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Research Article

**Antibacterial and probiotic properties of lactic acid
bacteria from traditional sorghum beer production
in Côte d'Ivoire**

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ABSTRACT

Antibacterial activity of 117 lactic acid bacteria isolated from different steps of *tchapalo* production, a traditional sorghum beverage, was tested using the agar-well diffusion method. Strains showing antibacterial activity were identified by biochemical characteristics based on API 50CH, and their resistance to the digestive tract conditions was tested through the determination of pH and bile salts tolerance. As results, most of isolates inhibited indicator strains by organic acids production. *Bacillus cereus*, *Lactobacillus delbrueckii*, *Listeria innocua* and *Pseudomonas aeruginosa* were the most sensitive to this production. Besides, 27 lactic acid bacteria produced bacteriocins which inhibited *Lb. delbrueckii* F/31. Among them, four strains displayed also inhibitory activity against *Enterococcus faecalis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp, *Enterococcus faecalis* CIP 105042, *Enterococcus faecium* ATCC 51558 and *Listeria innocua*. These 27 lactic acid bacteria were identified as *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus pentosus* and *Pediococcus acidilactici*. *Pediococcus acidilactici* strains At1BE22 and At34E21 which had good antibacterial potential were tolerant against acidic conditions and bile salts. Therefore, they could survive in the gastrointestinal tract environment. So, they could be used for *tchapalo* preservation and also to protect *tchapalo* consumers' health.

Key words: Antimicrobial activity, probiotic, lactic acid bacteria, *tchapalo* production.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive, non-spore forming and catalase negative bacteria. They represent a heterogeneous species which common feature is the production of lactic acid and belong to various genus such as *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus*, *Aerococcus*, *Carnobacterium* and *Weissella*¹. Lactic acid bacteria produce various types of compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins during fermentation. Bacteriocins are a heterogeneous family of small, heat-stable peptides with potent antimicrobial activity produced by many bacterial

species^{2, 3, 4}. Those produced by Gram-positive bacteria have a bactericidal or bacteriostatic effect on other genus and species, but their activities are usually limited to other Gram-positive bacteria. Studies on bacteriocins from LAB have been carried out at large scale over the last decades, including the use of bacteriocins or producer organisms as natural food preservatives and their potential utility in human health applications^{3, 4}.

Some LAB strains called probiotics are able to protect and have a beneficial effect on the host related health^{3,5,6}. Indeed, probiotics are live microorganisms defined by the Food and Agricultural Organization / World Health Organization⁷ as: "Live

microorganisms whose administration in adequate amount to the body is able to confer a health beneficial effect on the host". The probiotic bio-therapeutic effect is multifactorial, including: maintaining the intestinal microbial balance in human and animal hosts, prevention and treatment of diarrhea, improving lactose tolerance, suppression of cancer, improving digestion, reduction in serum cholesterol levels and modulation of the host immune system^{8, 9, 10}. Probiotic strains survive harsh conditions in the gastrointestinal tract and adhere to intestinal epithelial cells. They also have some defense mechanisms against pathogenic microorganisms. Thus, probiotics are increasingly used in the food industry. Some probiotic LAB strains are already consumed as part of fermented food products or as dietary supplements¹¹. The most important contribution of these bacteria to fermented products is to improve the nutritive qualities of the raw material and to inhibit the growth of spoilage and pathogenic bacteria².

Tchapalo is a traditional opaque, sour alcoholic beverage produced in West Africa and particularly in Côte d'Ivoire. It contains a large amount of insoluble materials with continuous fermentation and it has a nutritional value that significantly contributes to improve the diet of consumers^{12, 13, 14}. *Tchapalo* is very well appreciated by the public and its consumption is getting more and more widespread. According to consumers *tchapalo* has some therapeutic virtues. But these virtues have not been scientifically proven yet, whereas two successive steps of fermentation occur at ambient conditions during its production^{12, 13}. The first one, which is a spontaneous fermentation, is mainly provided by LAB such as *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Enterococcus*¹⁴. The second one, which is an alcoholic fermentation, is performed by the sweet wort inoculum made of dried yeast as well as LAB harvested from previous *tchapalo*. The presence of these LAB confers the sour taste and storage longevity. These bacteria might have also probiotic properties. However, their antimicrobial and probiotic activities are not well documented. In this work, the antagonistic activity of LAB isolated during *tchapalo* production was investigated against spoilage and food-borne pathogens. The isolates having antimicrobial activity were identified based on phenotypic characteristics and their resistance to the conditions of the digestive tract were also tested.

MATERIALS AND METHODS

Sampling and bacteria strains isolation:

Sampling was carried out on different products during nine *tchapalo* production. Samples were collected from three municipalities of Abidjan

(Abobo, Attecoubé and Yopougon) in the district of Abidjan (Southern of Côte d'Ivoire). A total of 9 samples of sorghum, sorghum malt, sorghum malt flour, mash, cooked sediment, wort, sour wort, sweet wort, traditional starter and *tchapalo* were collected from these municipalities according to Aka et al.¹² in sterile small bottles, labelled and then transported to the laboratory in a box containing a freezing pack. Ten milliliters of each sample were aseptically added into 90 ml of sterile 0.9% NaCl solution and mixed. Serial dilutions (10^{-1} to 10^{-7}) are performed and 0.1 ml aliquot of the appropriate dilution is directly inoculated in duplicate on Man Rogosa Sharpe agar¹⁵; then incubated anaerobically at 30°C, 37°C and 45°C for 48 hours for enumeration of LAB. A total of 117 LAB strains were isolated characterized by cell morphology, catalase and oxidase tests. Cultures were maintained as frozen stocks at -80°C in MRS broth containing 20% glycerol. Before experiments, cultures were propagated twice in MRS at 37°C, the transfer inoculum was 1% (v/v) of 16 h culture grown in fresh medium.

