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Characterization of exopolysaccharide producing LAB isolated from *Zoom-koom*, a cereal-based traditional beverage from Burkina Faso

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Abstract

Zoom-koom is a traditional fermented beverage from Burkina Faso produced from millet or sorghum grains. A total of 33 Lactic acid bacteria (LAB) that produce exopolysaccharides (EPS) were isolated from *zoom-koom* and characterized in order to select the most efficient as starter cultures to improve the quality of *zoom-koom*. Physiological and morphological characteristics were determined using growth tests and microscopic observation, respectively. Activities of starch degrading enzymes (amylases) were performed using biochemical methods. Antimicrobial activities were determined using disc diffusion assay and overlay method. Genotypic characterization and identification of selected LAB EPS synthetizing isolates which were able to produce antimicrobial compound were performed through 16S rRNA sequencing. EPSs Structures of selected LAB isolates were determined using NMR spectroscopy. Among LAB isolates76% was hetero-fermentative while the other was homo-fermentative. Some isolates (15%) could grow in the presence of bile and esculin and 12% were able to grow on MRS-starch. Although all the isolates were able to produce EPSs, only 6% were efficient producers. Among the screened LAB isolates, 7had inhibitory activities against *Escherichiacoli*81 nr.149 SKN 541, *Pseudomonas aeruginosa* ATCC and *Salmonella typhimurium* O:1036340P/t49. In addition 6 isolates displayed antifungal activities against *Aspergillus fumugatus, Aspergillus flavus* and *Aspergillus niger*. The 16S rRNA sequencing showed that most of the isolates were clustered with *Weissella cibaria/confusa* and the others were clustered with *Lactobacillus plantarum/pentosus* and *Lactococcus lactis/garvieae*. The NMR spectroscopy showed that excreted EPSs are ramified glucanpolyaccharides containing α-(1→3) and α-(1→6) linkages, where the later are the main chains.

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Introduction

Zoom-koom is a popular beverage in Burkina Faso, based on cereals (millet, sorghum) and mainly produced by women and has important socio-economic implications (Soma *et al.,* 2017). In the traditional processing, the cereals used can be dehulled or not according to the producer. The traditional processing of *zoom-koom* involves grains soaking, milling, filtration and adding of sugar. Tamarind juice is usually added at the end of process to acidify the *zoom-koom* (Soma, 2014). In Burkina Faso, cereals-based beverages are considered as foods because of their nutritional value and their contribution to the diet of the populations (Sawadogo-Lingani *et al.,* 2008). Unfortunately, these beverages are always produced without pasteurization treatment, and therefore maycontain some potential pathogens such as enterobacteria (Tapsoba *et al.,* 2017). In fact, Besadjo-Tchamba *et al.,* (2014) identified enteropathogenic *Escherichia coli* (EPEC) serotypes in *zoom-koom* sold in Ouagadougou (Burkina Faso). Also, according to Barro *et al.,* (2007), *zoom-koom* is the street food which contains high amount of thermotolerant coliforms bacteria. Street-vended foods and beverages that the production process is dominated by hand intervention without adhering to good hygienic practices are susceptible to contamination (Sunday *et al.,* 2011; Tchamba *et al.,* 2014).

Recently, a new process of producing *zoom-koom* from whole grains including fermentation of the dough before filtering resulted in a reduction of the enterobacteria counts (Tapsoba *et al.,* 2017). In fact, lactic acid bacteria (LAB) are able to be produce antimicrobial substances like organic acids (lactic, acetic, formic, phenyllacticcaproic), carbon dioxide, hydrogen peroxide, ethanol and bacteriocins (Messens De Vuyst, 2002). In addition to their antimicrobial activity, LAB is also able to produce exopolysaccharides (EPS). The EPS are very important for a wide range of industrial applications, including baked goods (Katina 2009, Kajala *et al.,* 2015) and for the production of cereal-based functional beverages fermented by LAB (Zannini *et al.,* 2013).

Several health benefits have been attributed to the microbial EPS, such as immune-stimulatory and antitumoral effects or lowering blood cholesterol (Vinderola *et al.,* 2006; Caggianiello *et al.,* 2016). Thereby, these polymers are receiving increased attention for their potential application as prebiotics (Fusco *et al.,* 2015).

LAB have traditionally been associated with food and feed fermentations. Their uses around the world include improving the preservation, organoleptic characteristics, and nutritional values of a large variety of food and beverages products (Sawadogo-Lingani *et al.,* 2008; Caggianiello *et al.,* 2016; Zannini *et al.,* 2016). In last years, many studies have focused on selecting strains of nutritional or technological interest (Songre-Ouattara *et al.,* 2008; Sawadogo-Lingani *et al.,* 2008; Fusco *et al.,* 2015; Zannini *et al.,* 2016) with the aim of using them as starters to enhance the quality of fermented food products. The purpose of the study was therefore to find suitable starters cultures, which are able to produce EPS and antimicrobial compounds, to improve the rheological and hygienic quality of *zoom-koom* in controlled fermentation.

Materials and methods

Microorganisms and culture conditions

A total of 350 presumptive LAB isolates (Gram+, Catalase-, Oxidase-, no motile bacteria) were collected throughout the traditional processing of *zoom-koom* based on fermented whole millet or red sorghum dough in Ouagadougou(Burkina Faso) in Zogona (207 isolates) and Dassasgho (143 isolates) production sites (Tapsoba *et al.,* 2017). These isolates were tested for their EPSs production according the method previously described by Sawadogo-Lingani *et al.,* (2008). From this screening, 33 EPSs positives isolates were EPS positive isolates. These isolates were maintained at -20°C in MRS broth (Liofilchem, Spain) with 50% (v /v) glycerol (Merck, Darmstadt, Germany), and were cultured twice on MRS agar (Liofilchem, Spain) in anaerobic conditions at 37°C for 48 h, before characterization and identification.

The pathogenic strains used for antimicrobial screening: *Pseudomonas aeruginosa* ATCC, *Staphylococcus aureus* toxine A+B and *Aspergillus niger* were obtained from the culture collection of the Department of Food Science, Food Microbiology in Copenhagen University, Denmark (*Escherichia coli* 81 nr.149 SKN 541,); *Listeria monocytogenes* NCTC 9863, *Bacillus cereus* LMG13569 and *Salmonella typhimurium* O:1036340P/t49 were obtained from the culture collection of London Metroplitan University. *Aspergillus fumigatus* and *Aspergillus flavus* were previously isolated from rice and groundnut (Compaoré *et al.,* 2016), and *Candida albicans* was previously isolated from blood samples as described by Compaoré *et al.,* (2013).

