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Full Length Research Paper

Acid resistance, bile tolerance and antimicrobial properties of dominant lactic acid bacteria isolated from traditional "maari" baobab seeds fermented condiment

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Maari **is a fermented food condiment obtained by spontaneous fermentation of seeds from the baobab tree (*Adansonia digitata*). Nine dominant lactic acid bacteria (LAB) strains, isolated from traditional *maari* fermentation were examined for their resistance to pH 2.5, their tolerance to 0.3% bile and their antimicrobial activities against pathogenic bacteria. The agar spot test was used to screen the dominant LAB for antagonistic activity against a total of 21 indicator organisms including *Bacillus cereus* strains, *Salmonella* spp., *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* and *Micrococcus luteus.* It was observed that all LAB strains survived in 0.3% bile and exhibited antimicrobial activity against a broad spectrum of bacteria, including food spoilage and pathogenic organisms such as *B. cereus*, *Salmonella* spp., *L. monocytogenes*, *E. coli* and *M. luteus*. None of the tested LAB strains was active against *Y. enterocolitica* strains. At pH 2.5, *Pediococcus acidilactici* L87 survived over a period of 4 h whereas a slight decrease was observed for *Enterococcus faecium* L169. *E. faecium* L154 cell number decreased considerably. The rest of the tested strains did not survive at pH 2.5 over a period of 4 h. Based on the present results, *P. acidilactici* L87, *P. acidilactici* L169 and *E. faecium* L154 in addition to the *Bacillus* sp. which are the main microorganisms responsible of the fermentation of baobab seeds can be useful as starter cultures for improving *maari* quality and safety with respect to protection against food spoilage and pathogenic microorganisms.**

Key words: Baobab seeds, *maari*, fermentation, lactic acid bacteria, acid resistance, bile tolerance, antimicrobial activity.

INTRODUCTION

Maari is a traditional food condiment produced from the spontaneous and alkaline fermentation of baobab (*Adansonia digitata*) seeds. It is consumed in different regions of West Africa, including Burkina Faso, Benin, Mali and Nigeria under different names depending on the ethnic tribe such as *Dadawa Higgi* or *Issai* in Nigeria, *Dikouanyouri* in Benin, *N'Gono* in Mali and *Maari*,

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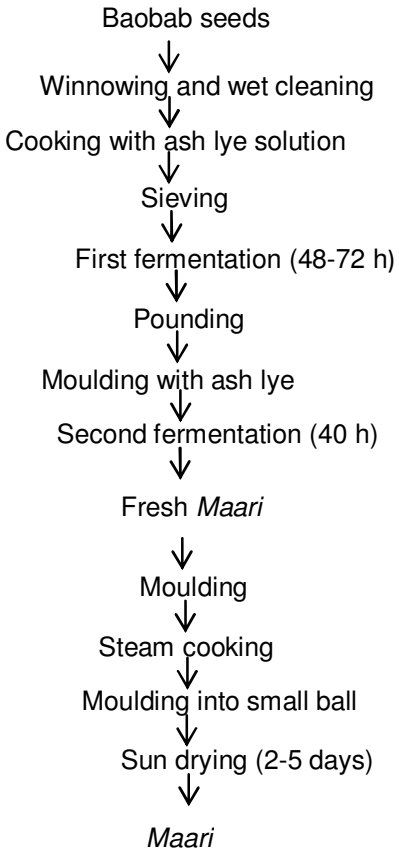

Figure 1. Diagram of traditional processing of baobab seeds into *maari* (Parkouda et al. 2010).

Mackaari, *Kaando*, *Thyou* or *Teed bikalgo* in Burkina Faso (Parkouda et al., 2010; Nkafamiya et al., 2007; Parkouda et al., 2009). For the traditional production of *maari*, seeds of *A. digitata* are cleaned and cooked till soft with ash lye solution used as traditional potash (Parkouda et al., 2010 as seen in Figure 1). Then a two-stage spontaneous fermentation process takes place. During the first fermentation for 48 h, the pH increase from 6.73 to 7.31. After the first fermentation step, the seeds are pounded and an alkalizing agent obtained by leaching ashes with water is added, resulting in an increase in pH up to 9. The second fermentation (40 h) takes place and the final product (pH 7.34) is sun dried, resulting in a dark brown and pungent-smelling condiment (Compaoré, 2009). The product which is similar in taste, color and smell to Soumbala (*Parkia biglobosa* fermented seeds) can be used as a taste enhancer in traditional dishes.