Indicator bacteria strains:

The indicator bacteria strains were obtained from national laboratory of public health cultures collection and our laboratory culture collection (CSRS microbiology laboratory). Their media and cultivation conditions used for antibacterial activities are presented in Table 1.

Antibacterial activities of lactic acid bacteria isolates:

Extraction of Cell-free Supernatants (CFS):

Antibacterial activities were assayed against indicator bacteria strains using an agar diffusion method described by Arici et al.¹⁶. Ten mL of MRS broth was inoculated with 1% (v/v) of 16 h culture of each LAB strain and was incubated at 37°C for 24 h. Then, cell-free solutions were obtained by centrifugation ($6000 \times g$, TGL-16 M) for 15 min at 4°C of the culture, followed by filtration of the supernatant through a 0.45 μ M pore size filter (Corning syringe filters, Sigma-Aldrich, Germany). Three portions of the sterilized CFS were transferred aseptically into sterile samples bottles. One of these portions was neutralized with 5 N NaOH (pH 6.5-7). One other of these portions was neutralized with 5 N NaOH (pH 6.5-7) and treated with 1 mg/mL of catalase (Sigma-Aldrich, Germany) to eliminate organic acid and hydrogen peroxide (H_2O_2) respectively.

Antibacterial activity of CFS:

The indicator test bacteria were incubated in Brain Heart Infusion (BHI) or nutrient broth or MRS broth at 37°C or 44°C for 18-24 h (Table 1).

Approximately 10^6 ufc/mL of the indicator bacteria to be tested for sensitivity were inoculated (1% v/v) into 20 mL of BHI or nutrient or MRS soft agar (0.9% agar) and the agar plates were allowed to dry. A 100 μ L sample of the un-neutralized and neutralized CFS were filled in 5 mm diameter sealed wells which were cut into the agar plates. The culture plates were pre-incubated at 4°C for 2 h and then incubated for 18 h and the zones of inhibition were measured in diameter (mm). Antibacterial tests were carried out in triplicate and the mean values recorded.

Phenotypic characterizations:

Cell morphology was observed using an optical microscope and isolates tested for catalase production. Twenty seven LAB which exhibited inhibitory effect against indicator strains were selected for identification to species level using the Api 50 CH galleries and Api 50 CHL medium (Bio Mérieux, l'Etoile, France). Tests of these galleries were performed according to the manufacturer's instructions. The APILAB Plus database (Bio Merieux, France) was used to interpret the results.

Resistance to the conditions of the digestive tract: Determination of optimal growth and pH resistance:

Resistance to pH 3 is often used *in vitro* assays to determine the resistance to stomach pH. Food usually stays in the stomach for 3 h and this time limit was taken into account¹⁷. For the determination of optimal growth and pH of LAB, a modified method of Hoque et al.¹⁸ was applied in this study; so 1% (v/v) fresh overnight culture of LAB were inoculated into MRS broth with varying pH ranging from 2.5 to 8.5. The pH was adjusted with 5 N HCl and 5 N NaOH. The inoculated broths were incubated at 37°C for 3 h. After incubation, growth of the bacteria was measured using a spectrophotometer, reading the optical density at 560 nm (OD₅₆₀) against the uninoculated broth.

Bile Tolerance test:

The mean intestinal bile concentration is believed to be 0.3% (w/v). The staying time of food in small intestine is suggested to be 4 h¹⁷. The experiment was carried out to this bile concentration for 4 hours. A modified method of Erkkilä and Petäjä¹⁹ was used to study the effects of bile salts. Briefly, 1% (v/v) overnight culture of LAB were inoculated into MRS broth with varying 0-3% bile acids (Merck, Germany). The inoculated broths were incubated at 37°C for 4 h. After incubation, growth of the bacteria was measured using a spectrophotometer, reading the optical density at 560 nm (OD₅₆₀) against the uninoculated broth containing 0-3% of bile acids.

RESULTS AND DISCUSSION

Antibacterial activities of lactic acid bacteria isolates

In fermented food, hygienic safety is generally due to potential of lactic acid bacteria to produce organic acids and bacteriocins¹⁷. In this study, 117 strains of LAB isolated from different steps of *tchapalo* production were evaluated for their antimicrobial activity against different spoilage and food borne pathogens. The whole results of non-neutralized CFSs inhibitions were indicated in Table 2. The inhibition diameters were ranged from 9 to 20 mm (Figure 1). When the LAB CFSs pH were not neutralized, 32.45% were able to inhibit the growth of *Bacillus cereus* DSM 31^T. This bacteria was the most sensitive indicator strains followed by *Lb. delbrueckii* F/31 which growth was inhibited by 23.07% of LAB CFSs. About 17.09% of LAB CFSs exhibited inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 and *Listeria innocua* ATCC 33090. Results showed also that 12.82%, 8.55% and 7.69% of LAB CFSs yielded inhibition zones against respectively *Salmonella typhimurium* ATCC 5066, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922. On the other hand, only 1.71% of LAB CFSs displayed bactericidal activity against *Enterococcus faecalis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp, *Enterococcus faecalis* CIP 105042 and *Enterococcus faecium* ATCC 51558. The CFSs of LAB isolated in dry malted sorghum inhibited most of indicators strains but those from sour wort inhibited indicators strains with highest diameters (inhibition diameters were from 12 and 20 mm, Figure 1). The inhibitory spectrum varied between isolates and for the same strain, the inhibitory spectrum varied between indicator strains. A total of 72.60% of LAB had inhibitory activity against one or more indicator strains when LAB CFSs pH was not neutralized. These inhibitory activities were due probably to either organic acids production. Indeed, the first role of lactic acid bacteria from *tchapalo* processing is to produce organic acids which reduced pH. The low pH inhibited the growth of pathogenic microorganisms^{20, 21, 22, 23}. So, it increased sweet wort and *tchapalo* shelf-life. Un-neutralized CFSs were active against both the Gram-positive and Gram-negative indicator strains as indicated also by Sourav and Arijit²⁴. When the pH was neutralized, the inhibitory activity against indicator strains reduced in most of CFS culture bacteria (Figure 2). Only 23.07% of LAB CFSs kept their entire inhibitory activity against *Lb. delbrueckii* F/31. About 1.71% of LAB CFSs displayed bactericidal activity against *L. innocua* ATCC 33090, *Enterococcus faecalis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp,