Pathogenic bacteria were maintained at -80°C in nutrient broth (Oxoid CM0001) supplemented with 20% (v/v) glycerol. The yeasts were maintained in yeast glucose peptone broth made of 1% (w/v) bactopeptone (211677; Becton, Dickinson, Sparks, MD, USA), 1% (w/v) glucose (Merck 38291142, Darmstadt, Germany), 0.5% (w/v) yeast extract (Oxoid LP0021), pH 5.6 ± 2 at -80°C, while the moulds were maintained in malt extract broth (Oxoid CM0057) supplemented with 20% (v/v) glycerol at -80°C.

Morphological, physiological and biochemical characterization

Pure cultures were characterized by cell morphology using phase contrast microscope, gas production from glucose using the semi-solid medium of Gibson and Abd-el-Malek or using MRS broth with Durham cloche. Growth at 10°C and 45°C; growth at pH 4.4 and 9.6; growth in MRS broth with 6.5% and 18% NaCl; growth on Bile Esculine Azide (BEA) medium; growth in MRS broth with 3% arginine.

Exopolysaccharides (EPS) production on the selected isolates

To evaluate efficiency of EPS production, the strains were grown on MRS agar supplemented with 2% sucrose as previously described (Maina *et al.,* 2008). The isolates were plated and incubated under anaerobic conditions, at 37°C for 24 h.

At the end of incubation, mucoid colonies were determined by visual appearance and ropiness was determined by touching them with a sterile inoculation loop (Maina *et al.,* 2008; Malang *et al.,* 2015). Colonies showing amucoid and/or ropy phenotype were selected for further studies.

Screening of isolates for amylase formation

The ability of the isolates to produce amylase was determined according to the method described in previous studies (Sanni *et al.,* 2002; Sawadogo-Lingani *et al.,* 2008). Three lines streak of each isolate were made with an inoculation needle on modified MRS agar without glucose but with 2% (w/v) potato starch as the only carbon source. The plates were incubated at 37°C for 48 h in anaerobiosis. The culture plates were covered by spraying with Lugol's iodine [0.33% (w /v) iodine (Prolabo), 0.66% (w/v) potassium iodide (Labosi, Paris, France)] to detect starch hydrolysis. Growth of the isolates on MRS-starch agar was also noted; the isolates which were able to grow on this media were selected and the experiment was performed again using other media such as MRS-2% (w /v) starch broth, MRS agar-2% starch with 0.05% (w /v) $CaCO_3$ (Labosi) and their growth was examined after incubation at 37°C for 48 h under anaerobic conditions.

Detection of the antibacterial activities of LAB isolates

A disc diffusion assay procedure with cell free supernatant (CFS) was used (Savadogo *et al.,* 2004; Abbas *et al.,* 2016). Each LAB isolates being investigated for anti-microbial properties was grown in 50 ml of MRS broth at 37 °C for 24h. The cell-free supernatant (CFS) was obtained by centrifuging the culture (8,000 G for 10 min at 4°C).For each isolate of LAB, three discs (5 mm) were used in duplicate: the first disc was dipped in the unadjusted Cell Free Supernatant (CFS) aliquot, the second disc was dipped in the remaining CFS which is adjusted to pH 6.0 with 1 $mol.l^{-1}$ NaOH in order to rule out possible inhibition effects due to organic acids. The third disc was dipped in neutralized CFS which is treated with 1 $mg.ml^{-1}$ of catalase at 25°C for 30 min to eliminate the possible inhibitory action of H_2O_2.

Before dipping, all the CFS was filtrated through a sterile 0.22 µm syringe filter Millipore to obtain cell free filtrate.

The indicator strains maintained at -80°C in nutrient broth with 20% (v/v) glycerol were cultured aerobically on Brain Heart Infusion (BHI) agar (Difco, Detroit, MI, USA) at 37°C, before use. A loopful of each indicator strain was inoculated in 10 ml of Brain Heart Infusion (BHI) broth (Difco, Detroit, MI, USA) and incubated at 37°C overnight (16-18h). Each disc was put on the Mueller-Hinton agar (Becton Dickinson, USA) previously inoculated (inundation method) with pathogen indicator bacteria from BHI broth (5×10^5 CFU/mL). The plates were incubated aerobically for 24h and 48h at 37°C. The inhibition zones were recorded in mm. If inhibitions zones were found in the third disk, the isolates were considered to be able to produce bacteriocin-like substance (BLS).

Detection of antifungal activities

Two methods were used for the detection of antifungal activities. The first method was the overlay method (Magnusson and Schnurer, 2001; Magnusson *et al.,* 2003). LAB isolates were streaked in 2 cm lines on mMRS agar (MRS-Maltose agar), and the plates were incubated at 37°C for 48 h anaerobically to allow the growth. The plates were then overlaid with 10 mL of soft malt extract (0.7% agar) containing 0.1 mL of spore suspension of fungi (10^5 spores/mL), incubated at 30°C for 48 h and the zones of inhibition were measured. The degree of inhibition was calculated as the area of inhibited growth in relation to the total area of the Petri dish. The scale was the following: - : no visible inhibition; +: no fungal growth on 0.1-3 % of plate area; ++: no fungal growth on 3-8 % of plate area; +++: no fungal growth on >8 % of plate area (Magnusson *et al.,* 2003).

The second method was the disc diffusion assay procedure (Savadogo *et al.,* 2004; Abbas *et al.,* 2016). Each tested isolate was cultured in 10 mL MRS broth at 37°C for 24 h and centrifuged at 8,000 G for 10 min. For each LAB isolates, one disc was used in duplicate.

The disc was dipped in the unadjusted CFS aliquot and put on the Mueller-Hinton agar previously inoculated (pour plate method) with fungal indicator strains (5×10^5 CFU/mL). The plates were incubated aerobically for 24h, 48h and 72h at 37°C. The inhibition zones were recorded in mm.

Genotypic characterization and identification of the selected LAB isolates

LAB isolates expressing antimicrobial activity and producing EPS were further identified.

