International Journal of Dairy Technology

SDT Society of Dairy Technology

doi: 10.1111/1471-0307.12340

ORIGINAL RESEARCH

Effect of *Lactobacillus plantarum* on the survival of acid-tolerant non-O157 Shiga toxin-producing *E. coli* (STEC) strains in fermented goat's milk

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The ability of goat's milk fermented with a Lactobacillus plantarum *strain B411, and in combination with commercial starter culture, to inhibit acid-adapted (AA) and non-acid-adapted (NAA) environmental non-O157 STEC strains was investigated. Acid-adapted and NAA non-O157 STEC strains were not inhibited in the* L. plantarum*-fermented goat's milk, while the goat's milk fermented with the combination of* L. plantarum *and starter culture inhibited AA more than NAA non-O157 STEC strains. Environmental acid-tolerant non-O157 STEC strains were not inhibited by* L. plantarum*, starter culture or combination of starter culture with* L. plantarum *unless they were subjected to prior acid adaptation such as backslopping.*

Keywords Acid adapted (AA), Non-acid adapted (NAA), Non-O157, STEC, *L. plantarum* B411, Goat's milk, Weaning food.

INTRODUCTION

Goat's milk plays an important role in nutrition and well-being of people in developing countries, where it provides basic nutrition and subsistence to rural people (Park and Haenlein 2007). It has higher digestibility, lower allergenic properties compared to cow's milk and also contains antibacterial characteristics (Haenlein and Wendorff 2006). Probiotics are defined as viable micro-organisms that following consumption with food have potential for improving the health and nutrition of the consumer (Gourbeyre *et al.* 2011). Bacterial probiotics include various species of *Lactobacillus*, *Bifidobacterium* and *Streptococcus*, as well as *Lactococcus lactis* and some *Enterococcus* species (Argyri *et al.* 2013).

Lactobacillus has a long history of safe use in food, and it plays a major role in fermented milk and other food products (Karska-Wysocki *et al.* 2010). Probiotics have been examined for their effectiveness in the prevention and treatment of a diverse spectrum of gastrointestinal disorders such as antibiotic-associated diarrhoea (Rolfe 2000). They have also been shown to aid in control of diarrhoea in children (McNaught and MacFie 2001), and the use of fermented milks containing *Lactobacillus rhamnosus* GG has been shown to shorten the duration of diarrhoea in infants (Marteau *et al.* 2001). Reduced incidence of diarrhoea was also reported in day care centres when *L. plantarum* was administered to the children (Vanderhoof 2000). Dairy fermented products, such as fermented milk and yoghurt including goat's milk (Argyri *et al.* 2013), have been regarded as the best matrices to deliver probiotics.

Goats have been regarded as a natural reservoir for both *E. coli* O157 and non-O157 shiga toxin-producing *E. coli* (STEC), and raw goat's milk may serve as a vehicle of such pathogen transmission (Rey *et al.* 2006). Non-O157 STEC strains have emerged as important foodborne pathogens worldwide (Wang *et al.* 2012), and the consumption of dairy products may represent an important route of non-O157 STEC infections in humans (Rangel *et al.* 2005). It has been shown that non-O157 STEC strains were not eliminated from lactic cheese made with raw goat's milk (Caro and Garcia-Armesto 2007) probably because they can tolerate the low pH of fermented food (Elhadidy and Mohammed 2013). This is because adaptation to acid by *E. coli* can significantly enhance their survival in acidic foods and alter other physiological characteristics of the cell (Rowan 1999).

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Non-O157 STEC infections may induce a range of illnesses from mild gastroenteritis to critical illnesses, including haemorrhagic colitis, haemolytic-uraemic syndrome (HUS) and death, either as sporadic cases or in outbreaks (Smith and Fratamico 2012). Although the survival of *E. coli* O157:H7 in fermented goat's milk (Dlamini and Buys 2009) and in yoghurt (Ogwaro *et al.* 2002) has been documented, there is paucity of information on the effect of *L. plantarum* on the non-O157 STEC strains in fermented goat's milk. Therefore, the aim of this study was to determine the effect of goat's milk fermented with a *L. plantarum* strain B411 on acid-tolerant non-O157 STEC strains from environmental sources.

