International Journal of Food Science +Technology

Institute of Food Science +Technology ifst

Original article

Potential for prevention of non-O157 Shiga toxin-producing *Escherichia coli* contamination in traditionally fermented African maize gruel by fermentative probiotic *Lactobacillus plantarum*

Olanrewaju E. Fayemi, John R. N. Taylor & Elna M. Buys*

Department of Food Science, University of Pretoria, Private Bag X 20, Hatfield, 0028 Pretoria, South Africa

(Received 26 August 2016; Accepted in revised form 20 December 2016)

Summary Non-O157 Shiga toxin-producing *Escherichia coli* (STEC) are a frequent cause of STEC-related infections such as diarrhoea. Fermentation by presumptive probiotic *Lactobacillus plantarum* strain B411 isolated from cereal fermentation was investigated to prevent the growth of acid-adapted (AA) and non-acid-adapted (NAA) non-O157 STEC in traditionally fermented maize gruel, a widely used complementary food in Africa. *L. plantarum* strain B411 possessed probiotic characteristics and antimicrobial activity against selected pathogenic bacteria. Growth of AA and NAA non-O157 STEC strains was substantially inhibited by 3.6 and 4.8 log reductions, respectively, in the maize gruel fermented with the *L. plantarum* B411, while their growth was only inhibited by 1.0 and 1.2 log reductions, respectively, by traditional fermentation alone. Inclusion of fermentative strains of *L. plantarum* exhibiting probiotic activity is a feasible method to ensure safety of traditionally fermented African cereal porridges through inhibition of non-O157 STEC.

Keywords Acid adapted, *Lactobacillus plantarum*, maize gruel, non-acid adapted, non-O157 STEC, probiotic.

Introduction

Shiga toxin-producing non-O157 *E. coli* (non-O157 STEC) serotypes are increasingly recognised as emerging foodborne pathogens worldwide, and their occurrence could be five times higher than that of STEC O157:H7 (Gould *et al.*, 2013). Non-O157 STEC is associated with both outbreaks and individual cases of severe illness such as diarrhoea, haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Hughes *et al.*, 2006; Mathusa *et al.*, 2010; Bettelheim & Goldwater, 2014). Non-O157 STEC serotypes pose just as great risk to public health as *E. coli* O157:H7 (Gould *et al.*, 2013), but their occurrence and survival in traditional fermented foods have been under-reported in Africa.

Contaminated food is the main principal vehicle for the transmission of STEC to humans (Erickson & Doyle, 2007). Traditionally fermented maize porridges are the most commonly used complementary foods in many rural communities across Africa (Chelule *et al.*, 2010). The ability of pathogenic bacteria to survive in traditionally fermented foods has raised issues concerning their safety (Oguntoyinbo, 2014). It has been shown that spontaneous fermentation cannot inactivate STEC O157:H7 in traditional African fermented foods such as maize porridge (Kwaw, 2014) and maize beverage (Tadesse *et al.*, 2005). Non-O157 STEC serotypes are highly resistant to acidic stress and can tolerate the low pH of fermented food products (Elhadidy & Mohammed, 2013). Exposure of pathogenic *E. coli* to the acidic conditions (pH 4.5) such as that found in fermented foods can induce acid adaptation and alter the physiological characteristics of the bacteria (Rowan, 1999). Acid-adapted STEC cells are more resistant to gastric acid and can more easily initiate foodborne infections than non-adapted cells (Bergholz & Whittam (2007). The ability of STEC to adapt to low acidic conditions contributes to its low infectious dose (<100 organisms) (Thorpe, 2004; Yuk & Marshall, 2004).

Contamination of traditionally fermented complementary foods during processing is one of the frequent causes of infant diarrhoea episodes. Utilisation of well-defined LAB starter cultures for the fermentation of traditionally fermented foods could be an important avenue for the treatment of diarrhoea especially in the rural communities in Africa. This study investigated the potential of a presumptive probiotic *L. plantarum* strain

*Correspondent: E-mail: elna.buys@up.ac.za

doi:10.1111/ijfs.13373

B411 to prevent the growth of non-O157 STEC serotypes in traditionally fermented African maize gruel.

Materials and methods

Spontaneous fermentation of maize gruel

White maize grain was purchased at Oba market, Akure, Nigeria. After sorting and cleaning, the grains (400 g) were processed to fermented maize gruel by steeping in potable tap water (1 L) and spontaneously fermented for 72 h at 30 °C. The fermented steeped grain was then wet-milled using a Waring blender and sieved through muslin cloth (approx 300-μm pore size). The slurry was then transferred to a closed container to sediment and ferment further (sour) for 48 h at 30 °C to pH < 4.5.