The fermentation of baobab seeds into *maari* is spontaneous and uncontrolled with sometimes inherent hygienic, nutritional and organoleptic defects, resulting in products inconsistent in quality attributes. To improve and optimize the production of *maari*, it is therefore necessary to control the fermentation by developing starter cultures. By the use of starter cultures it is possible to control fermentation of such condiments, avoiding growth of pathogenic and spoilage microorganisms, leading to a product of consistent taste and quality, as well as improved marketability (Ouoba et al., 2008).

Microbiological and physicochemical changes during the spontaneous fermentation of the seeds of *A. digitata* L. (baobab) into *maari* have been studied (Compaoré, 2009) with the identification of dominant microorganisms (Parkouda et al., 2010). The fermentation of baobab seeds was initiated by the aerobic mesophilic bacteria (AMB) identified as *Bacillus subtilis* (82% of AMB isolates). After 24 h of fermentation, LAB including *Enterococcus faecium, Enterococcus casseliflavus* and

Table 1. Dominant LAB isolated from traditional *maari.*

LAB strains	Code	Origin (reference)
Enterococcus faecium	L9	Isolated from traditional *maari* (Parkouda et al., 2010)
Pediococcus acidilactici	L87	
Enterococcus faecium	L104	
Enterococcus faecium	L117	
Enterococcus faecium	L134	
Enterococcus casseliflavus	L142	
Enterococcus casseliflavus	L152	
Enterococcus faecium	L154	
Pediococcus acidilactici	L169	

Pediococcus acidilactici appeared in the fermenting seeds and remained until the end of the fermentation, as the dominant LAB. Other microorganisms including *Bacillus cereus*, *Staphylococcus sciuri, Staphylococcus gallinarum* and *Corynebacterium sp.* were also isolated during the fermentation period (Parkouda et al., 2010). Although, the cooking of *maari* inactivates most of the contaminating micro-organisms, recontamination of the cooked *maari* may occur through handling and utensils. Moreover, the risks of cross-contamination, undercooking or persistence of heat-resistant microorganisms in the cooked product are reasons for concern. This may lead to microbial growth and possibly prove to be a health hazard depending on the nature and extent of the contamination as well as the storage conditions (Byaruhanga et al., 1999).

The aim of the present study was to investigate the antimicrobial properties as well as the acid resistance and bile tolerance of nine dominant LAB strains isolated from traditional *maari* in order to select the most suitable LAB as starter cultures for a controlled fermentation of baobab seeds.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Nine strains of LAB including five strains of *E. faecium*, two strains of *P. acidilactici* and two strains of *E. casseliflavus* were investigated (Table 1). Originally, the strains were isolated from different productions of *maari* and identified as described by Parkouda et al. (2010). The LAB strains were maintained as stock cultures at –80°C in de Man, Rogosa, Sharpe (MRS) broth (Oxoid CM0359, Basingstoke, Hampshire, England) with 20% (v/v) glycerol as a cryoprotectant. The LAB strains were subcultured in 10 ml MRS broth (Oxoid CM0359) at 37°C for 24 h before the cells were used. The antimicrobial activity was investigated against 21 indicator microorganisms representing Gram positive and Gram negative bacteria (Table 2). All indicator microorganisms were kindly supplied by the Department of Food Science, Food Microbiology in Copenhagen University, Denmark. The indicators were subcultured in 10 ml Brain Heart Infusion broth (BHI) (Oxoid CM0225, Basingstoke, Hampshire, England) at 37°C for 24 h before the cells were used.