The LAB strains were cultivated in MRS broth at 30°C for 24 h. Genomic DNA was extracted using a DNeasy® Blood and Tissue Kit (Qiagen, Germany) by following the manufacturer's instructions, with the addition of lysozyme (80 mg/ml, Sigma Aldrich, Canada) . To identify presumptive lactic acid bacteria, the primer pairs LacbF/LacbR were used to amplify 16S rRNA gene fragment of lactic acid bacteria. Reaction mixture and PCR conditions for primers were as described by (De Angelis *et al.,* 2006).

Electrophoresis was carried out on agarose gel at 1.5% (w/v) (Gellyphor, EuroClone) and amplicons were purified with GFX™PCR DNA and Gel Band Purification Kit (GE Healthcare). Molecular weight of the amplified DNA fragments was estimated by comparison with a 1 Kb Plus DNA Ladder (Invitrogen) ranging from 100 to 12,000 bp. Sequencing was carried out at GATC Laboratories, Germany. The identification queries were fulfilled by a BLASTn search in GenBank (Altschul *et al.,* 1997), (www.ncbi.nlm.nih.gov/genbank).

The phylogenetic tree was constructed using the Seaview version 4 software (Gouy *et al.,* 2010) from 16s rDNA sequences using the PhyML method (Maximum-Likelihood Phylogenies or maximum likelihood phylogeny). For this phylogenetic tree, only the probabilities of the estimated branches with the approximate likelihood ratio test (ALRT) greater than 70% were given at the branch points. The scale indicated a 100% sequence divergence.

Structural characterization of EPS by NMR spectroscopy.

EPS from LAB 1 and LAB 5 that showed the best production of EPS (+++) were isolated and their structure characterized by NMR spectroscopy. Isolation and NMR spectroscopy analysis were carried out according to Maina *et al.,* (2008). For EPS isolation, the strains were grown on MRS-Sucrose agar in anaerobic conditions at 30°C for five days. The cell mass was carefully removed from the plates and suspended in sodium phosphate buffer saline The suspensions were shaken for 10 min and allowed to stand for 30 min. Subsequently, the cells were separated by centrifugation at 10,000 rpm for 40 min. Supernatants were stored at 4°C and EPS were recovered from the supernatant by ethanol precipitation. For NMR spectroscopy analysis, samples (10 mg/mL) were exchanged once with D_2O, filtered, and then placed in NMR tubes (Wilmad NMR tubes, 5 mm, ultra-imperial grade, from Aldrich chemical company, Milwaukee, WI, USA). All the measurements were performed at 50°C, and the chemical shifts were referenced to acetone (1H = 2.225 ppm and 13C = 31.55 ppm), NMR analysis was carried out with a 600 MHz Bruker Avance III NMR spectrometer (BrukerBioSpin, Germany). 1D 1H spectra were obtained using Bruker 1D NOESY with prostration and spoil gradients pulse program (noesygppr1d).

Results

Physiological, morphological and biochemical characteristics of the dominant LAB isolates involved in the production process of zoom-koom.

The results obtained from the morphological, physiological and biochemical characterization tests allowed to group the 33 LAB isolates into 20 groups (Table 1).

Table 1. Morphological, physiological and biochemical characteristics of dominant LAB strains involved in the production process of fermented *zoom-koom*.

Code of isolates	Shapes	Fermentation type	Gas production from glucose	Bile Esculine	Arginine	NaCl 6.5%	NaCl 18%	pH 4.4	pH 9.6	45°C	10°C	EPS	Amylase
LAB 1 (1)	Short rods in pair and short chains	Hétéro	+ (1)	+ (1)	+ (1)	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	+++ (1)	- (1)
LAB 2 (2)	Cocci in pair and short chains	Homo	- (2)	- (2)	+ (2)	+ (2)	- (2)	+ (2)	- (2)	- (2)	+ (2)	++ (2)	- (2)
LAB 3 (6)	Short rods in pair and short chains	Hétéro	+ (6)	- (6)	- (6)	+ (6)	+ (6)	- (6)	+ (6)	+ (6)	+ (6)	++ (6)	- (6)
LAB 4 (3)	Short rods in pair and short chains	Hétéro	+ (3)	- (3)	- (3)	+ (3)	- (3)	+ (3)	+ (3)	+ (3)	+ (3)	++ (3)	- (3)
LAB 5 (1)	Short rods in pair and short chains	Hétéro	+ (1)	- (1)	+ (1)	+ (1)	- (1)	- (1)	+ (1)	+ (1)	+ (1)	+++ (1)	- (1)
LAB 6 (1)	Short rods in pair and short chains	Hétéro	+ (1)	+ (1)	+ (1)	- (1)	- (1)	- (1)	- (1)	+ (1)	+ (1)	++ (1)	- (1)
LAB 7 (1)	Short rods in pair and short chains	Hétéro	+ (1)	- (1)	+ (1)	- (1)	- (1)	- (1)	+ (1)	+ (1)	+ (1)	++ (1)	- (1)
LAB 8 (1)	Short rods in pair and short chains	Hétéro	+ (1)	+ (1)	- (1)	+ (1)	- (1)	+ (1)	- (1)	+ (1)	+ (1)	++ (1)	- (1)
LAB 9 (1)	Short rods in pair and short chains	Hétéro	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	- (1)
LAB 10 (1)	Cocci in pair and short chains	Homo	- (1)	- (1)	+ (1)	- (1)	- (1)	+ (1)	- (1)	- (1)	+ (1)	++ (1)	- (1)
LAB 11 (2)	Short rods in pair and short chains	Homo	- (2)	- (2)	- (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)	+ (2)	+ (2)	- (2)
LAB 12 (2)	Short rods in pair and short chains	Homo	- (2)	- (2)	- (2)	+ (2)	+ (2)	- (2)	+ (2)	+ (2)	+ (2)	++ (2)	- (2)
LAB 13 (1)	Short rods in pair and short chains	Hétéro	+ (1)	+ (1)	+ (1)	+ (1)	- (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	- (1)
LAB 14 (3)	Short rods in pair and short chains	Hétéro	+ (3)	- (3)	+ (3)	+ (3)	- (3)	- (3)	+ (3)	+ (3)	+ (3)	+ (3)	- (3)
LAB 15 (1)	Short rods in pair and short chains	Hétéro	+ (1)	- (1)	- (1)	+ (1)	- (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	- (1)
LAB 16 (2)	Short rods in pair and short chains	Hétéro	+ (2)	- (2)	- (2)	+ (2)	- (2)	- (2)	+ (2)	+ (2)	+ (2)	+ (2)	- (2)
LAB 17 (1)	Cocci in pair and short chains	Hétéro	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	++ (1)	+ (1)
LAB 18 (1)	Short rods in pair and short chains	Homo	- (1)	- (1)	+ (1)	+ (1)	- (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)
LAB 19 (1)	Long rods in pair and short chains	Hétéro	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+ (1)	+(1)
LAB 20 (1)	Short rods in pair and short chains	Hétéro	+ (1)	+ (1)	- (1)	+ (1)	- (1)	+ (1)	- (1)	+ (1)	+ (1)	+ (1)	+ (1)