Microbiological analysis of spontaneously fermented maize gruel

At 24-h intervals, the microbial population (cfu g^{-1}) of the total aerobic bacteria (TAC), lactic acid bacteria (LAB), Enterobacteriaceae and yeasts and moulds was determined using nutrient agar (NA) (Merck, Darmstadt, Germany), MRS (De Man *et al*., 1960), violet red bile glucose (VRBG) agar (Oxoid) and acidified potato dextrose agar (PDA) (Merck), respectively. The PDA was acidified with 10% w/v tartaric acid to pH 3.5 to inhibit the growth of bacteria. The NA and VRBG agar plates were incubated at 37 °C for 24 h. The yeast and mould plates were incubated at 25 °C for 3–5 days, and the MRS agar plates were incubated anaerobically using anaerobic jar together with Anaerocult system (Merck) at 37 °C for 48 h.

Characterisation and identification of dominant LAB in the maize gruel

The dominant LAB at different stages of traditional fermentation of the maize gruel were isolated and identified. Colonies were randomly picked from the highest dilution of MRS agar plates. The phenotypic characterisation such as cellular morphology, Gram staining, catalase reaction and motility test was performed as described by Collins *et al*. (1989). The LAB isolates were then identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). For strain classification, a score-oriented dendrogram was generated based on crosswise minimum spanning tree (MSP) matching using the standard settings of the MALDI Biotyper 3.0 software (Bruker Daltonics) to determine the relationship between the *L. plantarum* B411 and dominant LAB strains in maize gruel fermentation.

Probiotic potential of the *L. plantarum* strain used for the maize gruel fermentation

Twenty-five lactic acid bacteria isolated from traditional African cereal fermentations were screened for their probiotic potential (data not shown). Acid and bile tolerance was assayed according to Succi *et al*. (2005). Hydrophobicity as measured by the microbial adhesion to hydrocarbons (MATH), autoaggregation and coaggregation were determined as described by Kos *et al*. (2003). Of these, one *L. plantarum* strain (code B411) (obtained from the Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa) was selected on the basis of its superior performance among those screened and also in comparison with the literature data (Table 1).

Lactobacillus plantarum strain B411 was further evaluated for antimicrobial activity against non-O157 STEC serotypes and *E. coli* ATCC 25922 as described by Schillinger & Lucke (1989). Its adhesion to Caco-2 cells was assayed as described by Jacobsen *et al*. (1999) to confirm its applicable probiotic potential.

Acid adaption of non-O157 STEC strains

The presence of Shiga toxin 1 (*Stx* 1), Shiga toxin 2 (*Stx* 2) and intimin (*eae*) genes in the environmental non-O157 STEC strains used in this study had previously been determined (Aijuka *et al*., 2015). Stock cultures were stored in cryovial beads (Pro-Lab Diagnostic, Austin, TX) at −75 °C and were resuscitated in a tryptone soy broth (TSB) (Merck) for 18 h before inducing acid adaptation. The procedure of Buchanan & Edelson (1996) was used to prepare acid-adapted (AA) and non-acid-adapted (NAA) non-O157 STEC strains. Acid adaptation was induced in the non-O157 STEC strains by inoculating 1 mL working cultures into 100 mL TSB supplemented with 1% glucose (TSB+G) and with the pH adjusted to 4.5 (using 2 M lactic acid), while for NAA non-O157 STEC strains, TSB without glucose (TSB-G) buffered with 100 mM morpholino propanesulphonic acid (MOPS) to pH 7.4 was inoculated with 1 mL working cultures. Both were immediately incubated for 18 h at 37 °C. The viability of the AA non-O157 STEC was determined by plating on sorbitol MacConkey (SMAC) agar (Oxoid) and incubating at 37 °C for 24 h.

Preparation and inoculation of the maize gruel with presumptive probiotic *L. plantarum* strain and non-O157 STEC test strains

The three non-O157 STEC strains selected were serotyped: two were O138:K81 and the other was O83:K-. They were subjected to acid adaptation as described.

Table 1 Probiotic potential of *L. plantarum* strain B411 as determined by pH and bile salt tolerance, cell surface hydrophobicity (MATH), autoaggregation, coaggregation and adhesion to enterocyte-like Caco-2 cells in comparison with the literature data

			Microbial growth at low pH and subsequent exposure to 3% bile salt (Log 10 cfu mL^{-1})				Hydrophobicity (Microbial adhesion to hydrocarbon) %			Autoaggregation (%)			
			At pH 2.5		At 3% bile salt								
E. coil indicator serotype	Coaggregation (%)	Inhibition zone (mm)	0 h	2 h	3 h	7 h	Xylene	Chloroform	Ethyl acctatc	MRS	PBS	Adhesion to Caco-2 cell.	Reference
0138:K81	53.0^{a} ± 2.5	26.0^{b} ± 1.2	8.65 ± 0.10	6.51 ± 0.58	7.23 ± 1.03	7.59 ± 0.20	70.0 ± 3.5	50.0 ± 2.5	61.0 ± 1.4	92.0 ± 2.5	62.0 ± 3.1	68.0 ± 3.5	This study*
0138:K81	58.0^{ab} ± 1.2	29.0^{b} ± 0.8											
083:K-	64.0^{b} ± 2.8	28.0^{b} ± 1.2											
ATCC 25922	54.0^{a} ± 1.7	17.0^{a} ± 0.7											
L. rhamnosus GG		–	8.8	6.5	–	5.0	33.9	67.1	30.0	30.0	<30	–	Mirlohi *et al.*, 2009 & Pinto *et al.*, 2006;
		–	–	<4	<4	–	47	47	20	–	–	23.2	Xu *et al.*, 2009 & Succi *et al.*, 2005;
L. acidophilus LA-1 (against *E.* coli 0157:H7)		8.07	–	–	–	–	–	–	–	–	–	–	Gopal *et al.*, 2001;
L. acidophilus LA-1		–	–	–	–	–	–	–	–	–	–	17	Jacobsen *et al.*, 1999;
L. rhamnosus GG (ATCC 5310)		–	–	–	–	–	64.4	–	–	–	27.0	–	Collado *et al.*, 2008;
L. rhamnosus Lc-705 (Valio)		–	–	–	–	–	67.1	–	–	–	18.2	–	Collado *et al.*, 2008;
L. paracasei ATCC 25598		–	–	–	–	–	30	48	18	–	<40	11.9	Xu *et al.*, 2009