Enterococcus faecalis CIP 105042 and *Enterococcus faecium* ATCC 51558; and 0.85% of them showed inhibitory effect against *Bacillus cereus* DSM 31¹. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 5066 were inhibited respectively by 8.54%, 3.41% and 1.71% of LAB CFSs. This result confirmed that the inhibitory activity was attributed to organic acids produced by LAB isolates. Similar results were obtained by several authors^{10, 24, 25}. These findings corroborate also the findings by Kivanç et al.²⁶ who obtained 33 LAB isolates from *boza* having antimicrobial activity against *L. monocytogenes*, *B. cereus*, *B. subtilis*, *S. aureus*, *En. faecalis*, *E. coli*, *P. aeruginosa*, and *S. typhimurium* in a well diffusion assay. But, only 8 isolates kept their activities in the neutralized supernatant against all of the tested bacteria. Among these, 3 isolates, *Lactobacillus plantarum* had antimicrobial effect for *S. aureus*. The inhibitory activity of the other 25 isolates is due to organic acids. Alvarado et al.²⁷ also reported that about 26.6% of the isolated LAB strains from 27 artisan Mexican foods were capable of inhibitory activity, but only one strain (1.0%) showed bacteriocin production capacity. Diop et al.²⁸ also found that among two hundred and twenty colonies which displayed antibacterial activity against the indicator lawn, 20 of these strains produced antibacterial activity in the neutralized cell-free supernatant, whereas only 12 confirmed the activity when the CFSs were treated with catalase. The fact that the neutralized cell-free supernatants inhibited growth of indicator strains may be due to the production of hydrogen peroxide or bacteriocins because in the neutralized cell-free supernatants, organic acids are eliminated. Lactic acid bacteria have also the capacity to produce hydrogen peroxide which inhibits also indicator strains. Kivanç et al.²⁶ mentioned that hydrogen peroxide is one of the primary metabolites that may be produced by LAB and which may contribute to their antagonistic action and food preservation. It is produced by a large number of LAB which lack the enzyme catalase in particular by *Lactobacillus* spp. and inhibits other microorganisms such as *L. monocytogenes*, *B. cereus*, *B. subtilis*, *S. aureus*, *En. faecalis*, *E. coli*, *P. aeruginosa*, and *S. typhimurium*.

When the catalase was added in neutralized CFSs, the antagonistic activity against indicator strains was lost except for those against Gram-positive indicator strains that were closely related to isolated bacteria (Figure 3). The CFSs of 27 LAB exhibited inhibitory effect against *Lb. delbrueckii* F/31. Among these, 2 LAB strains had inhibitory activity against *Listeria innocua*, and 2 other LAB strains displayed also inhibitory activity against *Listeria innocua*,

Enterococcus faecalis, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp, *Enterococcus faecalis* CIP 105042, *Enterococcus faecium* ATCC 51558. The neutralized cell-free supernatants treated with catalase which inhibited the growth of indicator strains indicate that antibacterial activity is due to the production of bacteriocins^{6, 10, 25, 29, 30}. Gram-positive indicator strains were much more sensitive to tested LAB strains bacteriocins while Gram-negative indicator bacteria showed resistance to bacteriocins. These results indicated that tested LAB had an inhibitory spectrum towards closely related Gram-positive bacteria including pathogens. The resistance of Gram-negative bacteria is attributed to the particular nature of their cellular envelope and mechanisms of action described for bacteriocins^{24, 25}. Other authors reported also that some LAB species from various foods produced a broad spectrum of activity against various pathogenic bacteria^{27, 31}. Todorov and Dicks³² found that bacteriocin produced by *Lactobacillus plantarum* ST194BZ, a strain isolated from *boza*, a traditional drink produced by the fermentation of different cereals, inhibits the growth of *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecalis*. Our findings corroborate also the findings by earlier workers³³. For these authors, *Lactobacillus plantarum* F1 and *Lb. brevis* OG1 isolated from Nigerian fermented food products, produced bacteriocins that had broad spectrum of inhibition against both pathogenic food and spoilage organisms and various LAB as *Enterococcus faecalis*. In their study performed on antimicrobial activities of LAB strains involved in Burkina Faso fermented milk, Savadogo et al.² showed that the biggest diameter of inhibition (12 mm) is obtained with the extract of *Lactobacillus fermentum* S1 on *Enterococcus faecalis*. The inhibitory effect of our LAB strains acted differently on indicator strains. Bacteriocins are bactericidal agents as claimed by Klaenhammer³⁴, hence they may be used either as probiotic or as biopreservative indicated by Arokiyamy and Sivakumar³⁵.