+: growth or produce (amylase, EPS and gas production); -: not growth or not produce (amylase, EPS and gas production);
( ): number of isolates.

From the results it appears that76% of the LAB isolates were hetero-fermentative compared to 24% which were homo-fermentative. About 76% of LAB isolates were able to ferment the glucose with gas production and 15% of the isolates were able to grow in the presence of bile and esculin.

Physiological tests on the LAB isolates revealed that around 45% were able to grow in the presence of arginine; 90% and 33% were able to grow on 6.5% NaCl and 18% NaCl; 33% and 81% were able to grow at pH 4.4 and pH 9.6; 91%and 100% were able to grow at temperature of 45°C and 10°C.

However, only 12% of isolates were able to grow on modified MRS medium (MRS-starch). Therefore, these isolates are capable of producing starch-degrading enzymes such as amylases. Moreover, these isolates were able to use starch as the only source of carbon for growth, which confirmed amylolytic activity. The addition of $CaCO_3$ to the media to increase the production of amylase through Ca^{2+} ions, did not improve the efficiency of the isolates.

Table 2. Diameters of the inhibition zones (in mm) from antibacterial activities screening.

	S. typhimerium			*E. coli*			*P. aeruginosa*		
	S	AS	AS+C	S	AS	AS+C	S	AS	AS+C
LAB1	0	0	0	7	7.5	6.5	7	7.5	7
LAB4	6	6	6	0	0	0	6	6.5	6
LAB5	8	8.5	7	8	8.5	7	8	8.5	7.5
LAB8	7	9.5	9	6	6	6	7	7	7
LAB15	7	7	7.5	0	0	0	7	7	7
LAB16	7	7	7.5	0	0	0	7	7	7
LAB20	7	7.5	7	7	6.5	6.5	7	7	7

S: supernatant; AS: Adjusted Supernatant; AS+C: Adjusted Supernatant + Catalase.

Table 3. Antifungal activities of LAB isolates.

Fungi LAB isolates	*A. flavus*	*A. niger*	*A.fumugatus*
LAB1	++	-	++
LAB4	+	-	-
LAB5	+	+	-
LAB15	+	-	-
LAB16	+++	-	-
LAB20	-	-	+++

-: No antifungal activities, +: no fungal growth on 0.1-3 % of plate area; ++: no fungal growth on 3-8 % of plate area; +++: no fungal growth on >8 % of plate area.

The screening of EPSs production showed clear differences among the isolates. From the 33 isolates screened, only 2 LAB isolates had a good production of EPSs (+++), 18 LAB isolates produced fairly EPSs (++) and 13 produced poorly EPSs (+) as shown in fig 1.

Antimicrobial activities

From the result of antibacterial activities screening (Table 2), it appears that only 35% (7 out to 20 isolates) of LAB isolates had shown activity against *E. coli, S. typhimurium* and *P. aeruginosa*. No antibacterial activity was observed against *B. cereus, S. aureus* and *L. monocytogenes*. All the isolates which had shown antibacterial activities were considered to be able to produce bacteriocin-like substance (BLS) according to the inhibition zone found with the third disk (AS+C) as shown in Fig. 2. The diameter of the inhibition zone for all the positive isolates ranged between 6 and 9 mm (Table 2).

Table 4. Genotypic identification of selected LAB isolates from the BLAST on NCBI.

Code of LAB isolates	Identification
LAB 1	*Weissella cibaria/confusa*
LAB 2	*Lactococcus lactis*/spp.
LAB 4	*Weissella cibaria/confusa*
LAB 5	*Weissella cibaria/confusa*
LAB 6	*Weissella cibaria/confusa*
LAB 8	*Lactobacillus plantarum/pentosus*
LAB 10	*Lactococcusg arvieae*
LAB 13	*Weissella cibaria/confusa*
LAB 15	*Weissella cibaria/confusa*
LAB 16	*Weissella cibaria/confusa*
LAB 20	*Lactobacillus plantarum/pentosus*

From the antifungal activities assay (Table 3) 6 LAB isolates were found to express activity against *A. flavus; A. fumuigatus* and *A. niger*. No effect was observed against *C. albicans* strain. Also, no activities were observed against fungal pathogenic strains using disk methods. Only the overlay method had shown antifungal activities (Fig. 3).

Genotypic identification of selected LAB isolates

A total of eleven (11) LAB isolates were selected on the base of their ability to produce EPS and their antimicrobial activity and subjected to 16S rRNA sequencing and identification. From the 11 LAB isolates, 7 were identified as *Weissella* spp., 2 as *Lactococcus* spp. and 2 belong to *Lactobacillus* spp.

