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Characterization of *Lactobacillus* Species Isolated from Mash, Sour Wort and *Tchapalo* Produced in Côte d'Ivoire

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ABSTRACT

A total of 146 *Lactobacillus* strains isolated in *tchapalo* processing were characterized by phenotypic methods. Of these strains, 82.88% were heterofermentative strains and 17.12% homofermentative strains. Among the 82 strains identified using multiplex PCR methods, 55 strains were identified including *L. fermentum* (63.63%), *L. brevis* (21.82%), *L. plantarum* (9.1%) and *L. hilgardii* (5.45%). Api 50 CH identification system tested on twenty *Lactobacillus* strains allowed also to identify *L. coprophilus* and *L. cellobiosus*. *L. fermentum* was the predominant species which appeared in all samples of mash, sour wort and *tchapalo*. Thirty-one strains were analyzed for acid production and antimicrobial activities against indicator strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25913). Results showed that the acid production by strains varied within strains of the same species and between species also. Four strains were able to inhibit the growth of most of indicator strains with a broad spectrum. The inhibition diameters were between 8 mm and 21 mm.

Keywords: acidification, antibacterial activities, *Lactobacillus* spp, PCR-multiplex identification; phenotypic characterization; traditional starter

Abbreviations: ATCC, American Type Culture Collection; **PCR**, polymerase chain reaction

INTRODUCTION

Foods preservation by canning or freezing are often too expensive for millions of the world's economically deprived people. In Africa, spontaneous fermentations are empirical and widely used for preserving and often enhancing organoleptic and nutritional qualities of vegetables foods (Çon and Karasu 2009; Kalui *et al*. 2010). Among these fermentations, appears lactic fermentation. It is carried out by a complex population of environmental microorganisms (Owusu-Kwarteng *et al*. 2010). *Tchapalo* is such a traditional sorghum beer produced in Côte d'Ivoire. Its processing involves a lactic fermentation that is very important because it determines organoleptic properties and the preservation of sweet wort. Unfortunately, this lactic fermentation comes down in poor hygienic conditions and depends on environmental or climatic conditions. Thereby the hygienic, nutritional and organoleptic qualities of the beer are not always guaranteed (Aka *et al*. 2008; Djè *et al*. 2008).

According to Asmahan and Muna (2009), use of starter cultures would be an appropriate approach for the control and optimization of the fermentation process in order to alleviate the problems of variations in organoleptic quality and microbiological stability observed in African indigenous fermented foods. Previous studies have shown that lactic acid bacteria (LAB) are involved in the African beverages processing, mainly during the spontaneous lactic fermentation. Identification and selection of species of these genera for the purpose of starter culture would be a great economic importance for the production of *tchapalo* at small-scale industrial and house home.

Various techniques have been used to characterize *Lactobacillus* species. These techniques include morphological, physiological and biochemical analyses (Bhatta *et al*. 2006); but these methods are not sufficiently discriminatory to differentiate *Lactobacillus* at the species and strains levels. The development of the polymerase chain reaction (PCR)-based molecular approaches offers new perspectives in microbial taxonomic and diagnostic studies (Settanni *et al.* 2005). Therefore, the use of phenotypic identification in combination with a genotypic method would be desirable (Settanni *et al.* 2005). The aim of this study was to characterize *Lactobacilli* at the species level that occur in *tchapalo* processing by using both phenotypic and PCR method. In this study we also investigated their antimicrobial and acidifying activities. The results will contribute to the development of starter cultures to improve the *tchapalo* production.

MATERIALS AND METHODS

Production of *tchapalo* and sampling

Tchapalo was produced by traditional brewers following the method previously described by Aka *et al*. (2008). Briefly, malted sorghum grain was milled and the flour obtained was mixed out with water containing a sticky substance which came from the bark of a shrub (*Anogeissus leo carpus*). The mixture obtained called mash was separated from supernatant and sediment. The sediment was cooked during 2-2 h 30 min; later mixed with the supernatant to give wort. The wort was left for a spontaneous fermentation during a night to give after percolation the sour wort. This wort was cooked during 5-6 h, cooled and inoculated with about 0.6 to 1.6% of dried yeast harvested from previous *tchapalo* for alcoholic fermentation during 9-12 h. The product obtained after alcoholic fermentation is *tchapalo*. Sampling was carried out on mash, sour wort and *tchapalo*. They were collected from three areas (Abobo, Attécoubé, Yopougon) randomly selected in the district of Abidjan. Sampling were collected in sterile small bottles,

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Table 1 PCR primers used for amplifications and their sequences.