METHODOLOGY

Source of the milk

Fresh Saanen goat's milk was sourced from the experimental farm of the University of Pretoria, Pretoria, South Africa. The goats were milked using standard milking machines under appropriate hygienic condition. The milk was collected in 1-L sterile Schott bottles immediately after milking and transferred to the laboratory within 30 min. Six portions (100 mL each) were then supplemented with skim milk (3%) (Oxoid, Basingstoke, UK) and gelatin (0.5%) (Davis, Gauteng, South Africa) for stability and pasteurised at 63 °C for 30 min.

Bacterial preparation and culture conditions

Acid adaption of the non-O157 STEC isolates

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). The stock cultures were stored in cryovial beads (Pro-Lab Diagnostic, Austin, TX) at −75 °C.

Seventeen (17) environmental non-O157 STEC strains were subjected to acid adaptation as follows; the non-O157 STEC strains were resuscitated in tryptone soy broth (TSB) (Merck, Darmstadt, Germany) for 18 h before inducing acid adaptation and subsequently acid tolerance. The working cultures were prepared by inoculating 1 mL of the resuscitated cultures into 100 mL of TSB buffered with 100 mM morpholino propanesulphonic acid (MOPS) (Merck) to pH 7.4 and incubated at 37 °C for 18 h. The procedure of Buchanan and Edelson (1996) was then 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 of the working cultures into 100 mL of TSB supplemented with 1% glucose (Merck) (TSB+G) and with the pH adjusted to 4.5 (using 2 M lactic acid). The TSB+G was held at 37 °C in a water bath shortly before inoculation with non-O157 STEC strains. While for NAA non-O157 STEC strains, TSB without glucose (TSB-G) buffered with 100 mM MOPS with a pH 7.4 was inoculated with 1 mL of the working cultures. Both were immediately incubated for 18 h at 37 °C. The viability was determined by plating on Sorbitol MacConkey (SMAC) agar (Oxoid), and the plates were incubated at 37 °C for 24 h.

Acid tolerance of the non-O157 STEC test strains

After acid adaptation for 18 h, 8 non-O157 STEC strains with high acid adaptation potential were selected and exposed to lethal acid shock. Cells were harvested by centrifugation at 1095 *g* for 15 min at 4 °C and resuspended in fresh brain–heart infusion (BHI) broth (Oxoid) previously acidified to pH 2.5 using 2 M lactic acid and then incubated at 37 °C for 2 h. The viability was determined after 0, 60, 90 and 120 min of exposure to lethal acid shock by plating appropriate dilutions on SMAC agar (Merck), incubated at 37 °C for 24 h, and the percentages survival were calculated. After acid tolerance, strains MPU(W)8(3), MPU(W)9(1) and MPU(W)5(2) were then selected for this study. The selection was based on the strains that had more than 50% survival after exposure to lethal acid shock for 2 h. The MPU(W)8(3) and MPU(W)9(1) non-O157 STEC strains were serotyped as O138:K81, while the MPU(W)5(2) strain was serotyped as O83:K-.

Starter culture and *L. plantarum* strains

A commercial starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) (Cape Food Ingredients, Noordhoek, South Africa) and *L. plantarum* strain B411 (obtained from the Council for Scientific and Industrial Research [CSIR], Pretoria, South Africa) were used for this study. The probiotic characteristics of *L. plantarum* B411 had been determined (data not published). The *L. plantarum* B411 and starter stock culture were activated in MRS broth (De Man *et al.* 1960) incubated at 37 °C for 18 h to obtain stationary-phase cells. The 18-h cultures of *L. plantarum* B411 and starter culture were centrifuged at 1095 *g* for 15 min at 4 °C and standardised using McFarland Standard ampules (BioMerieux, Marcy l'Etoile, France) to obtain cells at 10^8 cfu/mL before suspending in the pasteurised and chilled goat's milk.