Phenotypic characterization

A total of twenty seven LAB which exhibited inhibitory activities were phenotypic identified based on the carbohydrate utilization and the results are presented in Figure 4. These isolates were grouped into four species of *Lactobacillus* and one species of *Pediococcus*. Eight isolates were characterized as *Lactobacillus fermentum*, six belonged to *Lactobacillus acidophilus*, six was identified as *Lactobacillus pentosus*, one was identified as *Lactobacillus brevis* and six isolates were assigned to *Pediococcus acidilactici*. Some strains of these

species are well documented in the literature for their production of bacteriocins^{9, 29, 30, 31, 36, 37, 38}.

Resistance to the conditions of the digestive tract

Resistance to low pH in the stomach and bile salts in the small intestine is one of the major important properties of probiotic bacteria⁵. During this study, the four LAB (*Lactobacillus fermentum* Ab3BE41, *Lactobacillus brevis* Ab3BE42 and *Pediococcus acidilactici* At1BE22 and At34E21) which had produced inhibitory activity against two or more indicator bacteria had been selected for their resistance to conditions of the digestive tract. The pH resistance of these four strains was conducted in MRS broths whose pH varied between 2.5 and 8.5 for 3 h as the time of digestion in the stomach is 3 h. The results showed that all strains were resistant to the pH of culture medium. However, their optimal growth varied with pH and strains themselves (Figure 5). Optimal growth is observed at pH 6 for strain Ab3BE41, at pH 6.5 for strains At1BE22 and Ab3BE42 and at pH 6 and pH 8.5 for strain At34E21. In addition, the strain At1BE22 and At34E21 had higher capacity for survival in acid medium at pH 2.5 to pH 3.5 than others. The results of bile salts tolerance for our four LAB were observed in Figure 6

in different MRS broths which level of bile salts varied between 0% and 3%. All strains were resistant to bile salts, but most of them lost their viability at 3% bile salts concentration. At 0.15% bile salts, strains At1BE22 and At34E21 were able to survive more than 70% and 50% of their initial count respectively. Strains At1BE22 and At34E21 had better survived withstand different concentrations of bile salts than strains Ab3BE41 and Ab3BE42. At 0.5% bile salts concentration, less than 10% of the strains survived except strain At1BE22. Strains At1BE22 and At34E21 had better resistance to bile salts and were able to survive in acidic digestive tract conditions. This result is similar to the result obtained by Erkkilä and Petäjä¹⁹ who showed that strains of LAB such as *Pediococcus acidilactici* proved to be more acid tolerant, but 0.30% bile salt was the critical bile salt concentration for screening tolerant strains. It is also mentioned that the resistance to bile salts varies a lot among LAB species and even between strains themselves³. Therefore, LAB strains can survive in gastrointestinal tract environment which has characteristic features of having acidic pH and high concentrations of bile salts as indicated by Pelinescu et al.⁸ and Oluwajoba et al.¹⁶.

Table 1
Indicator bacteria strains with their Media and culture conditions

Indicator bacteria strains	Sources	Media and culture conditions
Gram-positive bacteria		
<i>Listeria innocua</i>	ATCC 33090	Nutrient broth, 18-24 h, 37°C
<i>Bacillus cereus</i>	DSM 31 ^T	Nutrient broth, 18-24 h, 37°C
<i>Staphylococcus aureus</i>	ATCC 25923	Nutrient broth, 18-24 h, 37°C
<i>Enterococcus faecalis</i>	ATCC 29212	Brain Heart Infusion, 18-24 h, 37°C
<i>Enterococcus faecalis</i>	CIP 105042	Brain Heart Infusion, 18-24 h, 37°C
<i>Enterococcus faecium</i>	ATCC 51558	Brain Heart Infusion, 18-24 h, 37°C
<i>Enterococcus faecalis</i>	Clinical LNSP	Brain Heart Infusion, 18-24 h, 37°C
<i>Streptococcus</i> sp	Clinical LNSP	Brain Heart Infusion, 18-24 h, 37°C
<i>Lactobacillus delbrueckii</i>	F/31	Man Rogosa Sharp, 18-24 h, 44°C, anaerobiosis
Gram-negative bacteria		
<i>Salmonella typhi</i>	ATCC 5066	Nutrient broth, 18-24 h, 37°C
<i>Escherichia coli</i>	ATCC 28170	Nutrient broth, 18-24 h, 37°C
<i>Pseudomonas aeruginosa</i>	ATCC 27853	Nutrient broth, 18-24 h, 37°C

Table 2
Number of test LAB having antimicrobial activity against indicator strains at un-neutralized pH

Number of LAB isolated from different steps during <i>tchapalo</i> production (n=117)	Gram positive indicator bacteria									Gram negative indicator bacteria		
	<i>L. ino</i>	<i>B. c3</i>	<i>S. a</i>	<i>E. f</i>	<i>E. f2</i>	<i>St. sp</i>	<i>E. fO</i>	<i>E. ff151</i>	<i>Lb. d</i>	<i>S. t5</i>	<i>E. c28</i>	<i>P. a27</i>
Sorghum (n= 10)	1	6	0	0	0	0	0	0	0	0	0	1
Dry malted sorghum (n=22)	4	8	2	2	2	2	2	2	3	2	0	4
Flour dry malted sorghum (n=12)	4	4	3	0	0	0	0	0	1	4	2	4
Mash (n=17)	2	7	2	0	0	0	0	0	4	2	1	1
Sediment (n=6)	0	1	0	0	0	0	0	0	0	1	0	1
Wort (n= 19)	5	4	0	0	0	0	0	0	6	4	3	4
Sour wort (n=21)	4	5	2	0	0	0	0	0	8	2	2	3
Sweet wort (n=1)	0	0	0	0	0	0	0	0	0	0	0	0
Traditional starter (n=8)	0	2	0	0	0	0	0	0	5	0	0	1
<i>Tchapalo</i> (n=1)	0	1	1	0	0	0	0	0	0	0	1	1
Total number of LAB isolates having antimicrobial activity	20	38	10	2	2	2	2	2	27	15	9	20
Percentage of LAB isolates having antimicrobial activity (%)	17.09	32.45	8.55	1.71	1.71	1.71	1.71	1.71	23.07	12.82	7.69	17.09