	Groups/species	Primers	Sequences (5' – 3')	PCR product size (bp)
Grouping-multiplex PCR	Group 1	LB1*	GCAGTTTTAATCAACTGTTACC	340
	Group 2	LB2*	AGGGTTGTAGGACTGATGTTG	180
	Group 3	LB3*	GGGGAACTGAAACATCTAAGTACCC	280
		LB4*	GGGGAACTGAAACATCTCAGTACCC	
	Group 4	LB5*	GGCAACGAGCGCAACCCTTGTTAC	1100
Goup1-multiplex PCR	*L. paraplantarum*	LB6**	GTCACAGGCATTACGAAAAC	107
	L. pentosus	LB7**	CAGTGGCGCGGTTGATATC	218
	L. plantarum	LB8**	CCGTTTATGCGGAACACCTA	318
	Reverse	LB9**	TCGGGATTACCAAACATCAC	
Group2-multiplex PCR	*L. farciminis*	LB10*	CTACTTTCACATGATCGTAGC	2007
	L. mindensis	LB11*	GGTAGGATGATGCGTAAGCAT	671
	L. paraalimentarius	LB12*	GACGAAAGTCATGGCAAATTG	521
	L. alimentarius	LB13*	TAGTTGAGATAGCTGAACAGC	147
Group3-multiplex PCR	*L. hilgardii*	LB14*	CGGAAACCTACACAATGTCG	683
	L. brevis	LB15*	TTTGACGATCACGAAGTGACCG	502
	L. fructivorans	LB16*	CGACAGTGAATTCATAGCTGTCG	319
	L. sanfranscensis	LB17*	TGAAGTAGTTGGGAAGCTACA	134
Group4-multiplex PCR	*L. pontis*	LB18*	TCAAAACCACATGGTTTTGATTTC	2019
	L. frumenti	LB19*	CACCGCGTTATTTTGAGTTGT	548
	L. panis	LB20*	CCAACTTAGTCGTTGGTTATC	341
	L. fermentum	LB21*	AAGAATCAGGATGTCGAAGTG	148
	Reverse	LB22*	GCCTTGGGAGATGGTCCTC	
	Reverse	LB23*	GCCTTGCGAGATGGTCCTC	

* : Settanni *et al*. 2005; ** : Torriani *et al*. 2001

labelled and then transported to the laboratory in a box containing a freezing pack. A total of ninety mash samples, ninety sour wort samples and ninety *tchapalo* samples were collected from these areas.

Determination of pH

The pH was determined with a pH-meter (Consort P604, Bio Block, France) and two independent measurements were made on each sample.

Enumeration of microorganisms

Ten millilitres of each sample were aseptically added into 90 ml of sterile 0.9% NaCl solution and mixed. Serial dilutions were performed and 0.1 ml aliquot of the appropriate dilution was directly inoculated in duplicate on Man Rogosa Sharpe agar (AFNOR, NF ISO 15214), was incubated anaerobically at 30°C for 48 h for enumeration of LAB on Sabouraud-chloramphenicol agar (AFNOR, NF ISO 7954) and was incubated at 25°C for 2-5 days for enumeration of yeasts.

Phenotypic characterizations

Cell morphology was observed using an optical microscope and isolates tested for catalase production. One hundred and forty-six (146) colonies picked randomly from plates of MRS were purified by repeated plating. Isolates were cultivated in MRS broth and preserved in MRS broth using 20% (v/v) glycerol at -80°C. Growth at 15, 45 and 51°C in MRS broth was determined by visual turbidity after 1-5 day(s) of incubation. Gas production from glucose was assessed in MRS broth containing inverted Durham tubes. The ability to grow at different pH (3, 3.9 and 9) was tested. The salt tolerance was done in MRS broth containing 4 and 6.5% (w/v) NaCl. Twenty isolates of MRS were selected for identification of the species using the Api 50 CH galleries (Bio Mérieux, l'Etoile, France). Tests were performed according to the manufacturer's instructions. The APILAB Plus database (Bio Mérieux) was used to interpret the results.