Inoculation of the goat's milk with non-O157 STEC strains

The 3 acid-tolerant non-O157 STEC strains (MPU[W]8[3], MPU[W]9[1] **and** MPU[W]5[2]) selected for the study were subjected to acid adaptation as previously described to obtain AA and NAA cells. After 18 h, the resulting cell suspensions were centrifuged at 1095 *g* for 15 min at 4 °C and suspended in 0.1% buffered peptone water (BPW) (Merck). A cocktail of the AA or NAA non-O157 STEC strains was standardised with McFarland Standard ampules

(BioMerieux) to obtain cells at final inoculum level 10^6 cfu/mL after suspending in the chilled goat's milk.

Fermentation of goat's milk and enumeration of lactic acid bacteria (LAB) and non-O157 STEC strains during survival studies

Goat's milk (100 mL) was inoculated with 10^6 cfu/mL of the commercial starter culture. The second portion (100 mL) of the pasteurised milk was inoculated with 10^6 cfu/mL of commercial starter culture in combination with the *L. plantarum* B411, while a third portion was only inoculated with *L. plantarum* B411 (10^6 cfu/mL). Each treatment was prepared in duplicate and inoculated with a cocktail of either AA or NAA non-O157 STEC strains to obtain final inoculum level 10^6 cfu/mL and incubated at 30 °C for 6 h. The inoculation of non-O157 STEC strains was performed when the pH of the milk reached 4.5. The non-O157 STEC strains and lactic acid bacteria (LAB) were enumerated at 0, 2, 4 and 6 h of incubation on SMAC and MRS agar, respectively. The SMAC agar plates were incubated at 37 °C for 24 h, while MRS agar plates were incubated anaerobically using anaerobic jar together with anaerocult system (Merck) at 37 °C for 48 h.

Changes in the pH during the survival of AA and NAA non-O157 STEC strains in the fermented goat's milk

The changes in the pH of the fermented goat's milk were determined using a Digital pH meter, Hanna pH meter 211 (Hanna instruments, USA).

Statistical analysis

Multifactorial analysis of variance (ANOVA) was used to determine whether factors such as fermentation treatment, acid adaptation and time affected the survival and growth of non-O157 STEC strains significantly (at 5% level of significance). All samples were analysed with Statistica software for Windows, version 12 (Stat-soft, Tulsa, OK).

RESULTS

Acid tolerance of the non-O157 STEC strains

There were significant ($P < 0.05$) differences in the level of survival of non-O157 STEC strains that were challenged at pH 2.5. All the strains exhibited acid adaptation at higher pH 4.5 (data not shown) but after 120 min of exposure at pH 2.5, three of the strains did not survive while the percentage survival of the remaining strains ranged between 29% and 57% (Table 1).

Effect of *L. plantarum* B411 on AA and NAA non-O157 STEC strains in fermented goat's milk

The goat's milk fermented with the *L. plantarum* B411 did not inhibit the growth of either AA or NAA non-O157 STEC strains. The initial counts of AA non-O157 STEC strains in the goat's milk fermented with the *L. plantarum* B411 increased from 5.3 ± 0.3 $\log_{10}$ cfu/mL to 6.8 ± 0.1 $\log_{10}$ cfu/mL after 6 h of incubation. Similarly, the viable counts of the NAA non-O157 STEC strains in the goat's milk fermented with *L. plantarum* B411 also increased significantly from 5.6 ± 0.2 $\log_{10}$ cfu/mL to 6.6 ± 0.1 $\log_{10}$ cfu/mL after 4 h of incubation and then remained constant up to 6 h (Figure 1). The presence of acid-adapted cells had no substantial effect on the pH as there was no notable difference in the pH of *L. plantarum* B411-fermented goat's milk inoculated with AA or NAA non-O157 STEC strains (Figure 2). The initial pH of 5.7 for both AA and NAA non-O157 STEC strains decreased to pH 5.4 and 5.5, respectively, after 6 h of incubation at 30 °C.