n : number of samples, *L. ino* : *Listeria innocua* : 33090, *B. c3* : *Bacillus cereus* DSM 31^T, *S. a* : *Staphylococcus aureus* ATCC 25923, *E. f* : *Enterococcus faecalis*, *E. f2* : *Enterococcus faecalis* ATCC 29212, *St. sp* : *Streptococcus* sp, *E. fO* : *Enterococcus faecalis* CIP 105042, *E. ff151* : *Enterococcus faecium* ATCC 51558, *Lb. d* : *Lb. delbrueckii* F/31, *S. t5* : *Salmonella typhimurium* ATCC 5066, *E. c28* : *Escherichia coli* ATCC 28170, *P. a27* : *Pseudomonas aeruginosa* ATCC 27853



Figure 1
Inhibition of *P. aeruginosa* 27853 by un-neutralized cell-free supernatants of LAB strains isolated during *tchapalo* processing. 1: AT34E21, 2: AT23E65, 3: AT2BE72, 4: AT2BE78, 5: AT33E41, 6: AB1BE73.

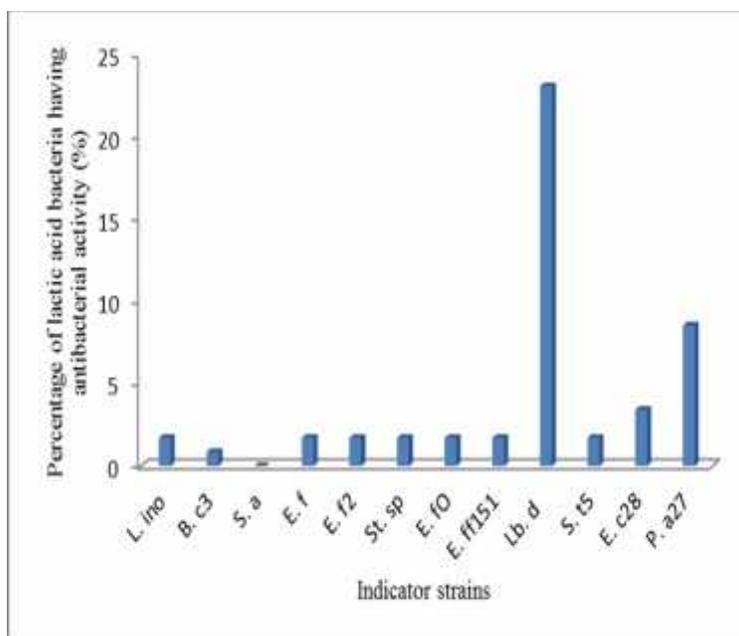


Figure 2

Percentage of LAB isolates having antibacterial activity against indicator strains at neutralized pH. *L. ino* :

Listeria innocua : 33090, *B. c3* : *Bacillus cereus* DSM 31^T, *S. a* : *Staphylococcus aureus* ATCC 25923,

E. f : *Enterococcus faecalis*, *E. f2* : *Enterococcus faecalis* ATCC 29212, *St. sp* : *Streptococcus* sp,

E. fo : *Enterococcus faecalis* CIP 105042, *E. ff151* : *Enterococcus faecium* ATCC 51558, *Lb. d* : *Lb. delbrueckii* F/31, *t5* : *Salmonella*

typhimurium ATCC 5066, *E. c28* : *Escherichia coli* ATCC 28170,

P. a27 : *Pseudomonas aeruginosa* ATCC 27853.

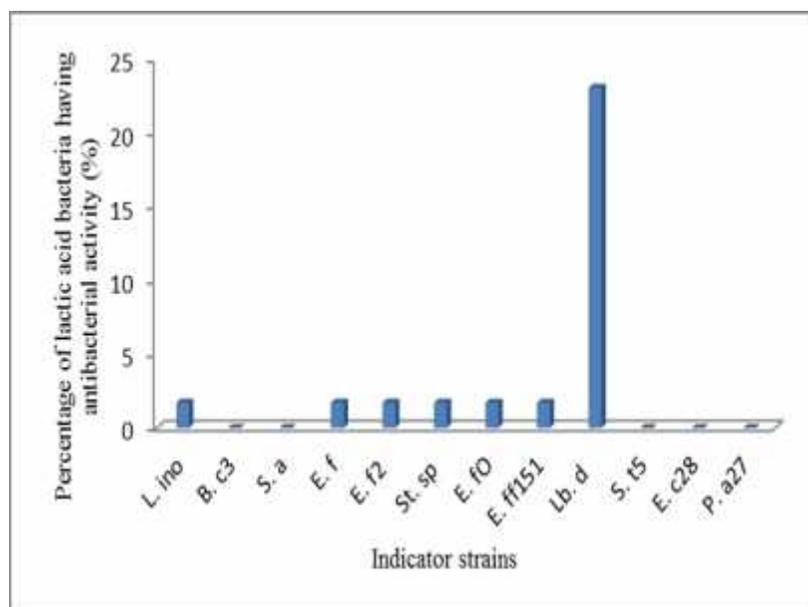


Figure 3

Percentage of LAB isolates having antibacterial activity against indicator strains at neutralized pH with

catalase addition. *L. ino* : *Listeria innocua* : 33090, *B. c3* : *Bacillus cereus* DSM 31^T, *S. a* : *Staphylococcus*

aureus ATCC 25923, *E. f* : *Enterococcus faecalis*, *E. f2* : *Enterococcus faecalis* ATCC 29212, *St. sp* :