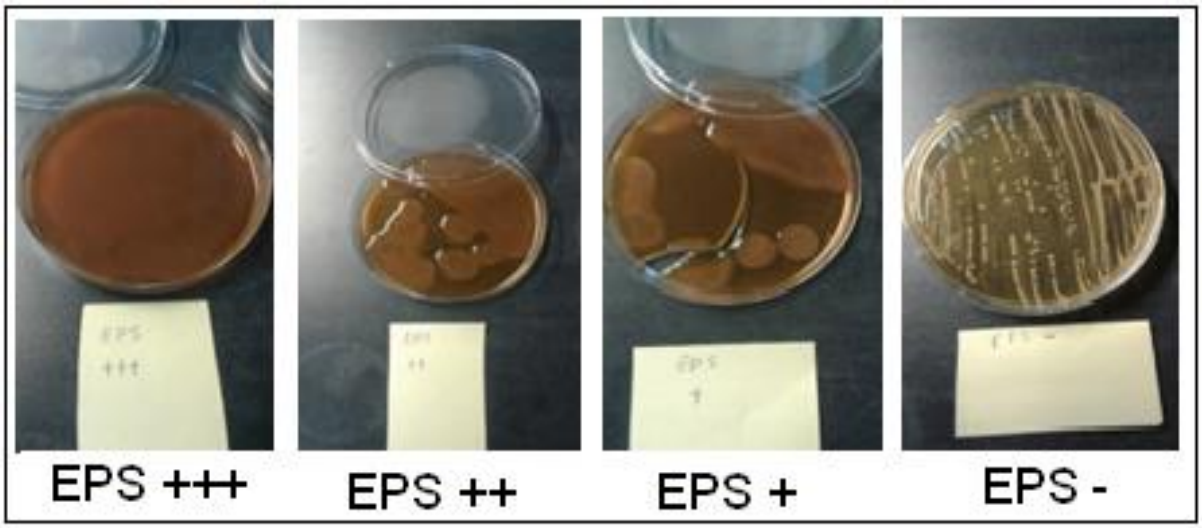

Fig. 1.EPSs production screening on MRS + 2% of sucrose.

The BLAST showed 99% of similarity for all LAB isolates except for LAB 16 which had 98% of similarity with *Weissella* spp. as shown in Table 4.From the dendrogram (Fig. 4), it appears that the isolates LAB 2 and LAB 10 clustered with the genus of *Lactococcus* and could belong to the species *Lactococcus lactis or Lactococcus garvieae* based on their 16S rRNA gene sequence (71% similarity). The isolates LAB 8 and LAB 20 could belong to the specie *Lactobacillus plantarum* based on their 16S rRNA gene sequence (79% similarity).

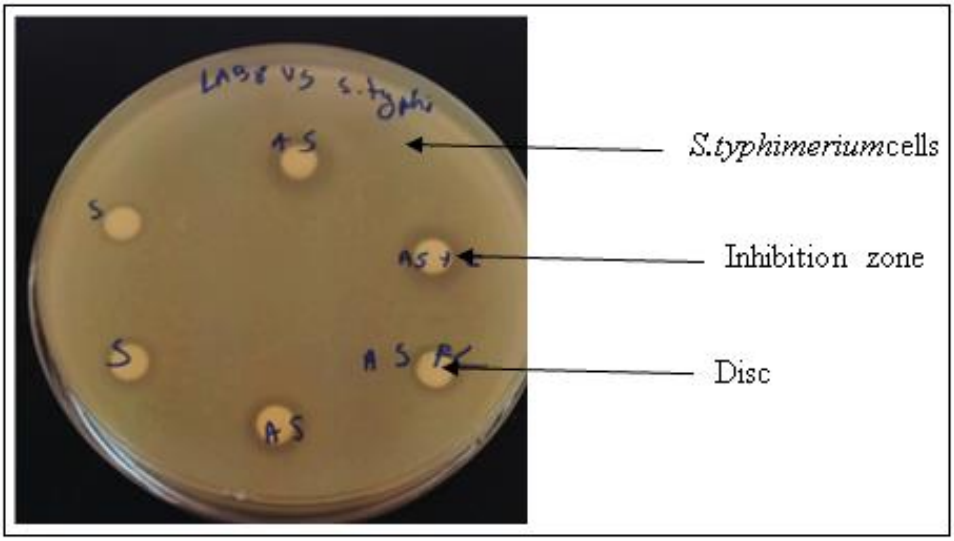

Fig. 2. Antibacterial activities of LAB 8 against *S. typhimerium* using disc methods; S: supernatant; AS: Adjusted Supernatant; AS+C: Adjusted Supernatant + Catalase.

The isolates LAB 1, LAB 4, LAB 5, LAB 6, LAB 13, LAB 15 and LAB 16 could belong to the species *Weissella cibaria* or *Weissella confuse* with 97% of similarity based on their 16S rRNA gene sequence. The isolates LAB 8 and LAB 20, belonging to the genus of *Lactobacillus* were closer to each other (73%) as shown in Fig. 4. These isolates were mainly collected from grain of millet, end of soaking (*Lactococcus* spp.); water, end of millet grain soaking (*Weissella* spp.), dough of millet, start of fermentation (*Weissella* spp.), fermented *zoom-koom* of millet (*Lactobacillus* spp.), fermented *zoom-koom* of red sorghum (*Lactobacillus* spp.) and unfermented *zoom-koom* of millet (*Weissella* spp. and *Lactococcus* spp.).

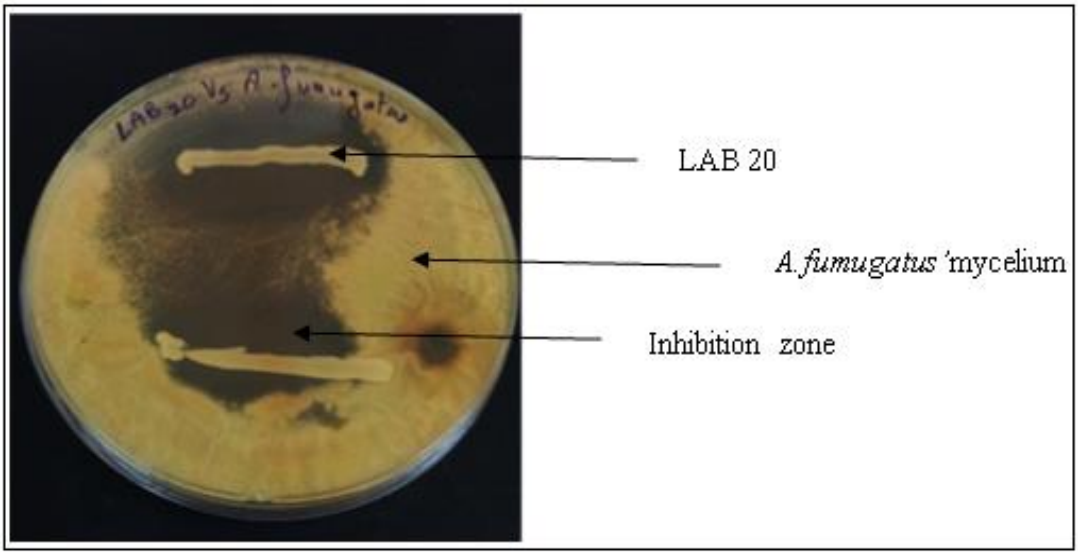

Fig. 3. Antifungal activities of LAB 20 against *A. fumugatus* using overlay method.