PCR-multiplex identification

Identification was performed with the modified method of Settanni *et al*. (2005). The 82 *Lactobacillus* strains were first typed by a multiplex PCR assay named grouping-multiplex-PCR. Subsequently, four multiplex PCR assays named group 1-, group 2-, group 3- and group 4-multiplex PCRs were performed in order to identify each group at the species level. PCR mixtures contained from four up to seven primers listed in **Table 1**. Amplification reactions were carried out in a total volume of 25 µl of a solution containing 10% (vol/vol) of 10 × PCR buffer, 5 mM $MgCl_2$, 200 µM of each 2'-deoxynucleoside 5' triphosphate, 1 U of *Taq* DNA polymerase, 10 µl of bacteria suspension and different primer concentrations, depending on the multiplex PCR assay: 0.6 µM of primer LB1, 0.5 µM of primer LB2, 0.25 µM of primers LB3/LB4, 1 µM of primer LB5 and 0.75 µM of reverse primers LB22/LB23 for grouping-multiplex PCR; 0.5 µM of primers LB6, LB7, 0.25 µM of primer LB8 and 0.5 µM of reverse primer LB9 for group 1-multiplex PCR; 0.5 µM of each group 2-multiplex PCR primers and reverse primers LB22/LB23; 1 µM of primer LB17 and 0.5 µM of all other group 3-multiplex primers and reverse primers LB22/LB23; 1.5 µM of primer LB18, 0.15 µM of primer LB19, 0.5 µM of primers LB20, LB21 and reverse primers LB22/LB23 for group 4-multiplex PCR. PCR amplification was performed using the thermocycler (Hybaid OmniGene TR3 CM220, MWG-Biotech, UK).

The applied PCR conditions comprised an initial template denaturation step for 10 min at 95°C, followed by 36 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 55°C for group 1- and group 2-multiplex PCRs and at 60°C for grouping-, group 3- and group 4-multiplex PCRs and elongation for 1 min at 72°C. The final extension step was for 10 min at 72°C. The yeast strain ATCC 9763 was used as negative control. PCR reactions using one single primer forward and its reverse counterpart were performed as positive controls. PCR products were separated by electrophoresis on 1.5% (w/v) agarose gel containing ethidium bromide (0.5 µg ml^{-1}) and the DNA was detected by UV transillumination.

Acidification of sorghum malt broth

Sorghum malt flour was mixed with an aqueous solution of *Anogeissus leo carpus* in the ratio of 16.5% (w/v) and let to settle for 35 h. The supernatant was collected, centrifuged (6000 × g for 15 min, Jouan CR412) and filtered (Sartorius AG, Goëttingen, Germany). The supernatant obtained constituted the sorghum malt broth which was distributed in flasks (200 ml per flask), autoclaved at 121°C for 15 min and cooled at room temperature (25°C). The broth was then inoculated with 1×10^6 cfu ml^{-1} of each *Lactobacillus* and was incubated at 30°C for 24 h. Every 4 h, 10 ml was sampled under aseptic conditions and pH was determined. The sorghum malt broth was used as negative control. The rate of aci-

Table 2 Counts (cfu/ml) of fermented microflora and pH throughout *tchapalo* processing.

	Mash	Sour wort	*Tchapalo*
pH	5.65 ± 0.37	3.71 ± 0.24	3.51 ± 0.15
LAB	$6.5 \times 10^8 \pm 4.6 \times 10^8$	$1.3 \times 10^9 \pm 6.1 \times 10^8$	$1.1 \times 10^4 \pm 4.7 \times 10^3$
Yeast	$1.2 \times 10^7 \pm 1.8 \times 10^7$	$2.9 \times 10^6 \pm 0.3 \times 10^5$	$1.5 \times 10^8 \pm 2.9 \times 10^8$

Table 3 Multiplex PCRs identification of *Lactobacillus* species involved in *tchapalo* processing.