Streptococcus sp, *E. fo* : *Enterococcus faecalis* CIP 105042, *E. ff151* : *Enterococcus faecium* ATCC 51558, *Lb.*

d : *Lb. delbrueckii* F/31, *S. t5* : *Salmonella typhimurium* ATCC 5066, *E. c28* : *Escherichia coli* ATCC 28170, *P.*

a27 : *Pseudomonas aeruginosa* ATCC 27853

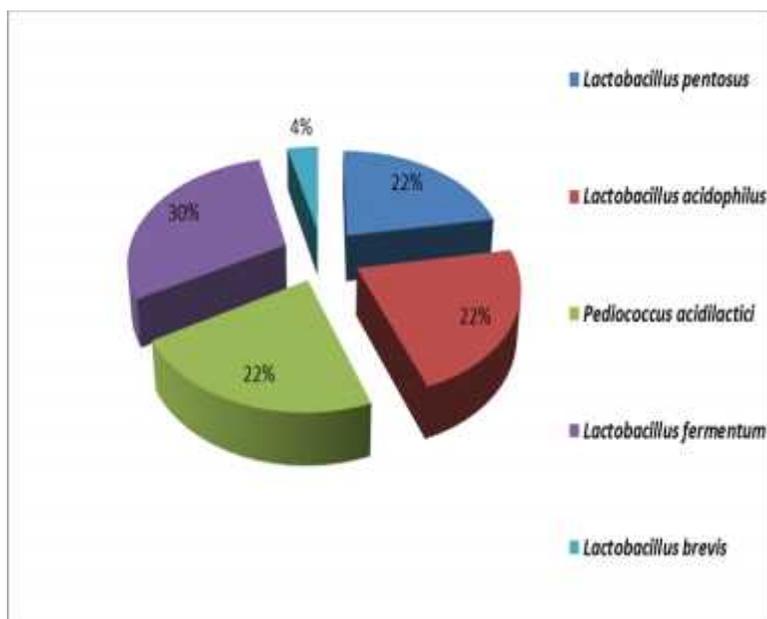


Figure 4

Lactic acid bacteria species involved in the *tchapalo* processing having antibacterial activity against indicator strains.

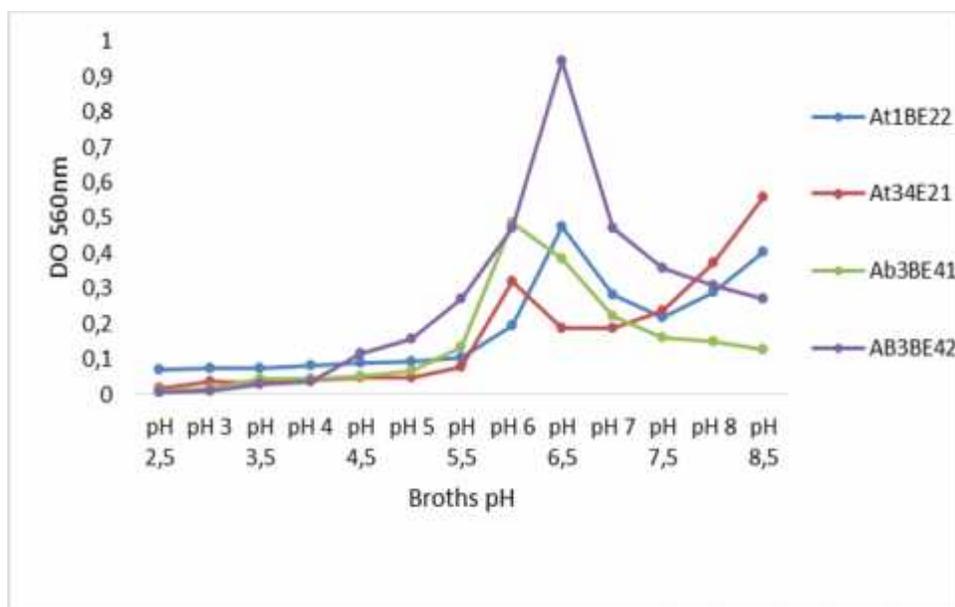


Figure 5

Growth of LAB strains at different pH of MRS Broth. At1BE22: *Pediococcus acidilactici*, At34E21: *Pediococcus acidilactici*, Ab3BE41: *Lactobacillus fermentum*, AB3BE42: *Lactobacillus brevis*