Structure of LAB 5 and LAB 1 EPSs

The ^{1}H spectra of EPSs of LAB 5 and LAB 1 were similar and indicated that the strains produced a dextran with low branching degree similar to *W. confuse* E392 (Fig 2 and 3).

The 1H NMR spectra showed a typical signal for an anomeric proton of α-(1→6)-linked glucosyl units centered at 4.98 ppm.

Furthermore, the spectra showed that the main chain contained α-(1→3)-linked branches. As previously described, the broad signal for this α-(1→3)-linked branches (5.31-5.32 ppm) indicated that the branches can be single unit or elongated, possibly by α-(1→6)-linked glucosyl units, thus forming a ramified structure.

Integration of anomeric signals showed a proportion of ca. 97 % α-(1→6) linkages (main chain) and ca. 3% α-(1→3) linkages.

Discussion

Physiological, morphological and biochemical characteristics of the dominant LAB isolates involved in the production process of zoom-koom.

The purpose of this study was to find suitable starters cultures, which are able to produce EPS and antimicrobial compounds, to improve the rheological and hygienic quality of *zoom-koom*in controlled fermentation and identified the most efficient. Two of our isolates which were *cocci* shape (LAB 2, LAB 10) were homo-fermentative without gas production from glucose and were identified as *Lactococcus* spp. These characteristics are specific of *Lactococcus, Tetrageno coccus, Vagococcus, Streptococcus, Pediococcus* and *Aerococcus* genera (Matamoros, 2008; Liu *et al.,* 2014). LAB isolates which were able to grow at acid pH (pH 4.4) are important for the production of fermented foods products and is particularly relevant for *zoom-koom*, which has a final pH close to 4 which ensures the safety of the final product.

In this study, 5 LAB isolates were able to grow on arginine and esculin. The ability of some LAB isolates of hydrolyzing of arginine and esculin and to grow in the presence of bile is common to *Weissella* spp. and few of *Lactobacillus spp*. (Liu *et al.,* 2014; Fusco *et al.,* 2015). Only 15% of the LAB isolates were able to produce amylase, according to their growth on MRS-starch agar. Amylases are of technological interest for cereal-based fermented foods.

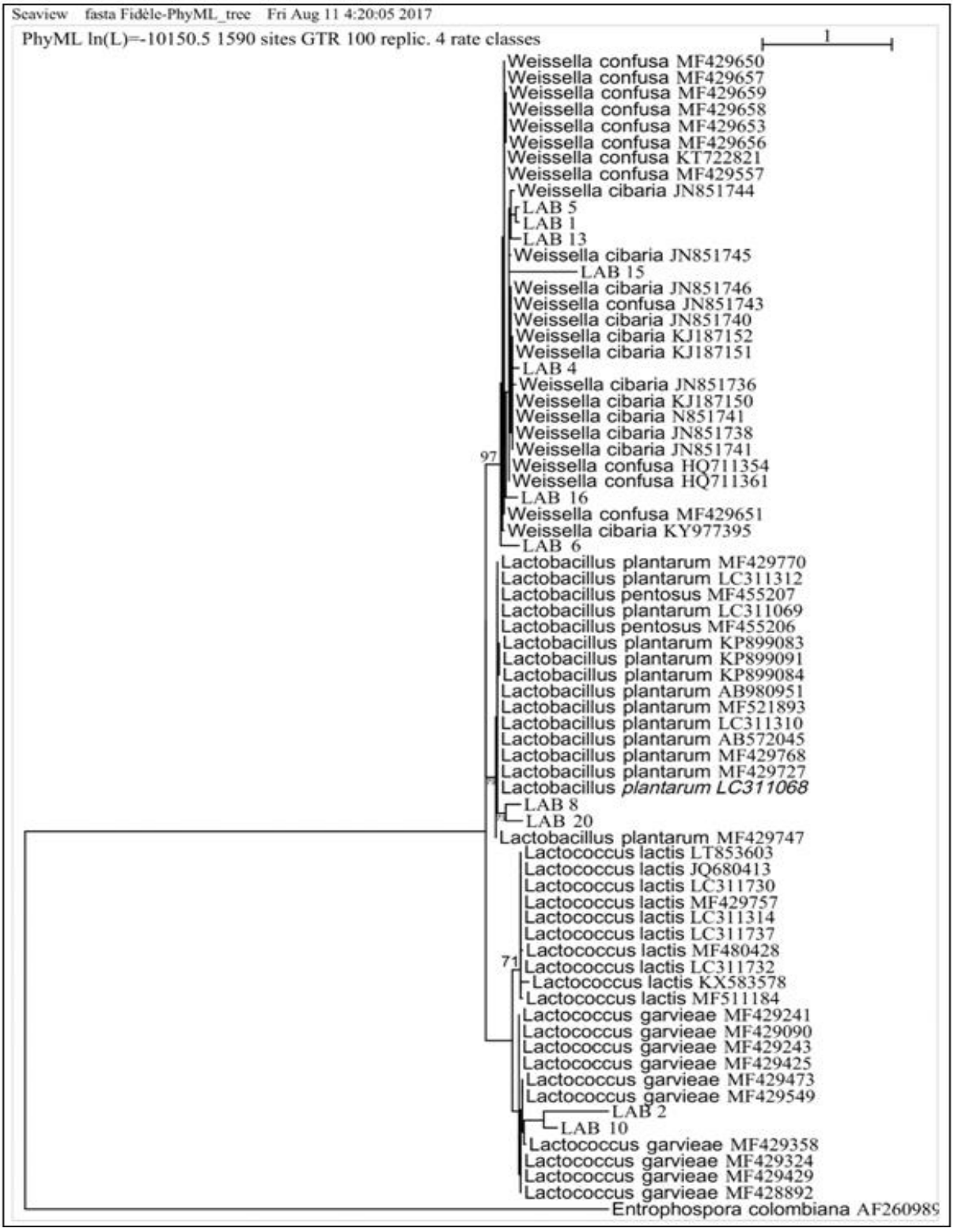

Fig. 4. PhyML phylogenetic tree based on 16S rDNA sequences of selected LAB isolates and GenBank related reference strains.