*Values are the means and standard deviations of three replicate experiments ($n = 3$).
Means with different superscripts in the same column are significantly different at $P \leq 0.05$.
– No data.

The cell suspensions were centrifuged at 5000 ***g*** for 15 min at 4 °C and suspended in 0.1% buffered peptone water. A cocktail of the three non-O157 STEC strains was then prepared by mixing 1 mL of each of the strains after centrifugation and standardised using McFarland Standard ampules (BioMerieux, Marcyl-l' Etoile, France) before inoculating the steeped maize grains to obtain final inoculum level at 10^6 cfu mL^{-1}.

The *L. plantarum* B411 stock culture was activated in MRS broth incubated at 37 °C for 18 h to obtain stationary-phase cells. The resulting cell suspension was then centrifuged at 5000 ***g*** for 15 min at 4 °C and standardised using McFarland Standard before inoculating the steeped maize grains to obtain final inoculum level at 10^6 cfu mL^{-1}. This level of inoculum is required for a fermentative organism/starter culture in a fermented food to outcompete or inhibit the growth non-O157 STEC.

Enumeration of LAB and non-O157 STEC strains during maize gruel fermentation

The survival of both AA and NAA non-O157 STEC strains was determined during maize gruel fermentation and processing by steeping maize grains in four separate closed containers. The first two containers were inoculated with presumptive probiotic *L. plantarum* plus either AA or NAA non-O157 STEC strains, while the other two containers were inoculated only with AA or NAA non-O157 STEC strains. Fermentation was at 30 °C for 72 h followed by other unit operations (Table 2). The non-O157 STEC strains and LAB were enumerated at 24-h intervals on SMAC and MRS agar media, respectively. SMAC agar plates were incubated at 37 °C for 24 h, while MRS agar plates were incubated as described. Titratable acidity (% lactic acid equiv.) and pH of the fermenting maize gruel were determined at 24-h intervals.

Statistical analysis

All experiments were performed three times, and the data were analysed using multifactor analysis of variance (ANOVA) for comparison between the treatments at each time interval. Fisher's least significant difference test (LSD) was used to determine significant differences between the treatments at $P \leq 0.05$.

Results and discussion

Microbial profile during maize gruel fermentation without inoculation with non-O157 STEC

The predominant microorganisms throughout the fermentation of the maize gruel were LAB, with $>10^8$ cfu g^{-1} at the end of the fermentation (Table 2). There was also a high level of yeasts and moulds in the later stages of the fermentation, $>10^7$ cfu g^{-1}. Lactic acid bacteria and yeasts had been identified as the most predominant microorganisms involved in the fermentation of maize gruel (Banwo *et al.*, 2012; Oyedeji *et al.*, 2013). High level of yeasts in the fermentation may be due to the amylolytic potentials of some of the yeast strains which enhance the breaking down of maize starch to simple sugars for other fermenting organisms such as LAB to use (Omemu *et al.*, 2007). Appreciable numbers of Enterobacteriaceae survived the fermentation ($>10^3$ cfu g^{-1}) despite the low pH 4.4 and high acidity 0.22% lactic equiv. The high level of Enterobacteriaceae in the final product confirms that traditionally fermented African maize-type foods could pose a health risk to consumers especially when used as a complementary food.

Table 2 Effect of processing steps on the microbiological profile of traditionally fermented African maize gruel without inoculation with non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC)