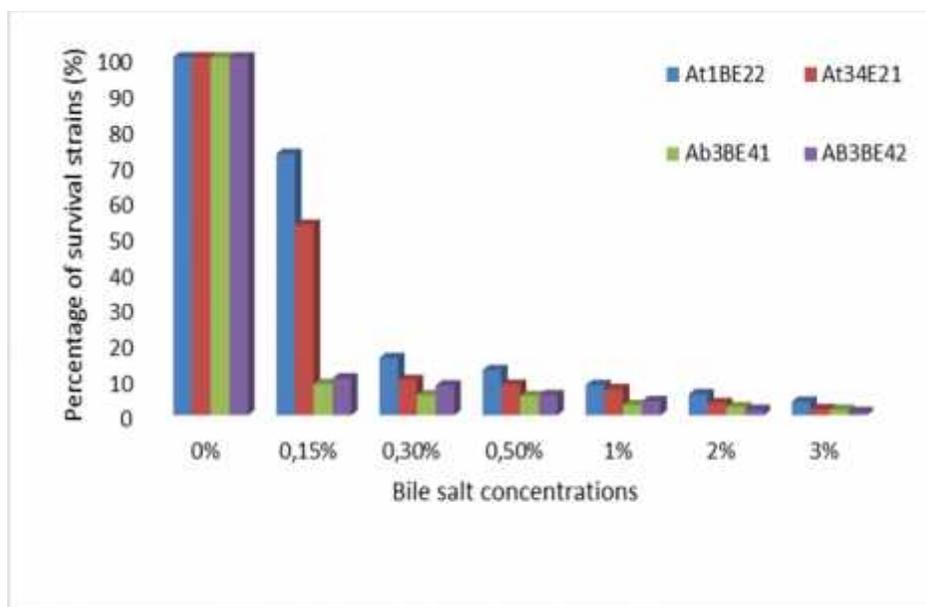


Figure 6

Percentage of survival LAB strains in presence of different bile salt concentrations. At1BE22: *Pediococcus acidilactici*, At34E21: *Pediococcus acidilactici*, Ab3BE41: *Lactobacillus fermentum*, AB3BE42: *Lactobacillus brevis*

These results represent great advantages for the survival of these bacteria, once introduced in the gastrointestinal tract. Bile resistance of some strains is related to specific enzyme activity bile salt hydrolase (BSH) which helps to hydrolyze conjugated bile, thus reducing its toxic effect. However high concentration of bile up to 2.0% was reported and the average concentration was about 0.3%⁵.

CONCLUSION

The results of this study showed that most of LAB, isolated from *tchapalo* production, inhibited indicator strains by organic acids production. *Bacillus cereus*, *Lactobacillus delbrueckii*, *Listeria innocua* and *Pseudomonas aeruginosa* are the most sensitive to this production. Besides, 27 LAB produced bacteriocins which inhibited *Lb. delbrueckii* F/31. Among them, four strains displayed also inhibitory activity against *Listeria innocua* ATCC 33090, *Enterococcus faecalis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp, *Enterococcus faecalis* CIP 105042, *Enterococcus faecium* ATCC 51558. *Pediococcus acidilactici* strains which had good antibacterial potential were tolerant against acidic conditions and bile salts. They could be used for *tchapalo* preservation and also to protect *tchapalo* consumers' health. These food bacterial strains will be therefore selected for further studies on genotypic characterizations.

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REFERENCES

1. Kocková M, Gereková P, Petruláková Z, Hybenová E, Šturdík E, Valík L. Evaluation of fermentation properties of lactic acid bacteria isolated from sourdough. *Acta Chimica Slovaca*. 2011 ; 4(2): 78-87.
2. Savadogo A, Ouattara CAT, Bassole IHN, Traore SA. Antimicrobial activities of lactic acid bacteria strains isolated from Burkina Faso fermented milk. *Pakistan J. Nutr.* 2004; 3(3): 174-179.
3. Kacem M, Karam N-E. In vitro preselection criteria for probiotic *Lactobacillus plantarum* strains of fermented olives origin., *Int. J. Probiot. Prebiot.* 2006; 1(1): 27-32.
4. Meera NS, Devi MC. Partial characterization and optimization of parameters for bacteriocin production by probiotic lactic acid bacteria. *J. Microbiol. Biotechnol. Res.* 2012; 2(2): 357-365.