Preparation of inocula of LAB strains

From MRS agar (Oxoid CM0361, Basingstoke, Hampshire, England) plates incubated anaerobically (GasPak system; BBL Microbiology Systems, Cockeysville, Md.) for 48 h at 37°C, the LAB strains were subcultured for 18 h at 37°C in 10 ml of MRS broth, pH 5.7. The cultures were centrifuged at 5000 g (4°C) for 15 min and the pellet re-suspended in 10 ml of sterile diluent (Oxoid CM0733, Basingstoke, Hampshire, England), pH 6.5. The count of the LAB cells was estimated by microscope using a counting chamber (Neubauer, Wertheim, Germany) and dilutions were done in sterile diluent (Oxoid CM0733) to obtain approximately 10^7 CFU/ml which were used as inoculum during the different assays.

Screening of dominant LAB strains for acid resistance

The method described by Klingberg et al. (2005) was used. The acid resistance was examined in MRS broth adjusted with hydrochloric acid (HCl) 1N to obtain a final pH of 2.5. 100 µl of cell suspensions of LAB cultured for 18 h (approximately 10^7 CFU/ml) were inoculated into MRS broth (Oxoid CM0359) previously adjusted to pH 2.5 with HCl 1N. The mixtures were incubated at 37°C. Samples were taken at various times (0, 1, 2, 3 and 4 h), serially 10-fold diluted using diluent (Oxoid CM0733) at pH 7, and plated in duplicate onto MRS agar (Oxoid CM0361). The plates were incubated at 37°C for 48 h under anaerobic conditions (GasPak system; BBL Microbiology Systems, Cockeysville, Md.). After the incubation period, viable bacterial colonies were counted and the number of LAB was calculated according to the standard ISO 15214 (1998). The survival rate was calculated as the percentage of LAB colonies grown on MRS agar (Oxoid CM0361) compared to the initial bacterial concentration. Three tests, each in duplicate, were made for each strain. The pH of the broth was recorded at the beginning of the experiment (pH_{0h}) and at the end of the experiment (pH_{4h}).

Screening of dominant LAB strains for bile tolerance

The method described by Klingberg et al. (2005) was used. The bile

Table 2. Indicator bacteria strains and their origins (references).

Indicator bacteria strains	Codes	Origin (reference)
Bacillus cereus MADM 1291	Bc11	
Bacillus cereus MADM 1561	Bc14	
Bacillus cereus NVH391-98	Bc98	
Bacillus cereus 007525	Bc00	
Bacillus cereus F4810-72	BcF	Culture Collection of Department of Food Science, Food Microbiology in Copenhagen University, Denmark
Bacillus cereus NC7401	BcNC	
Bacillus cereus Ba18H2	BcPA	
Listeria monocytogenes 057	Lm1	
Listeria monocytogenes L028	Lm2	
Listeria monocytogenes Scott A	Lm3	
Micrococcus luteus SKN 624	Ml	
Salmonella infantis SKN 557	Si	
Salmonella oranienburg SKN 1157	So	
Salmonella nigeria SKN 1160	Sn	
Salmonella thompson SKN 565	Sth	
Salmonella typhimurium SKN 533	St1	
Salmonella typhimurium SKN 1152	St2	
Salmonella typhimurium SKN 1155	St3	
E. coli 81 nr. 149 SKN 541	Ec	
Yersinia enterocolitica 6A28 SKN 599	Ye1	
Yersinia enterocolitica 8A30 SKN 601	Ye2	

tolerance was examined in MRS broth (Oxoid CM0359) containing 0.3% (w/v) oxgall bile (Sigma-Aldrich, Pcode: 30209037, Steinheim, Germany). A volume of 100 µl of cell suspensions of LAB cultured for 18 h (approximately 10^7 CFU/ml) were inoculated into MRS broth (Oxoid CM0359) pH7 without bile and into MRS broth (Oxoid CM0359) containing 0.3% (w/v) oxgall bile (Sigma-Aldrich, Germany). The mixtures were incubated at 37°C. Samples were taken at various times (0, 1, 2, 3 and 4 h), serially 10-fold diluted using diluent (Oxoid CM0733) pH 7, and plated in duplicate onto MRS agar (Oxoid CM0361). The plates were incubated at 37°C for 48 h under anaerobic conditions (GasPak system; BBL Microbiology Systems, Cockeysville, Md.). After the incubation period, viable bacterial colonies were counted and the number of LAB was calculated according to the standard ISO 15214 (1998). The survival rate was calculated as the percentage of LAB colonies grown on MRS agar compared to the initial bacterial concentration. The pH of the broth was recorded at the beginning of the experiment (pH_{0h}) and at the end of the experiment (pH_{4h}). Three tests, each in duplicate, were made for each strain.