Fermentation steps	pH	Titratable acidity (%)	Total aerobic bacteria (log_{10} cfu g^{-1})	Lactic acid bacteria (log_{10} cfu g^{-1})	*Streptococcus* and *Lactococcus* (log_{10} cfu g^{-1})	Enterobacteriaceae (log_{10} cfu g^{-1})	Yeasts moulds and (log_{10} cfu g^{-1})
Steeping period (h)							
0	$6.11^{d} \pm 0.04$	$0.01^{a} \pm 0.00$	$2.67^{a} \pm 0.22$	$2.61^{a} \pm 0.27$	$2.18^{a} \pm 0.06$	$1.10^{a} \pm 0.46$	$2.66^{a} \pm 0.20$
24	$5.64^{c} \pm 0.04$	$0.16^{b} \pm 0.01$	$5.15^{b} \pm 0.18$	$4.58^{b} \pm 0.11$	$3.66^{b} \pm 0.11$	$4.45^{e} \pm 0.17$	$3.32^{b} \pm 0.26$
48	$4.86^{ab} \pm 0.04$	$0.34^{c} \pm 0.04$	$7.34^{c} \pm 0.29$	$7.69^{c} \pm 0.05$	$4.56^{cd} \pm 0.10$	$3.43^{cd} \pm 0.05$	$5.00^{c} \pm 0.56$
72	$4.62^{ab} \pm 0.12$	$0.38^{c} \pm 0.02$	$7.72^{c} \pm 0.60$	$8.80^{d} \pm 0.03$	$5.60^{e} \pm 0.10$	$3.25^{c} \pm 0.05$	$6.72^{d} \pm 0.51$
Souring period (h) after wet milling and sieving with fresh potable water							
0	$6.18^{d} \pm 0.03$	$0.11^{ab} \pm 0.00$	$5.38^{b} \pm 0.33$	$7.47^{c} \pm 0.35$	$4.33^{c} \pm 0.26$	$2.27^{b} \pm 0.17$	$3.41^{b} \pm 0.10$
24	$4.66^{ab} \pm 0.11$	$0.14^{b} \pm 0.04$	$7.41^{c} \pm 0.38$	$8.47^{d} \pm 0.08$	$4.74^{d} \pm 0.10$	$4.04^{de} \pm 0.24$	$6.54^{d} \pm 0.30$
48	$4.36^{a} \pm 0.02$	$0.22^{bc} \pm 0.04$	$7.65^{c} \pm 0.28$	$8.48^{d} \pm 0.28$	$5.41^{e} \pm 0.20$	$3.28^{c} \pm 0.47$	$7.41^{e} \pm 0.01$

Values are the means and standard deviations of three replicate experiments ($n = 3$).
Means with different superscripts in the same column are significantly different at $P \leq 0.05$.

Classification of LAB isolated during maize gruel fermentation

Comparative cluster analysis (dendrogram) for the *L. plantarum* strains isolated from the fermented maize gruel revealed two distinct clusters (A1 and A2) with an 80% degree of relatedness (Fig. 1). The *L. plantarum* strains at the later stages of the fermentation had higher degree of similarity than those isolated at the early stages of the fermentation. There was also high degree (90%) of relatedness between *L. plantarum* strain B411 and the *L. plantarum* strains in cluster A2.

Cluster analysis demonstrated that different strains of *L. plantarum* were involved in the fermentation of traditionally fermented African maize gruel. However, the high level of relatedness among the *L. plantarum* strains in the later stages of steeping and souring suggests that similar *L. plantarum* strains dominate the traditional fermentation of African maize gruel. This agrees with the work of Sanni *et al*. (2013) who found a high degree of similarity among the *L. plantarum* strains in spontaneous fermented maize gruel. The high level of similarity between *L. plantarum* strain B411 and the dominant *L. plantarum* strains isolated during fermentation of the maize gruel suggests that the strain was a suitable starter culture for effective fermentation of African maize gruel.

Probiotic potential of *L. plantarum* strain B411

The *L. plantarum* strain B411 had ability to coaggregate with non-O157 STEC strains and *E. coli* ATCC 25922 (Table 1). Further, the cell-free supernatant (CFS) of the *L. plantarum* B411 had antimicrobial activity against the pathogenic indicator strains. The most susceptible to antimicrobial activity of the *L. plantarum* B411 were non-O157 STEC strains, while the least inhibited was *E. coli* ATCC 25922. Additionally, the