Groups	Grouping-multiplex PCR (size in bp)	Species-specific-multiplex PCR (size in bp)	Number of isolates identified	Percentage (%)	Species
Group 1	340	318	5	9.1	*L. plantarum*
Group 3	280	502	12	21.82	*L. brevis*
Group 3	280	683	3	5.45	*L. hilgardii*
Group 4	1100	148	35	63.63	*L. fermentum*

dification was calculated as ΔpH according to Ayad *et al*. (2004). The strains were considered as fast, medium or slow acidifying when a ΔpH of 0.8U pH was achieved during 8 h, 8–16 h and >16 h, respectively. Duplicate trial fermentations were realized with 31 *Lactobacillus* strains. For each *Lactobacillus* species, the number of strains in each class (fast, medium, slow) was added and expressed in proportion.

Antibacterial activities

Antibacterial activities were assayed against *Escherichia coli* ATCC (American Type Culture Collection) 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 25913 using an agar diffusion method described by Arici *et al*. (2004). Thirty-one *Lactobacillus* strains were inoculated into MRS broth. After incubation at 30°C for 48h, cultures were centrifuged and the supernatant was filtered through a 0.45 µM pore size filter (Sartorius AG, Goëttingen, Germany). An aliquot of 100 µl of the final supernatant was spotted onto nutrient agar plate seeded with active growing cells of the pathogenic test bacteria (approximately 10^6 cfu ml^{-1}). The experience was also realized with final supernatant with 1 N NaOH to pH 6.5 and 0.01 g l^{-1} of peroxidase. Triplicate trial assays were performed.

Statistical analyses

The results were statistically evaluated by one way analysis of variance (ANOVA) and Duncan's multiple range test with the software Statistica, 99 Edition. Statistical differences with P values < 0.05 were considered significant.

RESULTS

Evolution of fermented microflora and pH during *tchapalo* processing

As shown in **Table 2**, pH of *tchapalo* (3.51) was significantly ($P < 0.05$) lower than that of sour wort (3.71) and mash (5.65). The counts of LAB ranged from 6.5×10^8 cfu ml^{-1} in mash to 1.3×10^9 cfu ml^{-1} in sour wort after spontaneous fermentation. This load was higher in the sour wort and the mash than in *tchapalo* (1.1×10^4 cfu ml^{-1}). On the other hand, yeasts were higher in *tchapalo* (1.5×10^8 cfu ml^{-1}) than in the sour wort (2.9×10^6 cfu ml^{-1}) and the mash (1.2×10^7 cfu ml^{-1}).

Phenotypic characterization of *Lactobacillus*

A total of 146 Gram-positive, catalase-negative, non-sporing and non-motile rods bacteria were isolated at different steps of *tchapalo* production (mash, sour wort and *tchapalo*). Most of isolates (82.88%) produced gas from glucose indicating heterofermentative metabolism. The majority of strains (96.6%) grew at 15°C; 95.2% grew at 45°C, but none grew at 51°C. All strains except two were able to grow at pH 3.9; 97.2% at pH 9 and only five were capable to grow at pH 3. Ninety-two strains shared the ability to grow in MRS supplemented with 4% NaCl but only 6 strains grew in MRS supplemented with 6.5% NaCl. *Lactobacillus* strains tested with Api 50 CH identification system were identified as *L. fermentum* (20%), *L. brevis* (10%), *L. plantarum* (5%), *L. coprophilus* (5%), *L. cellobiosus* (5%) and *Lactobacillus* spp (55%).