5. Buasai P, Nongpanga K. Screening of probiotic lactic acid bacteria from piglet feces, Kasetsart J. (Nat. Sci.) 2011; 45: 245-253.
6. Sifour M, Tayeb I, Haddar HO, Namous H, Aissaoui S. Production and characterization of bacteriocin of *Lactobacillus plantarum* F12 with inhibitory activity against *Listeria monocytogenes*, The Online J. Sci. Technol. 2012; 2(1): 55-61.
7. FAO/WHO, Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: report of a Joint Food and Agriculture Organization of the United Nations/ World Health Organization expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Tech. Rep., Food and Agriculture Organization/World Health Organization, Cordoba, Argentina, 2001.
8. Pereira DIA, Glenn RG. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from human gut. Appl. Environ. Microbiol. 2002 ; 68(9): 4689–4693.
9. Pelinescu et al., Selection and characterization of the probiotic potential of some lactic acid bacteria isolated from infant feces. Rom. Biotechnol. Lett. 2011 ; 16(3): 6178-6189.
10. Shokryazdan et al. Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some Human pathogenic strains. BioMed Res. Int. 2014 ; 2014: 1-16.
11. Uroi et al. Probiotic properties of lactic acid bacteria isolated from croatian fresh soft cheese and serbian white pickled cheese pickled cheese. Food Technol. Biotechnol. 2014; 52(2): 232-241.
12. Aka S, Camara F, Nanga YZ, Loukou YG, Dje KM. Evaluation of organic acids and sugars contents during the production of *tchapalo*, a traditional sorghum beer in Côte d'Ivoire. J. Food Technol. 2008; 6(5): 189-195.
13. Djè MK, N'Guessan KF, Djeni TN, Dadié TA. Biochemical changes during alcoholic fermentation in the production of *tchapalo*, a traditional sorghum beer. Int. J. Food Eng. 2008; 4(7): 1-15.
14. Djè MK, Aka S, Nanga YZ, Yao KC, Loukou YG. Predominant lactic acid bacteria involved in the spontaneous fermentation step of *tchapalo* process, a traditional sorghum beer of Côte d'Ivoire. Res. J. Biol. Sci. 2009; 4 (7): 789-795.
15. De Man JD, Rogosa M, Sharpe EA. Medium for the cultivation of *Lactobacilli*. J. Appl. Bact. 1960; 23:130-135.
16. Arici M, Bilgin B, Sagdic O, Ozdemir C. Some characteristics of *Lactobacillus* isolates from infant faeces. Food Microbiol. 2004; 21(1): 19-24.
17. Oluwajoba SO, Akinyosoye FA, Oyetayo VO. In vitro screening and selection of probiotic lactic acid bacteria isolated from spontaneously fermenting *kunu-zaki*. Adv. Microbiol. 2013; 3: 309-316.
18. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM, Islam KMD. Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. World J. Dairy Food Sci. 2010; 5(1): 39-46.
19. Erkkilä S, Petäjä. Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. Meat Sci 2000;55(3):297-300
20. Chelule PK, Mokoena MP, Gqaleni N. Advantages of traditional lactic acid bacteria fermentation of food in Africa. In: Current research, technology and education topics in applied microbiology and microbial biotechnology. Méndez-Vilas A, editor. Formatex. Spain. 2010, pp. 1160-1167.
21. Askari G Al, Kahouadji A, Khedid K, Charof R, Mennane Z. Screenings of lactic acid bacteria isolated from dried fruits and study of their antibacterial activity. Middle-East J. Sci. Res. 2012; 11(2): 209-215.
22. Nyanzi R, Jooste PJ. Cereal-based functional foods. In: Probiotic. (editor) Rigobelo E, InTech, 2012, pp. 161-196.
23. Aka S, Konan G, Fokou G, Dje KM, Bonfoh B., Review on African traditional cereal beverages. Am. J. Res. Commun. 2014; 2(5): 103-153.
24. Sourav B, Arijit D. Study of physical and cultural parameters on the bacteriocins produced by lactic acid bacteria isolated from traditional indian fermented foods. Am. J. Food Technol. 2010; 5(2): 111-120.
25. Saidi N, Hadadji M, Guessas B. Screening of bacteriocin-producing lactic acid bacteria isolated from west Algerian goat's milk. Global J. Biotechnol. Biochem. 2011; 6(3): 154-161.
26. Kivanç M, Yilmaz M, Çakir E. Isolation and identification of lactic acid bacteria from boza and their microbial activity against several reporter strains. Turk. J. Biol. 2011; 35:313-324.
27. Alvarado C, García ABE, Martin SE, Regalado C. Food-associated lactic acid bacteria with antimicrobial potential from traditional Mexican foods. Rev. Latinoam. Microbiol. 2006; 48(3-4):260-268.

28. Diop MB, Dubois-Dauphin R, Tine E, Destain J, Thonart P. Bacteriocin producers from traditional food products. *Biotechnol. Agron. Soc. Environ.* 2007; 11 (4): 275-281.
29. Desalegn A. Antimicrobial activity of lactic acid bacteria isolated from “Ergo”, Ethiopian traditional fermented milk. *Curr. Res. Microbiol. Biotechnol.* 2013; 1(6): 278-284.
30. Adebayo FA, Afolabi OR, Akintokun AK. Antimicrobial properties of purified bacteriocins produced from *Lactobacillus casei* and *Lactobacillus fermentum* against selected pathogenic microorganisms. *British J Med. Med. Res.* 2014; 4(18): 3415-3431.
31. Mollendorff JWV, Todorov SD, Dicks LMT. Comparison of bacteriocins produced by lactic acid bacteria isolated from *boza*, a cereal-based fermented beverage from the balkan Peninsula. *Curr. Microbiol.* 2006; 53: 209-216.
32. Todorov SD, Dicks LMT. Effect of growth medium on bacteriocin production by *Lactobacillus plantarum* ST194BZ, a strain isolated introduction from *boza*. *Biotechnol.* 2005 43(2): 165-173.
33. Ogunbanwo ST, Sanni AI, Onilude AA., Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *Afr. J. Biotechnol.* 2003; 2(8): 219-227.
34. Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* 1993; 12: 39-86.
35. Arokiyama A, Sivakumar PK. Antibacterial activity of bacterocin producing *Lactobacillus* sp., isolated from traditional milk products. *Curr. Bot.* 2011; 2(3): 05-08.
36. Tadesse G, Ephraim E, Ashenafi M. Assessment of the antimicrobial activity of lactic acid bacteria isolated from *borde* and *shamita*, traditional Ethiopian fermented beverages, on some food-borne pathogens and effect of growth medium on the inhibitory activity. *Internet J. Food Safety* 2005; 5: 13-20.
37. Ben Omar et al. Bacteriocin-producing *Lactobacillus* strains isolated from poto poto, a Congolese fermented maize product and genetic fingerprinting of their plantaricin operons. *Int. J. Food Microbiol.* 2008; 127: 18-25.
38. Anteneh T, Tetemke M, Mogessie A. Antagonism of lactic acid bacteria against foodborne pathogens during fermentation and storage of *borde* and *shamita*, traditional Ethiopian fermented beverages. *Int. Food Res. J.* 2011; 18(3): 1189-1194.