These enzymes allow the degradation of the starch and allow a better availability of the fermentable sugars. These results are in line with previous studies in which only few strains of *Lactobacillus plantarum, Lactobacillus cellobiosus* and *Lactobacillus manihotivorans* isolated from maize and cassava-fermented products, were identified as potential amylase producers (Morlon-Guyot *et al.,* 1998; Sanni *et al.,* 2002). Moreover, Kostinek *et al.,* (2005) did not find amylase-producing strains among the predominant LAB isolated from fermented cassava. Most of the LAB strains producing EPS isolated from *dolo* and *pito*, sorghum malt fermented beverages, were not able to produce amylase, and only few (15%) were able to grow on MRS-starch medium (Sawadogo-Lingani *et al.,* 2008).

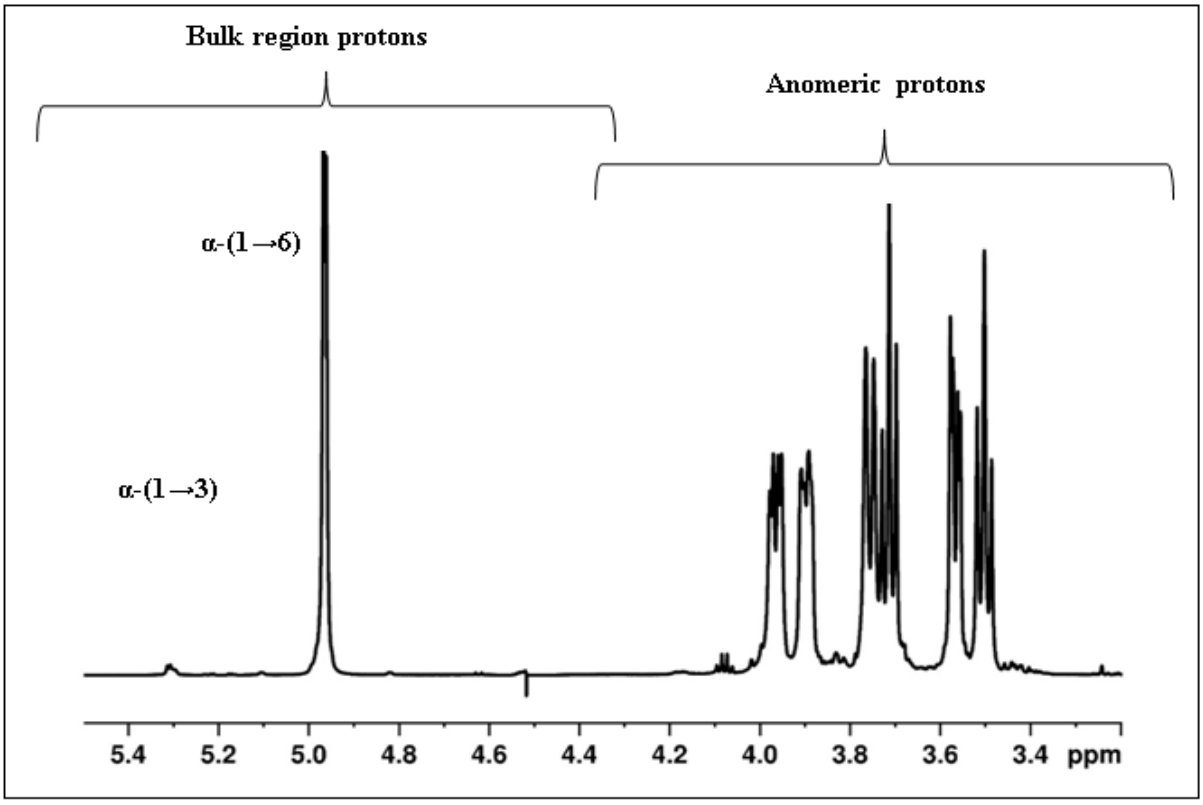

Fig. 5. 1D 1H NMR spectroscopy spectrum of EPS isolated from LAB 1 and LAB 5.

Antimicrobial activities

Some LAB isolates collected through *zoom-koom* processing showed antibacterial and antifungal activities. The antibacterial activities showed inhibitory effect on *E. coli, S. typhimerium* and *P. aeruginosa*but not on *S. aureus, B. cereus* and *L. monocytogenes*. These results are important for safety of *zoom-koom* production. Indeed, these pathogenic bacteria are often associated in many diseases, in particular intestinal disorders and diarrheal diseases (Mathara *et al.,* 2004; Besadjo-Tchamba *et al.,* 2014). Previous studies were already isolated these pathogenic bacteria in the unfermented *zoom-koom* (Barro *et al.,* 2007; Besadjo-Tchamba *et al.,* 2014).

Our results are similar to those of Olanrewaju (2007) who observed some LAB strains isolated from cow milk samples with antibacterial activities against E. *coli* and *Pseudomonas aerouginosa* but not against *Bacillus cereus* and *Staphylococcus aureus* using the agar well diffusion method. Also, Savadogo *et al.,* (2004) found LAB strains isolated from fermented milk which showed antimicrobial activities against *Bacillus cereus* 13569 LMG, *Staphylococcus aureus* ATCC 25293, *Escherichia coli* 105182 CIP using the agar drop diffusion test and the inhibition diameters obtained were between 8 mm and 12 mm. LAB can produce antimicrobial substances such as organic acids, hydrogen peroxide, diacetyl which are capable of inhibiting the growth of pathogenic and spoilage microorganisms (Yuksekdag and Aslim, 2010).

The difference found in the antimicrobial activities with the others authors could be explained by the concentration of antimicrobial compounds contained in the supernatant, the method used, the kind of LAB strains and pathogenic indicators strains used. The inhibition zone found with the adjusted supernatant + catalase disc in our study showed that the inhibition was not due to H_2O_2, and could therefore be attributed to other compounds such as bacteriocins or other metabolites. It was also observed in previous studies that the bacteriocin produced by *W. confusa* had a broad spectrum of antimicrobial activity inhibiting both Gram-positive and Gram-negative bacteria (Hweh and Koshy, 2015).

