bacteria isolates with probiotic potential by MALDITOF mass spectrometry

The results of the pheatmap treatment were used to group together the different probiotic properties of all isolates, which would have similar selection criteria. It was found that the LAB isolates T3.1, T3.7 and T3.4 presented the best probiotic profiles.The identification of the three lactic acid bacteria isolates with probiotic potential, by the MALDITOF-MS technique, revealed that the LAB T3.1, T3.4 and T3.7 isolates belong to the genus *Lactobacillus* (*Lactobacillus fermentum*). Probiotic bacteria play an important role in protecting the host from harmful microorganisms, enhancing the host immune system,

improving food digestibility and reducing metabolic disorders. The *L. fermentum* strains isolated from fermented maize dough in this study have very interesting antimicrobial activities against entero-invasive and foodborne pathogens such as *Escherichia coli*, *Salmonella enterica* serogroup O:8 and *Staphylococcus aureus*.

In addition to this activity, the study showed that they have strong probiotic potential, which would imply their beneficial action when supplemented with corn-based foods, with the simple aim of providing consumers with a food that is beneficial to health. Indeed, its supplementation would help to improve immunity, fight digestive diseases and even reduce LDL cholesterol. Well documented by scientists, this bacterial strain is said to produce antimicrobial, antioxidant and anti-inflammatory compounds (Marika and Mihkler, 2009; Kullisaar *et al.*, 2009).

Regarding its involvement in cholesterol reduction, recent studies by Simons *et al.* (2006) reveal that this bacterium, when supplemented with plant foods, would contribute to the reduction of dietary cholesterol. Also, another effect of the *Lactobacillus fermentum* strain is that it binds and breaks down lipid-rich bile salts, creating an effect similar to some of the original anti-cholesterol pharmaceutical drugs such as cholestyramine (Simons *et al.*, 2006). In view of the above and the results obtained in this study, the *Lactobacillus fermentum* species isolated from fermented maize pulp in Côte d'Ivoire has potential for food preservation and biomedical applications.

Conclusion

From the data of the present study, it can be concluded that the *Lactobacillus fermentum* strains isolated from fermented maize paste in Côte d'Ivoire, are tolerant to stressful gastro-intestinal conditions and showing a variable capacity to adhere to human epithelial cells, have known probiotic potentialities. Therefore, they could be used as a food supplement, with a view to improving both the nutritional and health aspects, which would contribute to the safety of food for human consumption.

References

Ashraf R, Shah N. 2011. Antibiotic resistance of probiotic organisms and safety of probiotic dairy products, Review article. International Food Research Journal **18(3),** 837-853.

Bakari D, Tatsadjieu NL, Mbawala A, Mbofung CM. 2011. Assessment of physiological properties of some lactic acid bacteria isolated from the intestine of chickens use as probiotics and antimicrobial agents against enteropathogenic bacteria. Innovative Romanian Food Biotechnology **8,** 33-40.

Behira B. 2012. Contribution à l'étude des espèces de lactobacilles à caractère probiotique isolées de la poule domestique (*Gallus gallus domesticus*) de l'ouest Algérien, Thèse pour l'obtention du diplôme de Doctorat, faculté des sciences, Département de Biologie- option microbiologie alimentaire, Université d'Oran (Algérie), 24-93.

Belarbi F. 2011. Isolement et sélection des souches de bactéries lactiques productrices des métabolites antibactériennes, Mémoire de magistère, faculté des sciences, Département de Biologie- option microbiologie alimentaire et industrielles. Université d'Oran Senia, (Algérie), 98-102.

Bielecka M, Babuchowski A, Boutard A, Dubeau H. 1993. Les probiotiques, Revue canadienne de microbiologie **12**, 1089-1095.

Boone PS, Charles JD, Wanzie LR. 2008. Évaluation sous régionale de la chaîne de valeurs du maïs, rapport technique, ATP n°1. Bethesda, MD : Projet ATP, Abt Associates Inc. 12515. May 29. Review, 329-348.

Boudouhi R, Ferreira C, Morel E, Szymanski A, Tizaoui S. 2005. Aliments fonctionnels « Réalité et/ou allégation », mémoire de these de doctorat, Université Lille 1, Sciences et Technologies Lille (France), 154-202.

Boukhalfi F. 2020. Contribution à l'évaluation de quelques caractères probiotiques et technologiques Du Bifidobactérie isolées à partir des selles de nourrissons et lait maternel ; Mémoire de master ; Université Mohamed Khider de Biskra Faculté des sciences exactes et sciences de la nature et de la vie, Département des sciences de la nature et de la vie, Biskra (Algérie), 38-79.

Bruno E. 2012. Sélection de bactéries probiotiques et amélioration de la survie et de la fonctionnalité d'une bactérie modèle, *Bifidobacterium bifidum*, par modification du potentiel d'oxydoréduction par bullage de gaz. Mémoire de master, Université de Bourgogne, Dijon (France), 33-76.

Bruns HA, Abbas HK. 2005. Réponses of short-season corn hybrids to a humid subtropical environment. Agronomy Journal **97 (2),** 446-451. http://dx.doi.org/10.2134/agroj2005.0446

CASFM. 2019. Comite comité de l'antibiogramme de la société Française de Microbiologie recommandation **1(0),** 225-144.

Cromwell GL, Calvert C, Cline TR, Crenshaw JD, Crenshaw TD, Easter RA, Ewan RC, Hamilton CR, Hill GM, Lewis AJ, Mahan DC, Miller ER, Nelssen JL, Pettigrew JE, Tribble LF, Veum TL, Yen JT. 1999. Variability among sources and laboratories in nutrient analyses of corn and soybean meal. Journal of Animal Science **77(12),** 3262-3273.