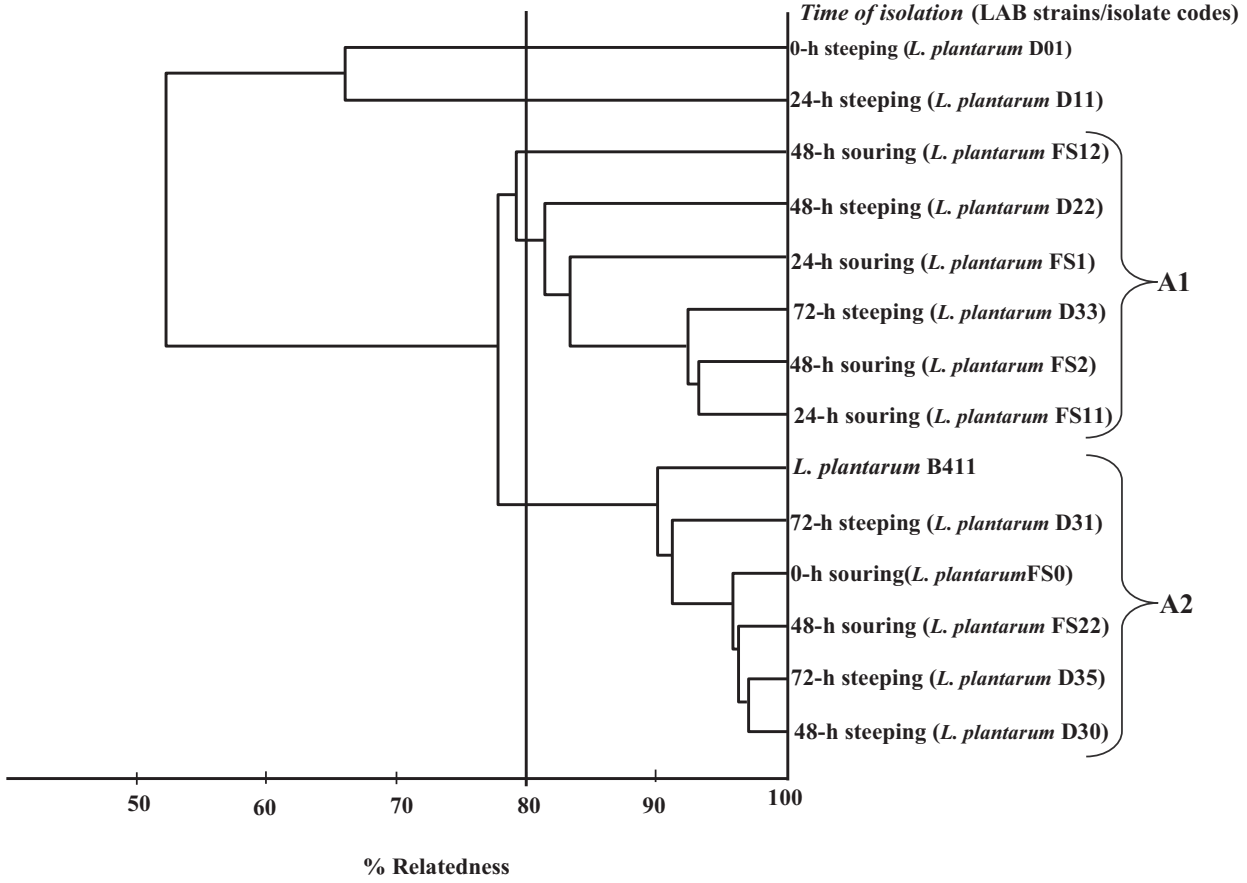

Figure 1 Score-oriented dendrogram showing genetic relationships between the dominant *L. plantarum* strains isolated during spontaneous fermentation of fermented African maize gruel and *L. plantarum* strain B411. The vertical line represents clusters of isolates that showed 80% strain similarity which was taken as the threshold for closely related isolates. *Isolate codes are next to the fermentation steps.* A1 and A2 indicate clusters of closely related *L. plantarum* isolates.

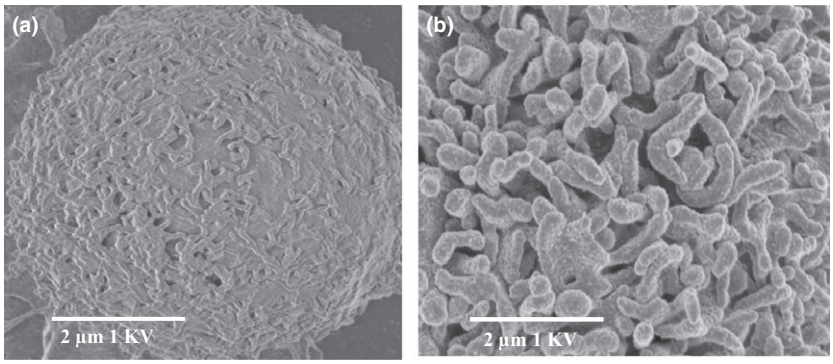

Figure 2 SEM showing (a) Caco-2 cells and (b) adherence of *L. plantarum* strain B411 to Caco-2 cells.

L. plantarum B411 exhibited a high level of tolerance at low pH and bile salt as well as high degree of hydrophobicity. It also demonstrated high level of autoaggregation and adhesion to enterocyte-like Caco-2 cells (Fig. 2).

Importantly, the levels of coaggregation of the *L. plantarum* B411 were higher than those reported for commercial probiotic *L. rhamnosus* GG, *L. rhamnosus* Lc-705 and *L. paracasei* ATCC 25598 (Collado *et al.*, 2008; Mirlohi *et al.*, 2009; Xu *et al.*, 2009). The ability of the *L. plantarum* B411 to coaggregate with potential gut pathogens indicates that it has a host defence mechanism against infection in the gastrointestinal tract (GIT), a property of probiotic bacteria (Kos *et al.*, 2003). Also importantly, the antimicrobial activity of the filtered and neutralised supernatant of the *L. plantarum* strain against non-O157 STEC and *E. coli* ATCC 25922 strains was not lost after treatment with catalase or adjustment of pH to 6.5 (Table 1). Further, the acid and bile tolerance of the *L. plantarum* B411 suggests its ability to survive through the acidic condition of the upper part of the gastrointestinal tract and exert its probiotic potential on the host. The adhesion to Caco-2 cells suggests that the *L. plantarum* B411 possessed the ability to competitively exclude the pathogen in the GIT by limiting the surface area available for the pathogen adhesion. Hence, the *L. plantarum* B411 was probably probiotic and was therefore used for fermentation of the maize gruel to complement the natural LAB flora which was dominated by similar stains of *L. plantarum*.