Screening for antimicrobial activity of LAB strains

For the detection of antimicrobial activity, the agar spot test described by Jakobsen et al. (1999) was carried out. A volume of 3 µl of cell suspensions of LAB cultured for 18 h was spotted on the surface of modified MRS agar containing only 0.2% glucose and 1.2% agar and incubated anaerobically (GasPak system; BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 30°C to develop the spots. The inhibitory effect of MRS was tested as negative control on each plate. 100 µl of cell suspensions of indicator microorganisms cultured for 18 h were mixed with 7 ml of soft MRS agar (0.7% agar) and poured over the plates. The plates were incubated aerobically at 37°C. After 24 h of incubation, inhibition zones were read. The presence of clear zone around the spot, which indicate an inhibition, was measured. The size of an inhibition zone (mm) was measured from the edge of the colony to the edge of the inhibition zone. Three tests, each in duplicate, were made for each strain.

RESULTS

Acid resistance

The effects of low pH (2.5) on the viability of the LAB strains are presented in Table 3. Considerable variations among the strains were observed. *E. faecium* L104 was the most acid sensitive of all the strains tested, losing its viability (<1 CFU/ml of viable cells) in less than 2 h at pH 2.5. The other sensitive strains which lost viability in acidic conditions were *E. faecium* strains L117, L134, L9 and *E. casseliflavus* strains L142 and L152. From all the tested strains, only *P. acidilactici* L87 survived over a period of 4 h at pH 2.5. A slight decrease in cell viability (96.2%) at pH 2.5 was observed with *P. acidilactici* L169. For *E. faecium* L154, the results showed a decrease in cell viability of around 28% within 4 h. MRS broth (Oxoid CM0359) pH 7 was used as positive control; as it is seen

Table 3. Acid resistance of dominant LAB isolated from traditional *maari*.

LAB strains		pH of MRS broth	Viable count [log (CFU/ml)]					Survival rate at 4 h (%)	pH of MRS broth	
			0 h	1 h	2 h	3 h	4 h		pH_{0h}	pH_{4h}
Enterococcus faecium	L9	7	6.00	6.46	7.15	8.08	8.53	142.17	7.03	6.21
		2.5	6.15	6.18	3.38	1.15	<1	0	2.51	2.51
	L104	7	6.30	6.46	7.15	8.08	8.53	135.40	7.02	5.53
		2.5	6.41	1.18	<1	<1	<1	0	2.53	2.43
	L117	7	6.30	6.46	7.45	7.72	7.81	123.97	6.99	5.72
		2.5	6.38	5.91	2.51	<1	<1	0	2.53	2.45
	L134	7	6.26	6.34	6.99	7.48	7.95	127.00	7.01	6.07
		2.5	6.15	4.41	1.26	<1	<1	0	2.52	2.51
	L154	7	6.23	5.94	6.30	6.95	7.73	124.08	6.97	5.82
		2.5	6.27	5.95	4.89	2.38	1.76	28.07	2.50	2.56
Enterococcus casseliflavus	L142	7	6.43	6.60	6.81	7.04	7.23	112.44	6.93	6.76
		2.5	6.30	6.11	5.48	< 1	< 1	0	2.50	2.55
	L152	7	6.28	6.36	6.76	7.30	7.54	120.06	6.99	6.83
		2.5	6.15	5.97	5.78	< 1	<1	0	2.52	2.59
Pediococcus acidilactici	L169	7	6.38	6.32	6.46	6.60	7.30	114.42	6.95	6.06
		2.5	6.32	6.11	6.15	6.11	6.08	96.20	2.51	2.50
	L87	7	6.34	6.97	6.82	6.98	7.48	117.98	7.01	6.80
		2.5	6.20	6.67	6.69	6.69	6.69	107.90	2.50	2.49

from Table 3, a good growth was observed for all the LAB strains tested in MRS broth pH 7 (Oxoid CM0359). From the beginning (0 h) to the end of the experiment (4 h), the pH varied from 2.43 to 2.59.