Table 1 The acid tolerance of acid-adapted non-O157 STEC strains in brain–heart infusion (BHI) broth at pH 2.5 and the percentage of survival after 2 h of exposure at 37 °C

	Microbial count (log_{10} cfu/mL)				
Strain	*0 min*	*60 min*	*90 min*	*120 min*	*% Survival after 120 min*
MPU(W)8(3)	6.60 ± 0.08^{a}	5.42 ± 0.01^{ab}	4.19 ± 0.01^{b}	3.28 ± 0.04^{d}	50 ± 3
MPU(W)9(3)	6.66 ± 0.01^{a}	5.64 ± 0.06^{ab}	3.11 ± 0.04^{a}	2.26 ± 0.03^{b}	34 ± 2
MPU(W)8(4)	6.73 ± 0.08^{a}	4.05 ± 0.07^{c}	nd	nd	0
MPU(W)5(3)	6.12 ± 0.01^{a}	5.60 ± 0.08^{ab}	3.31 ± 0.10^{a}	1.80 ± 0.03^{a}	29 ± 3
NW(W)5(1)	6.67 ± 0.02^{a}	4.31 ± 0.03^{cd}	nd	nd	0
MPU(W)9(1)	6.80 ± 0.06^{a}	5.30 ± 0.10^{a}	4.68 ± 0.10^{b}	3.89 ± 0.07^{c}	57 ± 3
MPU(W)5(7)	6.75 ± 0.02^{a}	6.04 ± 0.10^{b}	3.12 ± 0.10^{a}	nd	0
MPU(W)5(2)	6.84 ± 0.04^{a}	5.00 ± 0.03^{a}	4.49 ± 0.05^{b}	3.70 ± 0.10^{c}	54 ± 3

Values are the means and standard deviations of three replicate experiments ($n = 3$).

Means with different superscript in the same column are significantly different at $P < 0.05$.

nd, not detected.

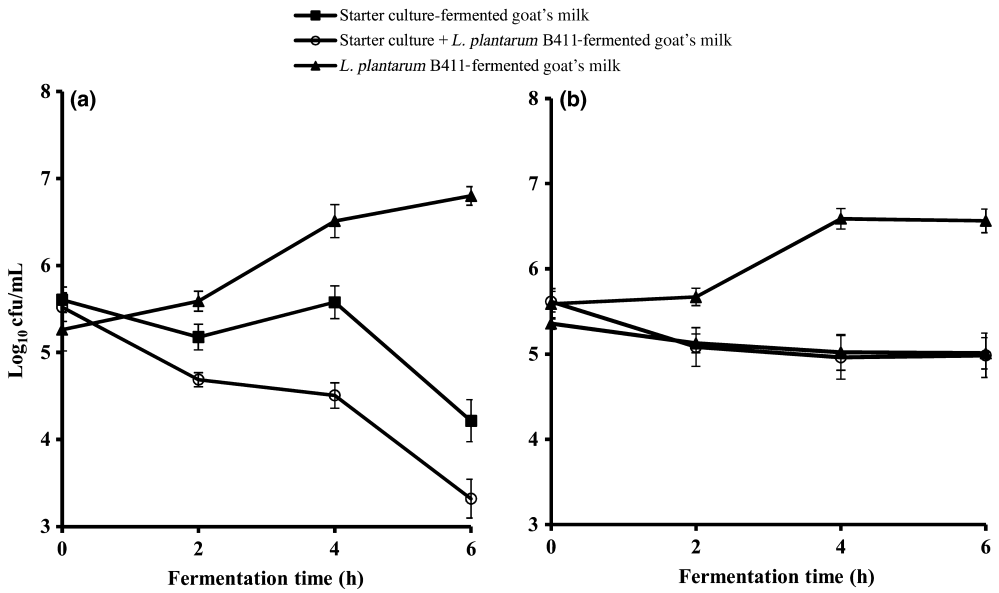

Figure 1 The effect of goat's milk fermented with *L. plantarum* B411, starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarus* subsp. *thermophilus)* and combination of starter culture with *L. plantarum* B411 for 6 h at 30 °C on (a) survival of acid-adapted and (b) non-acid-adapted environmental acid-tolerant non-O157 STEC strains. Results are expressed as mean ± standard deviation (n = 3).