Genotypic characterization of *Lactobacillus*

Among the 82 isolates which were characterized by multiplex PCR, 55 gave expected amplicons. Grouping-multiplex PCR generated the following expected major amplicons for group 1, group 3, group 4 of *Lactobacillus* reported in **Table 3**: 35 isolates gave 1.100 bp for group 4, 5 isolates gave 340 bp for group 1 and 15 isolates gave 280 bp for group 3. An example of grouping-Multiplex PCR electrophoresis gel is given in **Fig. 1**. There was not expected amplicon for group 2. Individual *Lactobacillus* strains belonging to group 1, group 3 and group 4 were successfully detected and discriminated at the species level by the specie specific-multiplex PCR. Group 1-multiplex PCR gave only one amplicon of 318 bp corresponding to *L. plantarum* (**Fig. 2**, lanes 1-4, **Table 3**). Furthermore, group 3-multiplex PCR was shown to distinguish *L. hilgardii* and *L. brevis*. The amplicon sizes corresponding to their species were respectively 683 bp and 502 bp (**Fig. 2**, lanes 6-9 for *L. brevis*, **Table 3**). The 35 isolates of group 4 tested with group 4-multiplex PCR primers were identified as *L. fermentum* (**Fig. 2**, lanes 11-14, **Table 3**). *L. cellobiosus* and *L. coprophilus* strains gave negative results with the multiplex PCR

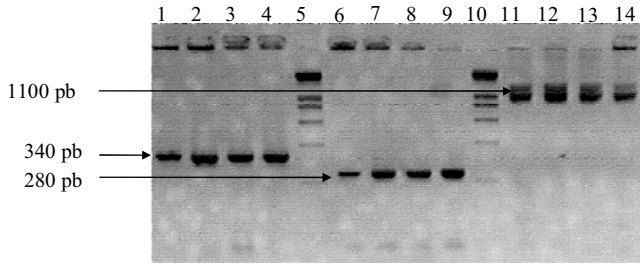

Fig. 1 Grouping-multiplex PCR (first step) assay. Lanes 1-4: PCR products with LB1; lanes 5 and 10: 10000 bp DNA molecular weight marker; lanes 6-9: PCR products with LB3/LB4; lanes 11-14: PCR products with LB5.

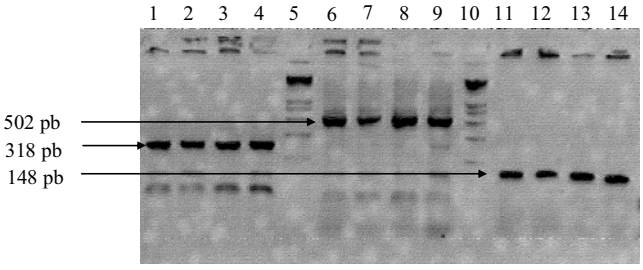

Fig. 2 Group 1-, group 3- and group 4-multiplex PCRs (second step) assays. Lanes 1-4: *L. plantarum*; lanes 5 and 10: 10000 bp DNA molecular weight marker; lanes 6-9: *L. brevis*; lanes 11-14: *L. fermentum*.

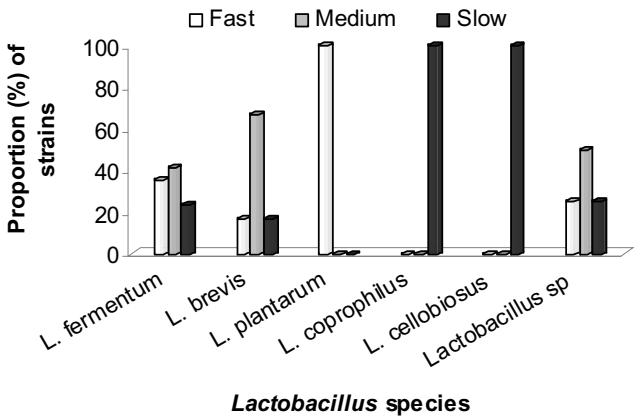

Fig. 3 Proportion of *Lactobacillus* species according to rate of acidification. The cultures were grouped as fast, medium or slow acidifying when a ΔpH of 0.8U pH was achieved during 8 h, 8–16 h and >16 h, respectively.

primers.

L. fermentum represented the predominant species (63.63%) of *Lactobacillus* strains which were found in every step of *tchapalo* processing, followed by *L. brevis* (21.82%) also found in every step of *tchapalo* processing. These two species appeared as the most important species identified during spontaneous fermentation. *L. hilgardii* and *L. coprophilus* were detected only in the mash before spontaneous fermentation; *L. plantarum* was found in the mash before spontaneous fermentation and after alcoholic fermentation in *tchapalo* but not in the sour wort. *L. cellobiosus* appeared only in *tchapalo* (results not shown).