Bile tolerance

Table 4 shows the effect of bile on the growth of dominant LAB strains isolated from *maari*. The growth of lactobacilli in MRS broth without bile was used as a positive control (Table 3). All the tested strains showed full tolerance to 0.3% bile. These results indicate that bile at 0.3% didn't affect the viability of all LAB strains and all isolates grew in the presence of 0.3 % bile. After

Table 4. Bile tolerance of dominant LAB isolated from traditional *maari*

LAB strains	Codes	Viable count [log (CFU/ml)] in 0.3% oxgall					Survival rate at 4 h (%)	pH of MRS broth	
		0 h	1 h	2 h	3 h	4 h		pH_{0h}	pH_{4h}
Enterococcus faecium	L9	6.25	6.99	7.75	7.81	8.04	128.64	7.01	5.98
	L104	6.41	6.75	7.57	8.04	8.63	134.63	7.03	6.46
	L117	6.23	6.43	6.98	7.60	7.78	124.88	7.01	5.40
	L134	6.28	6.80	7.95	8.08	8.46	134.71	6.99	5.82
	L154	6.26	6.08	6.52	6.76	7.60	121.41	7.03	5.91
Enterococcus casseliflavus	L142	6.30	6.41	6.53	6.66	6.98	110.79	7.04	7.01
	L152	6.23	6.53	6.78	7.04	7.40	118.78	7.05	7.03
Pediococcus acidilactici	L169	6.28	6.30	6.26	6.34	7.34	115.77	7.01	6.70
	L87	6.11	6.67	7.08	7.26	7.34	120.13	7.03	6.93

exposure to bile, the greatest viability was found

Antimicrobial activity spectrum of dominant LAB

An agar spotting method was used to assess the antimicrobial properties of dominant LAB strains isolated from traditional *maari* against a panel of Gram negative and Gram positive microorganisms, which included food spoilage and pathogenic bacteria (Table 5). The antimicrobial properties were variable according to the LAB strains. The results showed that all *E. faecium* strains (L9, L104, L117, L134 and L154), *E. casseliflavus* strains (L142 and L152) and *P. acidilactici* strains (L87 and L169) were able to inhibit seven strains of *B. cereus*, seven strains of *Salmonella spp.*, one strain of *E. coli*, three strains of *Listeria monocytogenes* and one strain of *M. luteus*. Furthermore, it is interesting to note that *E. faecium* strains as well as *P. acidilactici* strains were, in general, more effective against the pathogenic microorganisms than *E. casseliflavus* strains as regards the inhibition zones displayed. Among the tested LAB strains, the greatest inhibition zone (16 mm) was against *B. cereus* 11, and the least (1.5 mm), was against *Salmonella thompson* strain *and L. monocytogenes Scott A* strain. However, no inhibition was observed against *Yersinia enterocolitica* strains. None of the LAB strains tested was active against *Yersinia* strains. No inhibitory effect of MRS on any of the pathogenic strains tested was observed. As seen in Figure 2, large and clear inhibition zones were obtained from *E. faecium* L154 and *P. acidilactici* isolates (L87 and L169) using the spot assay.

DISCUSSION

In order to select starter cultures for controlled fermentation, the nine dominant LAB strains isolated from traditional *maari* including five strains of *E. faecium*, two strains of *P. acidilactici* and two strains of *E. casseliflavus* were examined for their antimicrobial activities against indicator microorganisms, their resistance to low pH (2.5) as well as their tolerance to 0.3% bile.