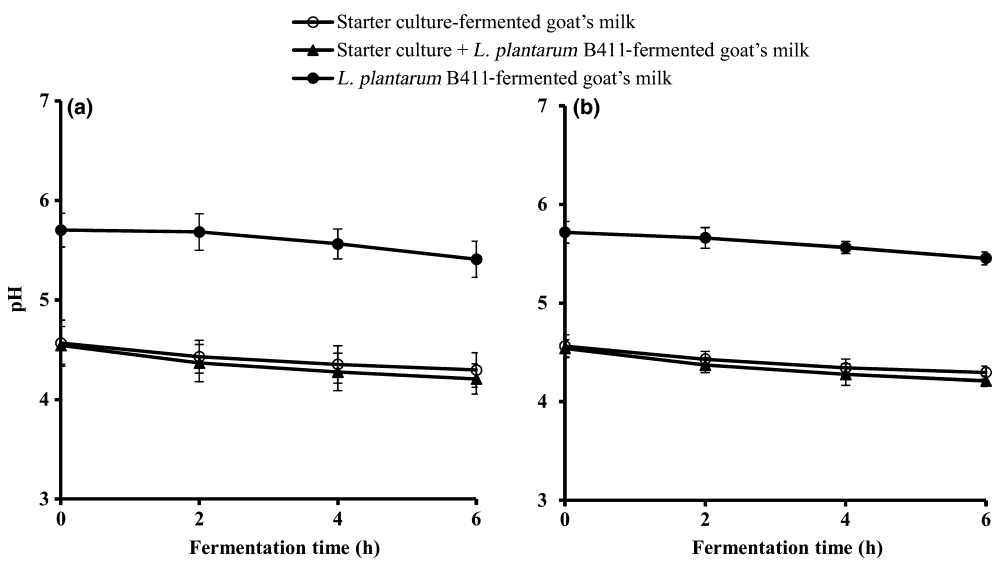

Figure 2 Changes in the pH during the fermentation of goat's milk with starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarus subsp. thermophilus)*, *L. plantarum* B411 and combination of starter culture and *L. plantarum* B411 for 6 h at 30 °C, inoculated with acid-adapted (a) or non-acid-adapted (b) environmental acid-tolerant non-O157 STEC strains. The pH of the goat's milk fermented with starter culture and combination of starter culture with *L. plantarum* B411 at the point of inoculating the non-O157 STEC strains was 4.5. Results are expressed as mean ± standard deviation (n = 3).

Effect of starter culture on AA and NAA non-O157 STEC strains in fermented goat's milk

Acid adaptation had a significant ($P < 0.05$) effect on the on the survival of the non-O157 STEC strains in the goat's milk fermented with the starter culture. The AA non-O157 STEC strains in goat's milk fermented with the starter culture decreased significantly ($P < 0.05$) from 5.6 ± 0.1 $\log_{10}$ cfu/mL to 4.2 ± 0.2 $\log_{10}$ cfu/mL after 6 h. However, the counts of NAA non-O157 STEC strains in the goat's milk fermented with the starter culture only decreased by 0.4 $\log_{10}$ cfu/mL after 4 h of inoculation and then remained constant up to 6 h (Figure 1). Similar to that which was observed in the goat's milk fermented with only *L. plantarum* B411, the presence of acid-adapted cells had no

substantial effect on the pH of the starter culture-fermented goat's milk inoculated with AA or NAA non-O157 STEC strains. The pH declined from 4.6 to 4.3 after 6 h for both starter culture-fermented goat's milk inoculated with AA and NAA non-O157 STEC strains (Figure 2).

Effect of starter culture combined with *L. plantarum* strain B411 on AA and NAA non-O157 STEC strains in fermented goat's milk

The addition of *L. plantarum* B411 and acid adaptation had a significant ($P < 0.05$) effect on the survival of non-O157 STEC strains in the goat's milk fermented with the combination of the starter culture and *L. plantarum* B411 after 6 h of exposure. A significant ($P < 0.05$) reduction from 5.5 ± 0.2 $\log_{10}$ cfu/mL to 3.3 ± 0.2 $\log_{10}$ cfu/mL was recorded for the counts of AA non-O157 STEC strains after 6 h of inoculation, while the counts of NAA non-O157 STEC strains only decreased by 0.5 $\log_{10}$ cfu/mL after 6 h in the goat's milk fermented with the combination of starter culture and *L. plantarum* B411 (Figure 1). The reduction in the pH was similar to that recorded in the goat's milk fermented with the starter culture. The initial pH 4.6 decreased to 4.2 after 6 h, for goat's milk fermented with the combination of starter culture and *L. plantarum* B411, inoculated with AA or NAA non-O157 STEC strains (Figure 2).