Growth of LAB and survival of AA and NAA non-O157 STEC during maize gruel fermentation

Lactic acid bacteria counts in the maize gruel fermented with *L. plantarum* B411 inoculated with AA and NAA non-O157 STEC were higher than those in the solely spontaneously fermented maize gruel by 1.4 and 1.2 $\log_{10}$ cfu g^{-1}, respectively, after 24 h of maize steeping (Table 3). This indicates that *L. plantarum* B411 actively grew and fermented the maize gruel. However, there was no significant difference ($P \geq 0.05$) in LAB growth in the fermented maize gruel inoculated with AA or NAA non-O157 STEC strains during the remaining period of fermentation.

Table 3 Growth of lactic acid bacteria during fermentation of maize gruel by spontaneous fermentation alone and in combination with *L. plantarum,* both in the presence of acid-adapted (AA) or non-acid-adapted (NAA) non-O157 STEC

	Traditionally fermented maize gruel inoculated with ($\log_{10}$ cfu g^{-1})		***L. plantarum* fermented maize gruel inoculated with ($\log_{10}$ cfu g^{-1})**	
Fermentation steps	**Acid-adapted non-O157 STEC**	**Non-acid-adapted non-O157 STEC**	**Acid-adapted non-O157 STEC**	**Non-acid-adapted non-O157 STEC**
Steeping period (h)				
0	$3.08^{a} \pm 0.60$	$3.22^{a} \pm 0.14$	$6.31^{b} \pm 0.09$	$6.28^{b} \pm 0.10$
24	$5.71^{a} \pm 0.57$	$6.08^{ab} \pm 0.12$	$6.86^{b} \pm 0.57$	$6.77^{b} \pm 0.68$
48	$8.76^{a} \pm 0.61$	$8.73^{a} \pm 0.55$	$8.48^{a} \pm 0.01$	$8.68^{a} \pm 0.13$
72	$8.70^{a} \pm 0.31$	$8.68^{a} \pm 0.24$	$8.83^{a} \pm 0.30$	$8.86^{a} \pm 0.41$
Souring period (h) after wet milling and sieving with fresh potable water				
0	$6.87^{a} \pm 0.75$	$6.92^{a} \pm 0.82$	$6.75^{a} \pm 0.52$	$7.19^{b} \pm 0.21$
24	$7.47^{a} \pm 0.60$	$7.85^{ab} \pm 0.51$	$7.70^{ab} \pm 0.19$	$8.14^{b} \pm 0.09$
48	$8.44^{ab} \pm 0.60$	$8.08^{a} \pm 0.40$	$8.76^{b} \pm 0.06$	$8.82^{b} \pm 0.40$

Values are the means and standard deviations of three replicate experiments ($n = 3$).
Means with different superscripts in the same row are significantly different at $P \leq 0.05$.

Table 4 Survival of acid-adapted and non-acid-adapted non-O157 STEC strains during the fermentation of maize gruel by spontaneous fermentation alone and in combination with *L. plantarum*

	Traditionally fermented maize gruel inoculated with (log_{10} cfu g^{-1})		*L. plantarum* fermented maize gruel inoculated with (log_{10} cfu g^{-1})	
Fermentation steps	**Acid-adapted non-O157 STEC**	**Non-acid-adapted non-O157 STEC**	**Acid-adapted non-O157 STEC**	**Non-acid-adapted non-O157 STEC**
Steeping period (h)				
0	$4.10^{a} \pm 0.25$	$4.22^{a} \pm 0.05$	$4.26^{a} \pm 0.27$	$4.23^{a} \pm 0.20$
24	$5.53^{ab} \pm 0.18$	$4.93^{a} \pm 0.42$	$5.43^{ab} \pm 0.13$	$6.09^{b} \pm 0.47$
48	$4.73^{ab} \pm 0.23$	$4.49^{a} \pm 0.04$	$4.23^{a} \pm 0.22$	$5.29^{b} \pm 0.11$
72	$3.62^{ab} \pm 0.24$	$3.48^{ab} \pm 0.15$	$3.15^{a} \pm 0.39$	$4.16^{b} \pm 0.24$
Souring period (h) after wet milling and sieving with fresh potable water				
0	$2.43^{b} \pm 0.20$	$2.26^{ab} \pm 0.12$	$1.95^{a} \pm 0.18$	$3.28^{c} \pm 0.13$
24	$3.15^{b} \pm 0.22$	$3.86^{c} \pm 0.15$	$2.27^{a} \pm 0.16$	$2.42^{a} \pm 0.22$
48	$4.57^{c} \pm 0.20$	$3.73^{b} \pm 0.07$	$1.80^{a} \pm 0.18$	$1.33^{a} \pm 0.25$

Values are the means and standard deviations of three replicate experiments ($n = 3$).
Means with different superscripts in the same row are significantly different at $P \leq 0.05$.