Rate of acidification of the isolates

Monocultures of the different species identified of *Lactobacillus* were effective in fermenting the sorghum malt broth as judged from the changes in pH; however, the acid production varied within strains of the same species and between species also. Most of *L. fermentum* (35.29%) tested were fast acidifying strains; while 41.82% of the strains were medium acid producers and 16.67% showed a slow acidification activity (**Fig. 3**). Among the *L. brevis*, 66.66% of strains showed a medium acidification activity. Only 16.67% of the strains of this specie had fast acidification activity. On the other hand, all *L. plantarum* showed the highest acidification activity during fermentation of the sorghum malt broth. *L. coprophilus*, *L. cellobiosus* and 25% of *Lactobacillus* spp. strains showed a slow acidification activity.

Antibacterial activities of *Lactobacillus* strains

A well diffusion method was used to screen 31 *Lactobacilli* isolates for antagonistic activity. **Table 4** gives the results of inhibition indicators strains (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25913). The inhibition diameters were between 8 mm and 21 mm. Most of the strains were found to produce an inhibition zone against some pathogenic bacteria. *P. aeruginosa* ATCC 27853 was the most sensitive indicator strain and *S. aureus* ATCC 25913 was the most resistant one. *L. fermentum* Yop P2P41, *L. brevis* Abo P10P24, *L. plantarum* Abo P4P112, *L. plantarum* Abo P9P34 and *Lactobacillus* sp. Aben MP16 produced a broad spectrum against all indicators strains.

When the pH value of the supernatants was adjusted to 6.5 and catalase was added, there was a reduction of inhibition against indicators strains and a reduction of number of strains which produced inhibition against pathogen bacteria (**Table 4**). Only eleven strains exhibited inhibition activities, indicating that antibacterial activities of most of *Lactobacillus* strains analyzed were due to acid production. *P. aeruginosa* ATCC 27853 was indicator strain most sensitive to these eleven strains. *L. fermentum* Att P3P13, *L. fermentum* Yop P2P18, *L. brevis* Abo P10P24 and *L. plantarum* Abo P9P34 showed a weak inhibition activity against indicators strains. *L. fermentum* Yop P2P41, *L. brevis* MP33, *L. coprophilus* P3P22 and *Lactobacillus* sp. Aben MP16 showed a broad spectrum against the most indicators strains.

DISCUSSION

In the natural fermentation, the microbial was associated with raw material, utensils and equipment (Owusu-Kwarteng *et al*. 2010). In this study, mash contained yeasts and LAB, the fermented microflora presents prior the spontaneous fermentation. This microflora may have been also developed during the malting of the sorghum grain, thus explaining the higher initial count in the mash. During the spontaneous fermentation, the load of yeast decreased while LAB increased. A similar observation was reported in literature for traditional fermented food and beverages (Tamang *et al*. 2005). At the end of spontaneous fermentation, LAB counts were higher than yeast in the sour wort and the pH dropped from 5.65 in the mash to 3.71 in the sour wort. The low pH led to the inhibition of pathogenic microorganisms (Sawadogo-lingani *et al*. 2007; Aka *et al*. 2008). Acid production during the spontaneous fermentation increased the shelf-life of sweet sour (sour wort cooked) and *tchapalo*; it also conferred the souring taste expected by producers (Nanadoum *et al*. 2005; Aka *et al*. 2008).

In *tchapalo*, yeasts count constituted 99.9% of fermented microflora. The dominance of yeasts could be explained by the fact that in the lactic fermentation, microorganisms were brought by environment; while in the alcoholic fermentation, microorganisms were brought by inoculation of dried yeast harvested from previous *tchapalo* (Nanadoum *et al*. 2005; Djè *et al*. 2008). The higher initial yeasts count in traditional alcoholic fermentation allowed a quick growth and ethanol production. High ethanol content inhibits LAB growth. This may explain the low LAB counts obtained in *tchapalo*. But this finding disagrees with previous reports. According to Nanadoum *et al*. (2006), LAB and yeasts counts were practically identical in *bili bili*, a traditional sorghum beer produced in Chad. These authors found that the traditional starter contained as many yeasts as LAB. Even if LAB were found in reduced number in *tchapalo*, they were not fortuitous contaminants because they were also involved in African traditional beverages from cereal-based (Nanadoum *et al*. 2006; Sawadogo-lingani *et al*. 2007).