Enumeration of LAB in fermented goat's milk inoculated with either AA or NAA non-O157 STEC strains

Fermentation of the goat's milk with starter culture in combination with the *L. plantarum* B411 had a significant ($P < 0.05$) effect on the LAB counts. The LAB counts in the goat's milk fermented with only *L. plantarum* B411 inoculated with NAA non-O157 STEC strains increased from 7.7 ± 0.3 $\log_{10}$ cfu/mL to 8.4 ± 0.3 $\log_{10}$ cfu/mL after 6 h of inoculation and incubation at 30 °C. The LAB counts in the *L. plantarum* B411-fermented goat's milk inoculated with AA non-O157 STEC strains also increased from 7.7 ± 0.2 $\log_{10}$ cfu/mL to 8.3 ± 0.2 $\log_{10}$ cfu/mL after 4 h and remained constant after 6 h of incubation (Figure 3). Similarly, there was no difference in the LAB counts of the goat's milk fermented with starter culture inoculated with AA or NAA non-O157 STEC strains. The LAB counts in the goat's milk fermented with the starter culture inoculated with AA and NAA non-O157 STEC strains increased slightly from 8.5 ± 0.2 $\log_{10}$ cfu/mL to 8.6 ± 0.2 $\log_{10}$ cfu/mL and from 8.4 ± 0.3 $\log_{10}$ cfu/mL to 8.6 ± 0.3 $\log_{10}$ cfu/mL, respectively, after 6 h of inoculation and incubation at 30 °C (Figure 3). Similar to that which was observed in the goat's milk fermented with the *L. plantarum* B411 and starter culture, there was no notable difference between the LAB counts in the goat's milk fermented with the combination of the starter culture and *L. plantarum* B411, inoculated with AA or NAA non-O157 STEC strains. The LAB counts in the goat's milk fermented with the combination of the starter culture and *L. plantarum* B411 inoculated with AA and NAA non-O157 STEC strains decreased from 8.8 ± 0.2 $\log_{10}$ cfu/mL to 8.6 ± 0.3 $\log_{10}$ cfu/mL and from 8.8 ± 0.2 $\log_{10}$ cfu/mL to 8.6 ± 0.2 $\log_{10}$ cfu/mL, respectively, after 6 h of incubation at 30 °C (Figure 3).

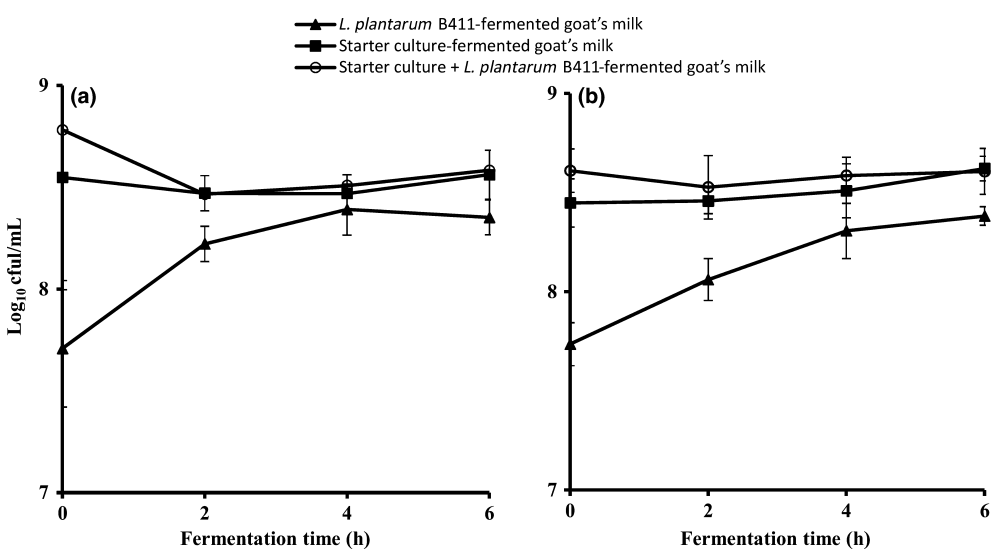

Figure 3 Lactic acid bacteria counts during the fermentation of goat's milk with *L. plantarum* B411, starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarus* subsp. *thermophilus*) and combination of starter culture with *L. plantarum* B411 inoculated with acid-adapted (a) or non-acid-adapted (b) environmental acid-tolerant non-O157 STEC strains for 6 h at 30 °C. Results are expressed as mean ± standard deviation ($n = 3$).