The results show that all the tested LAB strains

Table 5. Antimicrobial activity spectrum of dominant *LAB* isolated from traditional *maari*

Indicators / LAB strains	*Enterococcus faecium*					*Enterococcus casseliflavus*		*Pediococcus acidilactici*	
	L9	L104	L117	L134	L154	L142	L152	L87	L169
Bacillus cereus 11	++	++	++	++	++++	++	+	++	++
Bacillus cereus 14	+++	++	++	++	++	+	+	+++	+++
Bacillus cereus 391-98	+++	+++	+++	+++	+++	++	++	+++	++
Bacillus cereus 007525	++	++	++	++	++	+	+	++	+
Bacillus cereus F 4810-72	++	++	++	++	++	+	+	+++	+
Bacillus cereus NC 7401	+++	++	++	++	++	+	+	+++	+
Bacillus cereus PA24	++	+	+	+	++	+	+	++	++
SKN 557 Salmonella infantis	++	++	++	++	++	+	+	++	+++
SKN 1157 *Salmonella oranienburg*	++	++	++	++	++	+	+	++	+++
SKN 1160 *Salmonella nigeria*	++	++	+	++	++	+	+	++	++
SKN 565 Salmonella thompson	++	++	++	++	++	+	+	++	++
SKN 533 Salmonella typhimurium	++	++	++	++	++	+	+	+++	++
SKN 1152 Salmonella typhimurium	+++	++	++	++	++	+	+	++	++
SKN 1155 Salmonella typhimurium	+++	++	++	++	++	+	+	++	++
SKN 541 E. coli 81 nr. 149	++	++	++	++	++	+	+	++	++
SKN 599 Yersinia enterocolitica 6A28	-	-	-	-	-	-	-	-	-
SKN 601 Yersinia enterocolitica 8A30	-	-	-	-	-	-	-	-	-
SKN 1018 Listeria monocytogenes 057	++	++	++	++	++	+	+	++	++
SKN 1009 Listeria monocytogenes L028	+	++	+	++	++	+	+	++	++
SKN 1014 Listeria monocytogenes Scott A	++	+	++	++	++	+	+	++	++
SKN 624 Micrococcus luteus	++	+	++	++	++	+	+	++	++

Diameters of inhibition zones (mm): each value is the average of 2 repeated measurements from 2 independently replicated experiments, -: no inhibition; +: 0< Inhibition Zone (IZ) ≤5mm; ++: 6mm≤ IZ≤10mm; +++: 11mm≤ IZ≤15mm; ++++: IZ≥16mm

demonstrated an antagonistic activity against a wide range of pathogenic bacterial strains. Similar results reported the ability of *E. faecium, E. casseliflavus* and *P. acidilactici* strains to display antibacterial activity against the strains of *B. cereus*, *Salmonella* spp., *E. coli*, *Listeria* spp. and *M. luteus* (Osmanagaoglu et al., 1998; Kučerová et al., 2009; Sukumar and Ghosh, 2010; Bhakta et al., 2010; Yuksekdag and Aslim, 2010). Moreover, the antilisterial activity of enterococci is well known and can be explained by a close phylogenetic relationship between *Enterococcus* spp. and *Listeria* spp. (Kučerová et al., 2009). Lactic acid bacteria 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). This study concluded that all the isolates used have no inhibitory effects regarding Gram-negative indicator species *Y. enterocolitica*. Similar results were reported by Yuksekdag and Aslim (2010) who showed that LAB strains were not able to inhibit *Y. enterocolitica*. Visser et al.,(1996) reported that the plasmid-encoded factor YadA contributes to the resistance of *Y. enterocolitica* to the killing by antimicrobial polypeptides.

In the present study, it was observed that *P. acidilactici* L87 survived over a period of four hours at pH 2.5 whereas a slight decrease was observed for *E. faecium* L169. *E. faecium* L154 decreased considerably at pH 2.5. Therefore,