Lactobacillus species identified in this study were *L. fermentum*, *L. plantarum*, *L. brevis*, *L. coprophilus*, *L. cellobiosus* and *Lactobacillus* sp. *L. fermentum* was the predominant species involved in all steps of *tchapalo* processing. Sawadogo-lingani *et al*. (2007) found also that *L. fermentum* was the predominant LAB which was involved in *dolo* and *pito* wort for the production of sorghum beer in Burkina Faso and Ghana. This specie plays an important role in the spontaneous fermentation. The dominance of *L. fermentum* at late stages of the wort fermentation was attributed to its high acid tolerance (Sawadogo-Lingani *et al*. 2008).

L. brevis was also found in all steps of *tchapalo* processing. They were reported as one of the dominant LAB in several fermented cereal products (Kebede 2007). In this study, it came at the second position after *L. fermentum*. Some strains of *L. brevis* had antimicrobial activities. For example, *L. brevis* OG1 isolated from *ogi*, a Nigerian traditional product, produced a bacteriocin that exhibited a large inhibition spectrum against pathogenic food spoilage microorganisms (Ogunbanwa *et al*. 2003). However, *L. brevis* usually belong to the unwanted or spoilage microflora in wine and beer fermentation (Sakamoto *et al*. 2001).

No strains of *L. plantarum*, generally reported as dominating LAB at the final step of spontaneous fermentation of most plant foods, were isolated in the sour wort. They were isolated in the mash and they were involved in the alcoholic fermentation of *tchapalo*. They were reported as predomi-

Table 4 Inhibition spectra of *Lactobacillus* species isolated in *tchapalo* processing.

Strains names	Unneutralized supernatants				Neutralized supernatants			
	E.c	P.a	B.s	S.a	E.c	P.a	B.s	S.a
Lf Yop $P_4P_2$3	-	-	-	-	-	-	-	-
Lf Att $P_3P_1$3	++	++	+	++	-	+	-	-
Lf Abo $P_2P_1$10	-	-	-	-	-	-	-	-
Lf Att $P_1P_2$4	+	-	-	-	-	-	-	-
Lf Yop $P_3P_2$2	-	-	-	-	-	-	-	-
Lf Att $P_2P_2$1	-	-	+	-	-	-	-	-
Lf Yop $P_2P_4$1	++	++	++	++	+	++	-	++
Lf Abo $P_1P_2$6	+	+	++	-	-	-	-	-
Lf Aben $KP_2$4	++	++	-	-	-	-	-	-
Lf Abo $P_{10}P_2$10	++	+	+	-	-	-	-	-
Lf Bon $KP_4$2	-	+	+	-	-	-	-	-
Lf Yop $P_2P_2$3	++	++	-	-	+	++	-	-
Lf Yop $P_4P_2$7	-	++	++	-	-	-	-	-
Lf Bon $KP_2$2	-	-	-	-	-	-	-	-
Lf Abo $P_9P_2$7	+	+	-	-	-	-	-	-
Lf Yop $P_2P_1$8	+	++	++	-	-	+	-	-
Lf Abo $P_9P_3$1	-	-	-	-	-	-	-	-
Lb Aben $MP_3$3	++	++	+	-	++	+	+	+
Lb Aben $KP_1$12	-	+	-	-	-	-	-	-
Lb Abo $P_1P_2$7	++	++	+	-	-	-	-	-
Lb Yop $P_2P_4$6	++	++	-	-	-	++	-	-
Lb Att $P_1P_3$3	+	-	-	+	-	-	-	-
Lb Abo P10P2 4	++	++	++	++	-	+	+	-
Lp Abo $P_4P_1$12	++	++	+	++	-	++	-	-
Lp Abo $P_9P_3$4	++	++	++	++	-	+	-	-
Lc Aben $MP_4$1	-	+	++	-	-	-	-	-
Lcop Att $P_2P_1$1	++	++	++	-	++	+	-	+
Lsp Att $P_3P_2$2	-	+	+	-	-	-	-	-
Lsp Bon $KP_2$8	-	+	-	-	-	-	-	-
Lsp Bon $Kp_2$12	-	+	+	-	-	-	-	-
Lsp Aben $MP_1$6	++	++	++	++	+	++	++	++