DISCUSSION

Acid tolerance potential of the non-O157 STEC strains

The survival of the 3 strains selected for this study at pH 2.5 for 2 h suggests the ability of these non-O157 STEC strains to pass through the stomach acidity barrier and possibly initiate infection (Gorden and Small 1993). These strains can be regarded as highly acid tolerant based on their survival at pH 2.5. This is in accordance with the study of Benjamin and Datta (1995) who grouped EHEC into highly acid-tolerant (50–100% survival), moderately acid-tolerant (10–50% survival) and slightly acid-tolerant strains (10% survival) based on their survival at pH 2.5. The observed variations in acid tolerance of the non-O157 STEC strains at pH 2.5 are in agreement with the findings of Duffy *et al.* (2000) who reported that the behaviour of *E. coli* cells under acidic conditions varied among the strains of pathogenic *E. coli*.

Survival of AA and NAA non-O157 STEC strains in the goat's milk fermented with *L. plantarum* strain B411

The goat's milk fermented with only *L. plantarum* B411 did not inhibit the growth of both AA and NAA non-O157 STEC strains. This could be attributed to the high pH and low acidification during exposure which enhanced adaption of non-O157 STEC strains. According to Dlamini and Buys (2009), high pH enhanced the survival of *E. coli* O157:H7 for 3 days in fermented goat's milk amasi. Kingamkono *et al.* (1995) reported that ETEC inoculated in lactic-fermenting food when the pH was >5 developed an acid tolerance response (ATR) system that protects them against severe acid stress for a long period. The study of Gran *et al.* (2003) on the survival of *E. coli* in fermented milk products revealed that high pH and slow acid production induced acid adaptation and enhanced acid tolerance of acid-adapted cells present in the inoculum from backslopping. Backslopping is a process in which small portion of a previous batch of fermented food is used to inoculate another batch of food to be fermented. The non-O157 STEC strains used in this study possess a high level of acid tolerance at low pH, hence explaining the inability of the *L. plantarum* B411-fermented goat's milk with higher pH to inhibit the growth of both AA and NAA non-O157 STEC strains. This may be because the AA non-O157 STEC strains have developed acid tolerance during prior adaption to acid at lower pH thereby enhancing their survival at higher pH of the *L. plantarum* B411-fermented goat's milk while the NAA non-O157 STEC strains adapted to the changing pH due to their ability to tolerate and grow at lower pH as reported for *E. coli* O157:H7 in fermented milk products (Dlamini and Buys 2009).

Survival of AA and NAA non-O157 STEC strains in the goat's milk fermented with the starter culture

The goat's milk fermented with the starter culture inhibited AA and NAA non-O157 STEC strains more than *L. plantarum* B411-fermented goat's milk. The study of Dineen *et al.* (1998) on the survival of *E. coli* O157:H7 in the yoghurt production process reported that starter culture appeared to synergistically reduce *E. coli* O157:H7 beyond the capability of either culture alone. Ogueke (2008) also reported that the inhibitory level exhibited by the commercial starter culture-fermented milk on clinical *E. coli* isolates was higher than by the milk fermented with a single strain of *Lactobacillus* spp. The variation in the level of inhibition in that study was attributed to the higher amounts of antibacterial metabolites produced by the starter culture than by the individual strain of *Lactobacillus* spp when used for the fermentation of milk products. However, the inhibition of AA more than NAA non-O157 STEC strains in the goat's milk fermented with the starter culture could be attributed to the production of antimicrobial compounds by the starter culture coupled with the effect of prior adaptation to acid. Hsin-Yi and Chou (2001) reported lower survival of a population of acid-adapted *E. coli* O157:H7 ATCC 43889 than the non-acid-adapted cells in a fermented milk drink after 48 h of exposure. Studies have shown that LAB starter cultures produce antimicrobials such as organic acids, bacteriocins, hydrogen peroxide, ethanol and diacetyl which have potential to inhibit the growth of pathogenic bacteria during acidic fermentation (Stern *et al.* 2006). Furthermore, acid adaptation of non-O157 STEC strains in this study was performed with lactic acid before inoculation into the fermented goat's milk. This could have also enhanced the susceptibility of AA non-O157 STEC strains to the inhibition by other organic acids apart from lactic acid produced during the fermentation of the goat's milk with starter culture. This is in accordance with the findings of Ryu and Beuchat (1998) who suggested that the response of acid-adapted cells depends on the type of acidulant used to induce acid adaptation. In that study, acid induction of *E. coli* O157:H7 was performed with lactic acid before inoculation into apple cider and orange juice and this was reported to enhance the susceptibility of adapted cells to inhibition by other organic acids apart from lactic acid.