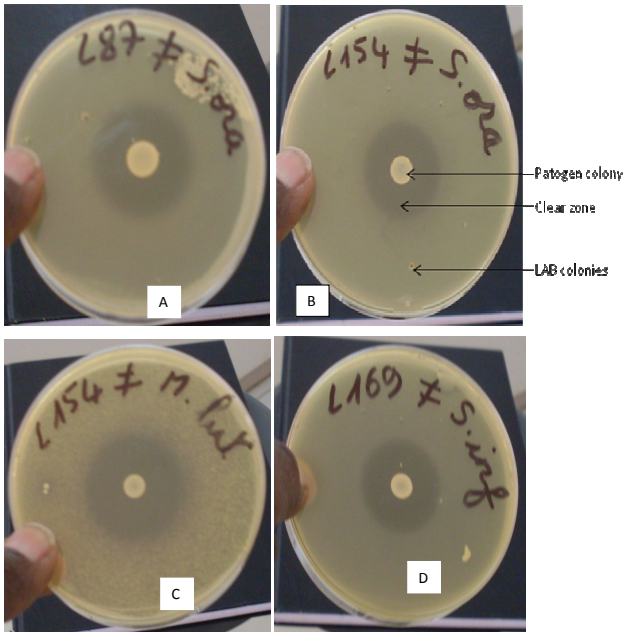

Figure 2. Spot assay illustrating the antagonistic activity of LAB strains against pathogenic indicators: A: Antagonistic activity of *Pediococcus acidilactici* L87 against SKN 1157 *Salmonella oranienburg.* B: Antagonistic activity of *Enterococcus faecium* L154 against SKN 1157 *Salmonella oranienburg.* C: Antagonistic activity of *Enterococcus faecium* L154 against *SKN 624 Micrococcus luteus*. D: Antagonistic activity of *Pediococcus acidilactici* L169 against *SKN 557 Salmonella infantis.*

these strains may be expected to survive acidic conditions that exist in stomach and intestinal juice. However, the exposure to pH 2.5 showed to be a discriminating factor with three out of the nine strains surviving after four hours of exposure. The fact that *P. acidilactici* L87 survived at pH 2.5 is in accordance with the results obtained by Erkkilä and Petäjä (2000). It has been recommended to use 0.3% bile as a suitable concentration for the selection of probiotics (Gilliland et al., 1984; Goldin et al., 1992). Even though several authors reported that this concentration of bile showed to be discriminatory (Chateau et al., 1994; Papamanoli et al., 2003), all the strains tested survived and grew in the presence of 0.3 % bile in the current study. Similar tolerances to 0.3% bile were reported among the strains tested by Sukumar and Ghosh (2010). It was considered that bile salt causes the increase in permeability of bacterial cell membranes, as the membranes are composed of lipids and fatty acids (Klayraung et al., 2008).

With regards to the results obtained, the use of *P. acidilactici strains* (L87 and L169) and *E. faecium* strain L154 is suggested to control the growth of undesirable microorganisms. This suggestion is strengthened by the fact that *P. acidilactici* and *E. faecium* strains have been already suggested as starter cultures for fermentations (Sukumar and Ghosh, 2010; de Castro et al., 2002; Leroy et al., 2003).

However, further characterizations have to be done to be sure of the harmlessness of *E. faecium* strain L154 because some strains of *E. faecium* can be pathogenic. Giraffa (2003) states that like other LAB, enterococci species may occasionally be involved in many nosocomial infections in hospitals, but that in spite of this, many strains are considered safe for use in foods. An example of such a strain is *E. faecium* K770, which was approved in 1996 in the United Kingdom for use in cultured dairy products.

Conclusion

The present study describes the antimicrobial activity, the acid resistance and bile tolerance of the dominant LAB

strains isolated from traditional baobab fermented seeds (*maari*). Two *P. acidilactici* strains (L87 and L169) and one *E. faecium* strain L154 exhibited antimicrobial activity against pathogenic bacteria such as *B. cereus* spp., *Salmonella* spp., *L. monocytogenes*, *E. coli* and *M. luteus* and they showed good ability to survive in acid and bile conditions. These properties make strains L87, L169 and L154 promising candidates to form part of a starter cultures for controlled fermentation of *maari,* as food safety concern. A consortium of starter cultures constituted by the selected *B. subtilis* strains (B3, B122 and B222, our previous study) and the selected LAB strains (L87, L169 and L154) can be proposed for controlled fermentation of *maari*; but the respective roles of these strains need to be understood during the fermentation. Then, it will be important to assess their performance under controlled fermentations and select the best strains for a consortium of starter cultures. Further characterizations have to be done to be sure of the harmlessness of *E. faecium* strain L154. Other studies including proteolytic, lipolytic, organoleptic properties should be associated to the final selection of the starter cultures.

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