Acid-adapted and non-acid-adapted non-O157 STEC grew in the maize gruel fermented spontaneously increasing by 1.3 and 0.7 log_{10} cfu g^{-1}, respectively, and by 1.2 and 1.8 log_{10} cfu g^{-1} in the maize gruel with *L. plantarum* B411 within the first 24 h of fermentation (Table 4). However, AA and NAA non-O157 STEC were substantially inhibited by 3.6 and 4.8 log reductions, respectively, in the final maize gruel fermented with the *L. plantarum* B411 compared to their highest levels after 24 h steeping, while their growth was also inhibited in the spontaneously fermented maize gruel, but only by 1.0 and 1.2 log reductions, respectively. Thus, *L. plantarum* strain B411 was effective at inhibiting the growth of AA and NAA non-O157 STEC.

There was, however, no notable difference between the survival of AA and NAA non-O157 STEC in the maize gruel fermented spontaneously or with *L. plantarum* B411 throughout the fermentation. Hence, acid adaptation appeared not to protect the STEC against inhibition by the LAB. The reduction in pH and increase in TA in all the fermented maize gruel inoculated with either AA or NAA non-O157 STEC was not different from those obtained during the spontaneous fermentation in the absence of non-O157 STEC shown in Table 1. Therefore, the inhibition of AA and NAA non-O157 STEC in maize fermented spontaneously or with *L. plantarum* B411 may not be as a result of the reduction in pH or increase in the acidity. This suggests that both the *L. plantarum* B411 exhibited true probiotic activity against the non-O157 STEC. According to Gagnon *et al.* (2004), the antagonistic activity of probiotic bacteria against STEC O157 is more related to the ability of the probiotic strain to produce various antimicrobial substances than acid production. Further, Rund *et al.* (2013) attributed the growth inhibition of non-O157 STEC strains when co-incubated with the probiotic bacteria to the production of bacteriocins and not organic acids. The true probiotic effect of *L. plantarum* B411 was indicated by its probable production of bacteriocins (crude supernatant) with antimicrobial activity against non-O157 STEC strains (Table 1).

Conclusions

Natural fermentation of maize gruel brings about some inhibition of non-O157 STEC but not enough to ensure the safety of such traditional African fermented foods. However, fermentative strains of *L. plantarum* that are associated with cereal fermentation which exhibit probiotic attributes can substantially increase inhibition of non-O157 STEC. Therefore, utilisation of such presumptive probiotic starter cultures could help prevent the growth of this important emerging pathogen and ensure the safety of traditional African cereal gruels, which are used as complementary foods.

References

Aijuka, M., Charimba, G., Hugo, C.J. & Buys, E.M. (2015). Characterization of bacterial pathogens in rural and urban irrigation water. *Journal of Water and Health*, **13**, 103–117.

Banwo, K., Sanni, A., Tan, H. & Tian, Y. (2012). Phenotypic and genotypic characterization of lactic acid bacteria isolated from some Nigerian traditional fermented foods. *Food Biotechnology*, **26**, 124–142.

Bergholz, T.M. & Whittam, T.S. (2007). Variation in acid resistance among enterohaemorrhagic *Escherichia coli* in a simulated gastric environment. *Journal of Applied Microbiology*, **102**, 352–362.

Bettelheim, K.A. & Goldwater, P.N. (2014). Serotypes of non-O157 Shigatoxigenic *Escherichia coli* (STEC). *Advances in Microbiology*, **4**, 377–389.

Buchanan, R.L. & Edelson, S.G. (1996). Culturing Enterohaemorrhagic *Escherichia coli* in the presence and absence of glucose as a