Demont M. 1997. Système de commercialisation des produits vivriers en Côte d'Ivoire : Étude pour le maïs *(Zea mays)* et l'arachide (*Arachis hypogaea*)". Rapport Technique Institut Des Savanes, Côte d'Ivoire, 22-58.

Ehrmann MA, Kurzak P, Bauer J, Vogel RF. 2002. Characterization of lactobacilli towards their use as probiotic adjunts in poultry. Journal of Applied Microbiology **92,** 966-975. http://dx.doi.org/10.10.1046/j.13652672.2002.01608.x

FAO/WHO. 1973. Aliments diététiques ou de régime (y compris les aliments destinés aux nourrissons et enfants en bas âge). Programme mixte sur les normes alimentaires. Commission du Codex Alimentarius. Rome **4,** 11-14.

Georgalaki MD, Paganelli M, Anastasiou R, Kalantzopoulos G, Tsakalidou E. 2002. Purification and characterization of the X-prolyl-dipeptidyl aminopeptidase (PepX) from *Streptococcus macedonicus* and cloning of the pepX gene. Dairy Science &Technology **82(6)**, 657–671. http://dx.doi.org/10.1051/lait:2002040

Gusils C, Oppezzo RP, Gonzalez S. 2003. Adhesion of probiotic lactobacilli to chick intestinal mucus. Canadian Journal of Microbiology **49(7),** 472-478. http://dx.doi.org/10.1139/w03-055

Hyung JK, Quinn BC, Madon T, Steve L. 2006. Graduate Student Mental Health: Needs Assessment and Utilization of Counseling Services. Journal of College Soudent Development **47(3),** 244 -266.

Kim EY, Kim YH, Rhee MH, Song JC, Lee KW, Kim SC. 2007. Selection of*Lactobacillus sp*. PSC101 that produces active dietary enzymes such as amylase, lipase, phytase and protease in pigs. The Journal of General and Applied Microbiology **53(2),** 111-117. http://dx.doi.org/10.2323/jgam.53.111

Klaenhammer TR. 2000. Probiotic Bacteria: Today and Tomorrow. Journal of Nutrition **130**(2s Suppl), 415–416. http://dx.doi.org/10.1093/jn/130.2.415S

Kullisaar T, Songisepp E, Aunapuu M, Kilk K, Arend A, Mikelsaar M, Rehema A, Zilmer M. 2010. Complete glutathione system in probiotic *Lactobacillus fermentum* ME-3. *Appl Biochem Microbiol* **46,** 481–486.

https://doi.org/10.1134/S0003683810050030

Marika M, Mihkel Z. 2009. *Lactobacillus fermentum* ME-3 – an antimicrobial and antioxidative probiotic, Microbial Ecology in Health and Disease **21(1),** 1-27.
https://doi.org/10.1080/08910600902815561

Moser S, Savage DC. 2001. Bile salt hydrolase activity and resistance to toxicity of conjugated bile salts are unrelated properties in lactobacilli. Applied Environment and Microbiology **67**, 3476-3480.
http://dx.doi.org/10.1128/AEM.67.8.34763480.2001

Nago CM. 1997. La transformation traditionnelle du mais au Bénin : Détermination des caractéristiques physico-chimiques des variétés en usage, relation avec l'obtention et la qualité des principaux produits dérivés, Thèse de Doctorat d'Etat es, Université Paris-7, Deris Diderot, Paris (France), 5-201.

Ogunbanwo ST, Sanni Al, Omilude AA. 2003. Characterization of lactobacilli in cheese. Journal of dairy research **25,** 431-438.

Psomas EL, Andrighetto C, Litopoulou TE, Lombardi A, Tzanetakis N. 2001. Some probiotic properties of yeast isolates from infant faeces and feta cheese. International Journal Food Microbiology **69,** 125-133.
http://dx.doi.org/10.1016/s0168-1605(01)00580-3

Simons LA, Amansec SG, Conway P. 2006. Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. Nutrition Metabolism and Cardiovascular Diseases **16(8),** 531-535.
https://doi.org/10.1016/j.numecd.2005.10.009

Taheri HR, Moravej H, Tabandeh F, Zaghari M, Shivazad M. 2009. Screening of lactic acid bacteria toward their selection as à source of chicken probiotic. Poultry Sciences **88,** 1586-1593.
http://dx.doi.org/10.3382/ps.2009-00041

Yobouet KS. 2020. Approche de lutte biologique contre les mycotoxines contaminant le maïs (*Zea mays*) par le criblage de bactéries lactiques à activité antifongique, Mémoire Présenté pour l'obtention du Diplôme de Master de Biotechnologies- Biosécurité - Bioressources de L'Université Félix HOUPHOUET-BOIGNY, Abidjan (Côte d'Ivoire), 12-50.

Van Tassell ML, Miller MJ. 2011, *Lactobacillus* adhesion to Mucus, Nutrient **3**, 613-636.
http://dx.doi.10.3390/nu3050613

Zhou JS, Pillidgec CJ, Gopal PK, Gill HS. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. International Journal of Food Microbiology **98(2),** 211- 217.
http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.011

Zuo L, Zhou T, Pannell BK, Ziegler AC, Best TM. 2015. Biological and physiological role of reactive oxygen species- the good, the bad and the ugly. Acta Physiology (Oxf), **214,** 329-348.
http://dx.doi.org/10.1111/apha.12515