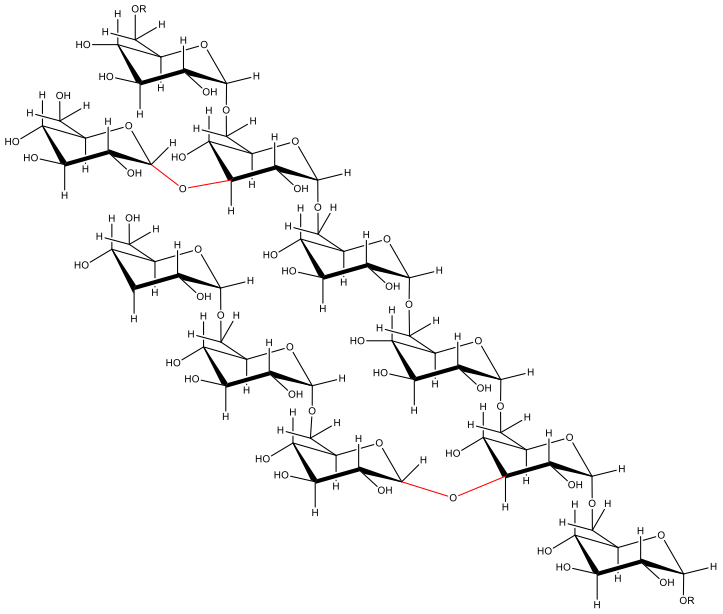

Fig. 6. Schematic representation of thedextrans isolated from LAB 1 and LAB 5.

The LAB isolates collected from *zoom-koom* also showed antifungal activity against *A. flavus, A. fumugatus* and *A. niger* but not on *C. albicans* using overlay method. These results emphasize the possibility to use these LAB isolates as starters to eliminate the molds in the *zoom-koom*. These fungi can produce some compounds such as aflatoxin which are dangerous for human health. Our results corroborated with previous studies on antifungal activities which found strains of *Lactobacillus plantarum* and *Lactobacillus farciminis* active against *Aspergillus* sp., *Fusarium roseum, Trichoderma* sp., *Penicillium* sp. and *Stemphillium* sp. using overlay method (Laref, 2014).

Genotypic identification of selected LAB isolates

The LAB isolates producing EPSs which were able to produce antibacterial and antifungal compounds were selected for further identification.

Three genera were found throughout 16s rRNA sequencing such as *Weissella* spp, *Lactobacillus* spp and *Lactococcus* spp. Most of the 11 LAB isolates selected for 16S rRNA sequencing belong to *Weissella cibaria/confusa*. The other LAB were identified as *Lactobacillus plantarum/pentosus* and *Lactococcus lactis/garvieae*. *Weissella*, *Lactobacillus* and *Lactococcus* genera were previously isolated from cereal based fermented beverages and cereals sourdoughs (Mathara *et al.,* 2004; Sawadogo-Lingani *et al.,* 2007; Minervini *et al.,* 2012; Sanni *et al.,* 2013; De Vuyst *et al.,* 2014; Osimani *et al.,* 2015; Kogno *et al.,* 2017). In our study, the genera *Weissella* and *Lactobacillus* were mainly collected during the fermentation process and fermented *zoom-koom*. The fermentation of African cereals is most typically dominated by LAB species belonging to the genera *weissella*, *Lactobacillus* and *Pediococcus* (Franz *et al.,* 2014).

Structure of LAB 5 and LAB 1 EPSs

The 33 strains collected from *zoom-koom* processing were able to synthetize EPS, in different levels. Many LAB are able to produce EPS such as *Weissella* spp., *Lactobacillus spp.* and *Lactococcus* spp. (Petronella *et al.,* 1999; Maina *et al.,* 2008; Sawadogo Lingani *et al.,* 2008; Fusco *et al.,* 2015). In our study the best EPSs producer LAB isolates from *zoom-koom* were LAB 1 and LAB 5 identified as *Weisella confusa/cibaria*. EPS produced by LAB are able to modify the rheological properties, texture, and mouth feel of food products; thus, they would find application in the food industry as viscosities', stabilizers, emulsifiers, or gelling agents (Caggianiello *et al.,* 2016; Zannini *et al.,* 2016). The availability of LAB starter cultures producing exopolysaccharides in situ during fermentation could be a suitable alternative for products whose polysaccharides addition requires the specification as food additives, which is a condition not much appreciated by consumers. Therefore, based on these results, strains with EPS production can be selected for tailored fermentation of *zoom-koom* in order to enhance the technological properties of the final product.

NMR spectroscopy analysis showed that the EPS produced by the most efficient produces (LAB1 and 5) were dextrans. Integration of the anomeric signals showed that the dextran contained only few branches (97% α-(1-6) linkages and 3% α-(1-3) branch linkages).*Our results corroborate with some previous studies which found that Weissella* produce dextrans that contain only few branches (Maina *et al.,* 2008, *Shukla et al., 2014*). Dextran-producing strains of *W. cibaria* and *W. confusa* are very appealing for a wide range of industrial applications, such as bakery (Katina *et al.,* 2009; Coda *et al.,* 2014; Wolter *et al.,* 2014; Fusco *et al.,* 2015) and fermented functional beverages (Zannini *et al.,* 2013). These polymers are also, receiving increased attention for their potential application as prebiotics (Fusco *et al.,* 2015). *In situ* production of dextran can therefore be used to improve the technological properties of *zoom-koom*, for example, in order to give it a homogeneous phase. EPSs contribute to the rheological behaviour and texture of the products (Sikkema and Oba, 1998; De Vuyst and Degeest 1999).

Conclusion

The present study allowed to characterize and identify selected LAB isolates, which could be used as starters' cultures to improve microbiological quality and the texture of *zoom-koom*. EPS-producing LAB are industrially important microorganisms in the development of functional food products and are used as starter cultures or coadjutants to develop fermented foods. The use of EPS-producing LAB "in situ" during the fermentation process may improve the rheological and sensorial properties of fermented food. The antimicrobial activities showed by the LAB isolates will allow keeping safe the product from the fermentation with these isolates as starters' cultures. The most efficient LAB isolates were *W. cibaria/confuse* and *Lactobacillus plantarum/pentosus*. The isolates of *W. cibaria/confuse* can be used as starter cultures to improve the quality of *zoom-koom* and kept safe the final product by their interesting technological property.

Conflict of interest

Authors declare that there is no conflict of interest.

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