simple means of evaluating the acid tolerance of stationary-phase cells. *Applied and Environmental Microbiology*, **62**, 4009–4013.
Chelule, P.K., Mbongwa, H.P., Carries, S. & Gqaleni, N. (2010). Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. *Food Chemistry*, **122**, 656–661.
Collado, M.C., Meriluoto, J. & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology*, **226**, 1065–1073.
Collins, C.H., Lyne, P.M. & Grange, J.M. (1989). *Collins and Lyne's Microbiological Methods*, 6th edn. London: Butterworths.
De Man, J., Rogosa, D. & Sharpe, M.E. (1960). A medium for the cultivation of *Lactobacilli*. *Journal of Applied Bacteriology*, **23**, 130–135.
Elhadidy, M. & Mohammed, M. (2013). Shiga toxin producing *Escherichia coli* from raw milk cheese in Egypt: prevalence, molecular characterization and survival to stress conditions. *Letters in Applied Microbiology*, **56**, 120–127.
Erickson, M.C. & Doyle, M.P. (2007). Food as a vehicle for transmission of Shiga toxin–producing *Escherichia coli*. *Journal of Food Protection*, **70**, 2426–2449.
Gagnon, M., Kheadr, E.E. & Le Blay, G. (2004). *In vitro* inhibition of *Escherichia coli* O157: H7 by bifidobacterial strains of human origin. *International Journal of Food Microbiology*, **92**, 69–78.
Gopal, P.K., Prasad, J., Smart, J. & Gill, H.S. (2001). *In vitro* adherence properties of *Lactobacillus rhamnosus* DR20 and *Bifidobacterium lactis* DR10 strains and their antagonistic activity against an Enterotoxigenic *Escherichia coli*. *International Journal of Food Microbiology*, **67**, 207–216.
Gould, L.H., Mody, R.K., Ong, K.L. *et al*. (2013). Increased recognition of non-O157 Shiga toxin producing *Escherichia coli* infections in the United States during 2000–2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathogens and Disease*, **10**, 453–460.
Hughes, J.M., Wilson, M.E., Johnson, K.E., Thorpe, C.M. & Sears, C.L. (2006). The emerging clinical importance of non-O157 Shiga toxin producing *Escherichia coli*. *Clinical Infectious Diseases*, **43**, 1587–1595.
Jacobsen, C.N., Nielsen, V.R., Hayford, A.E. *et al*. (1999). Screening of probiotic activities of forty-seven strains of *Lactobacillus* spp. by *in vitro* techniques and evaluation of the colonization ability of five selected strains in humans. *Applied and Environmental Microbiology*, **65**, 4949–4956.
Kos, B., Suskovic, J., Vukovic, S., Simpraga, M., Frece, J. & Matosic, S. (2003). Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology*, **94**, 981–987.
Kwaw, E. (2014). Effect of storage temperatures on the survival and growth of pathogens in semi preserved foods. *International Journal of Nutrition and Food Sciences*, **3**, 133–140.
Mathusa, E.C., Chen, Y., Enache, E. & Hontz, L. (2010). Non-O157 Shiga toxin–producing *Escherichia coli* in foods. *Journal of Food Protection*, **73**, 1721–1736.
Mirlohi, M., Soleimanian-Zad, S., Dokhani, S., Sheikh-Zeinodin, M. & Abghary, A. (2009). Investigation of acid and bile tolerance of native *Lactobacilli* isolated from faecal samples and commercial probiotics by growth and survival studies. *Iranian Journal of Biotechnology*, **7**, 233–240.
Oguntoyinbo, F.A. (2014). Safety challenges associated with traditional foods of West Africa. *Food Reviews International*, **30**, 338–358.
Omemu, A., Oyewole, O. & Bankole, M. (2007). Significance of yeasts in the fermentation of maize for ogi production. *Food Microbiology*, **24**, 571–576.
Oyedeji, O., Ogunbanwo, S.T. & Onilude, A.A. (2013). Predominant lactic acid bacteria involved in the traditional fermentation of fufu and ogi, two Nigerian fermented food products. *Food and Nutrition Sciences*, **4**, 40–46.
Pinto, M.V., Franz, C.M., Schillinger, U. & Holzapfel, W.H. (2006). *Lactobacillus* spp. with *in vitro* probiotic properties from human faeces and traditional fermented products. *International Journal of Food Microbiology*, **109**, 205–214.
Rowan, N.J. (1999). Evidence that inimical food preservation barriers alter microbial resistance, cell morphology and virulence. *Trends in Food Science and Technology*, **10**, 261–270.
Rund, S.A., Rohde, H., Sonnenborn, U. & Oelschlaeger, T.A. (2013). Antagonistic effects of probiotic *Escherichia coli* Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7. *International Journal of Medical Microbiology*, **303**, 1–8.
Sanni, A., Franz, C., Schillinger, U., Huch, M., Guigas, C. & Holzapfel, W. (2013). Characterization and technological properties of lactic acid bacteria in the production of "sorghurt", a cereal-based product. *Food Biotechnology*, **27**, 178–198.
Schillinger, U. & Lucke, F.K. (1989). Antibacterial activity of *Lactobacillus* sake isolated from meat. *Applied and Environmental Microbiology*, **55**, 1901–1906.
Succi, M., Tremonte, P., Reale, A. *et al*. (2005). Bile salt and acid tolerance of Lactobacillus rhamnosus strains isolated from Parmigiano Reggiano cheese. *FEMS Microbiology Letters*, **244**, 129–137.
Tadesse, G., Ashenafi, M. & Ephraim, E. (2005). Survival of *E. coli* O157:H7 *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella* spp. in fermenting Borde, a traditional Ethiopian beverage. *Food Control*, **16**, 189–196.
Thorpe, C.M. (2004). Shiga Toxin-Producing *Escherichia coli* Infection. *Clinical Infectious Diseases*, **38**, 1298–1303.
Xu, H., Jeong, H., Lee, H. & Ahn, J. (2009). Assessment of cell surface properties and adhesion potential of selected probiotic strains. *Letters in Applied Microbiology*, **49**, 434–442.
Yuk, H.G. & Marshall, D.L. (2004). Adaptation of *Escherichia coli* O157:H7 to pH alters membrane lipid composition, verotoxin secretion, and resistance to simulated gastric fluid acid. *Applied and Environmental Microbiology*, **70**, 3500–3505.