E.c: *E. coli* ATCC 25922, P.a: *P. aeruginosa* ATCC 27853, B.s: *B. subtilis* ATCC 6633, S.a: *S. aureus* ATCC 25913, *Lf: L. fermentum*, *Lb: L. brevis*, *Lp: L. plantarum*, *Lc: L. cellobiosus*, *Lcop: L. coprophilus*, *Lsp: Lactobacillus* sp, -: no inhibition, +: 6-10 mm diameter of inhibition, ++: 11-21 mm diameter of inhibition.

nant in *cachaça* and *hawked kunun*, traditional beverages (Amusa and Odunbaku 2009; Gomes *et al*. 2010) and in *akamu*, *fura* and *pozol*, fermented foods (Ben Omar *et al*. 2008; Nwachukwu *et al*. 2010; Owusu-Kwarteng *et al*. 2010). Kostinek *et al*. (2005) have shown that *L. plantarum* which was the most abundantly isolated species in the traditional fermented cassava dough, was shown to be a better acid producers and a faster ferment than *L. fermentum*. However, production of antagonistic substances such as H_2O_2 and bacteriocins was more common among *L. fermentum* strains.

L. hilgardii, *L. coprophilus* and *L. cellobiosus* constituted the minor population. It is unlikely that these species play a predominant role since they were not involved in the spontaneous and alcoholic fermentations apart *L. cellobiosus* which was found in *tchapalo*.

Many strains of *Lactobacillus* tested in this study showed the ability to acidify quickly the sorghum malt broth. There is a good reason to believe that the acidification is their major role in *tchapalo* processing. Their acidification power was tied up to the pH lowering of the broth. Other authors mentioned the highest acidification power of LAB in many fermented products (Mugala *et al*. 2003; Sawadogo-Lingani *et al*. 2008). In a monoculture of sorghum malt broth, *L. plantarum* acidified faster than other *Lactobacillus* tested. This strain was found in the spontaneous lactic fermentation of cereal-based-products where it was the predominant LAB (Mugala *et al*. 2003; Ben Omar *et al*. 2008).

Study of strains antibacterial activities was various. Results showed that when cultures were not neutralized, about 70% of the strains were found to produce an inhibition zone against some pathogenic bacteria. So, they inhibitory effects were tied up to their organic acids production capacity. The antibacterial activity was important for the consumers' safety and increase the products' shelf life (Bromberg *et al*. 2004; Ben Omar *et al*. 2008). When the pH value of the supernatants was adjusted to 6.5 and catalase was added, there was a reduction of the inhibition against indicators bacteria and only 35.4% of strains exhibited a weak inhibitory effect. This indicates that a few bacteria would be produced other compounds such as bacteriocins (Al-Allaf *et al*. 2009; Kalui *et al*. 2010). Other authors reported also that some *Lactobacillus* species from various foods produced a broad spectrum activity against various pathogenic bacteria (Ogunbanwa *et al*. 2003; Alvarado *et al*. 2006; Ben Omar *et al*. 2008). The inhibitory effect of the *Lactobacillus* strains in this study against the pathogenic indicated strains were different.

Tchapalo processing comprised a spontaneous fermentation dominated by LAB and an alcoholic fermentation dominated by yeast. Several *Lactobacillus* species were involved in these fermentations with a predominance of *L. fermentum* strains. Some *Lactobacillus* strains acidified quickly the wort and were able to inhibit the growth of most of indicator strains. Further research will be focus on organoleptic properties and trial fermentations with these microorganisms alone or in co-cultures. Bacteria with good fermentation behaviour will be selected and used to produce a starter for commercial sweet wort and *tchapalo*.

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