Survival of AA and NA non-O157 STEC strains in the goat's milk fermented with starter culture combined with *L. plantarum* strain B411

The goat's milk fermented with the starter culture combined with *L. plantarum* B411 inhibited the growth of AA non-O157 STEC strains more than the goat's milk fermented with either starter culture or *L. plantarum* B411 alone. This could possibly be as a result of the starter culture enhancing the growth of the *L. plantarum* thereby resulting in the

production and accumulation of various antimicrobial compounds and the weakening effects of prior adaptation to acid (Timmerman *et al.* 2004). Acid adaptation has been reported to increase susceptibility of *E. coli* O157:H7 to the antimicrobials produced by LAB starter cultures (Hsin-Yi and Chou 2001). The study of Buchanan and Edelson (1996) revealed that acid adaptation did not enhance acid tolerance in an extremely acid-tolerant *E. coli* O157:H7 strain due to the weakening effects of cellular damage during acid adaptation which exceeded the protective effect of acid-shock proteins or other protective metabolic changes induced by low pH. According to Leyer *et al.* (1995), acid adaptation of *E. coli* O157:H7 resulted in injured or damaged cells while producing protective acid-shock proteins leading to inability to survive when exposed to further harsh acidic environment in the presence of other antimicrobial metabolites.

Similar to the results observed in the starter culture-fermented goat's milk, the NAA non-O157 strains survived more than the AA non-O157 STEC strains in the goat's milk fermented with the starter culture combined with *L. plantarum* B411 after 6 h of exposure. This can be attributed to the fact that the non-O157 STEC strains in this study possess a high level of acid tolerance at low pH and this could have possibly enhanced the survival of NAA non-O157 STEC strains during fermentation due to gradual adaptation to the changing pH. However, the sudden shift of the AA non-O157 STEC strains to normal optimum growth conditions followed by the subsequent demand to re-adapt resulted in failure to acquire maximum adaptation as reported by Dlamini and Buys (2009). According to Ryu and Beuchat (1998), regardless of prior adaptation to acidic environment, *E. coli* O157:H7 will again undergo physiological changes during subsequent exposure in response to other organic acids and antimicrobial compounds produced. A similar trend of survival was observed in NAA non-O157 STEC strains inoculated into the goat's milk fermented with only starter culture or starter culture combined with probiotic. This could be attributed to the similar decrease in pH and increase in acidification levels of the two fermented goat's milk samples. Hence, the rate of adaptation of NAA non-O157 STEC strains to acid during the fermentation of the goat's milk with starter culture or with the combination of the starter culture and *L. plantarum* B411 seemed similar.

CONCLUSION

This study showed that non-O157 STEC strains from environmental sources vary in their acid tolerance ability and the acid-tolerant strains may not be inhibited either by *L. plantarum*, commercial starter culture or a starter culture and *L. plantarum* combination. However, prior adaptation to acid enhance the susceptibility of environmental acid-tolerant non-O157 STEC strains to inhibition for instance during backslopping as practised during traditional fermentation of goat's milk. Therefore, traditional practices such as backslopping may contribute to the safety of traditional fermented weaning food from environmental acid-tolerant non-O157 STEC strains.

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