International Food Research Journal 26(6): 1733-1740 (December 2019)

Journal homepage: *http://www.ifrj.upm.edu.my*

Antimicrobial characteristics of lactic acid bacteria in African yam bean-based drink

*Olaniyi, O. I., Adeniran, H. A. and Abiose, S. H.

Department of Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Article history

Received: 9 October, 2017
Received in revised form: 26 August, 2019
Accepted: 5 September, 2019

Keywords

Fermented beverage
African yam bean Non-dairy
LAB
Food pathogens
Inhibitory

Abstract

Fermented drinks containing strains of *Pediococcus pentosaceus* and *Lactobacillus paracasei* which were inoculated with strains of *Bacillus cereus* and *Pseudomonas aeruginosa* were studied for antagonistic features of the lactic acid bacteria (LAB). Antimicrobial activities of LAB isolates from local fermented foods against selected foodborne pathogens: *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhosa* were tested *in vivo* using agar spot assay. LAB strains that demonstrated high antimicrobial activities were selected to ferment two types of milk blends while the survival of these pathogens were monitored *in vitro*. LAB strains isolated from the local fermented foods were phenotypically identified. These strains inhibited the growth of the selected foodborne pathogens except *Lactococcus lactis* and *L. paracasei*. The results also showed that *L. paracasei* exhibited 51.5% inhibition against *B. cereus* while *P. pentosaceus* exhibited 54%. Their combination was also highly effective in inhibiting the pathogens.

Introduction

Despite improved manufacturing standard and effective quality control at critical processing units, the outbreaks of foodborne illness remain a public concern (Tharmaraj and Shah, 2009). The contamination and growth of psychotropic and pathogenic spoilage microorganisms in refrigerated foods is indeed a major risk in food industry. Consequently, the use of naturally produced antimicrobial agents without any adverse effect on human health to inhibit the proliferation of pathogenic microorganisms in foods has become a major area of research (Stiles, 1996).

Lactic acid fermentation of food has been found to reduce the risk of pathogenic growth in foods (Sahlin, 1999). Lactic acid bacteria (LAB) also contribute to the taste and texture of fermented products and inhibit food spoilage bacteria by producing growth-inhibiting substances (bacteriocins) and large amounts of lactic acid. The major metabolic products of LAB are organic acids, lactic acid, bacteriocins and hydrogen peroxide. Beneficial effects conferred by LAB, including the inhibition of pathogenic Gram-positive and Gram-negative bacteria, have been described by Maragkoudakis *et al*. (2006) and Charlier *et al*. (2008). A number of studies have found that LAB consumption could be useful in the treatment of many types of diarrhoea, including antibiotic-associated diarrhoea in adults, travellers' diarrhoea, and diarrheal diseases in young children caused by rotaviruses. The most commonly studied LAB species in this area of study have been found to be *Lactobacillus GG*, *L. casei*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus* (Oksanen *et al*., 1990; Isolauri *et al*., 1991).

Substantiating the antimicrobial activities of LAB will affirm their use in the development of functional foods for the betterment of the health of the consuming public (Chuayana *et al*., 2003). The isolation of 60 LAB strains from fresh vegetables which were grown in Man-Rogosa-Sharpe (MRS) broth exhibited remarkable antimicrobial activity against some selected pathogenic bacteria such as *Escherichia coli*, *Salmonella* typhi, *Shigella dysenteriae*, *Bacillus anthracis*, and *Staphylococcus aureus* (Amin *et al*., 2009).

Local fermented foods such as *nunu* and *wara* are rich sources of LAB (Oyewole, 1997, Akabanda *et al*., 2010). Pal *et al*. (2005) isolated 10 LAB from appam (a south Indian special *dosa*) batter which exhibited

*Corresponding author.
Email: oibipejuolaniyi@gmail.com

good antibacterial activity against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*) and Gram-negative (*Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*) bacteria.

The exploitation of underutilised legumes such as African yam bean (AYB), which belongs to the family *Fabaceae*, sub-family *Papilionoideae*, tribe *Phaseoleae*, sub-tribe *Phaseolinae* and genus *Sphenostylis* (Okigbo, 1973), as a LAB carrier could increase the diversity of functional foods. The centre of diversity of AYB is only within Africa. Nigeria is a very significant AYB production (Potter, 1992) where extensive cultivation had been reported in the eastern (Abbey and Berezi, 1988), western and southern (Saka *et al*., 2004) regions. The essential amino acid proportion in the protein of AYB is over 32%, with lysine and leucine being predominant (Onyenekwe *et al*., 2000). The crude protein content in the grains is up to 23.97% (Fasoyiro *et al*., 2006). AYB has been reported to be rich in minerals such as potassium, phosphorus, magnesium, calcium, iron, zinc, and manganese, while the levels of sodium and copper are low (Chinedu and Nwinyi, 2011).The presence of some antinutrients such as alkaloids, flavonoids, saponins, lectin, trypsin inhibitors, phytate, and oxalate have also been reported in the seeds of AYB (Asuzu and Undie, 1986; Ajibade *et al*., 2005).

The present work focused on the isolation and identification of LAB from *wara*, *nunu*, fermented sorghum, maize, and millet, and subsequent inoculation of sterile milk blends of AYB, coconut, and soybean milk with the isolated strains. The inhibitory effect of the LAB isolates was assessed *in vitro* and *in vivo* (in the LAB beverage) against two toxin-producing indicator microorganisms, *Pseudomonas aeruginosa* and *Bacillus cereus*. Their toxins are capable of surviving pasteurisation and ultra-high temperature treatment (de Victorica and Galván, 2001; Bartram *et al*., 2003). The present work thus aimed at incorporating health-promoting LABs in drinks for combatting food spoilage and poisoning.

Materials and methods

Materials

Maize (yellow and white varieties), sorghum (red and white varieties), millet used in *ogi* production, African yam bean (AYB), soybean, and coconut were purchased from a local market in Ile-Ife, while *nunu* and *wara* were purchased from a local market in Sabo. Strains of *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. typhosa* were obtained from the culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Isolation and identification of lactic acid bacteria from fermented foods

LABs were isolated from the supernatant of *ogi*, *wara* and *nunu*. Serial dilution of each product was carried out. The tubes were each shaken thoroughly for 10 s to ensure homogenisation. Each dilution (1.0 mL) was plated on MRS agar using the pour-plate technique (Harrigan and McCance, 1976; Harrigan, 1998). The poured molten agar was allowed to set, and the plates were inverted and incubated at 37 ± 2°C for 48 h. Pure isolates were identified according to the identification scheme and procedure described in Harrigan (1998). Gram reaction, catalase test, nitrate reduction, and other biochemical tests were performed to biochemically identify the isolates.

Antimicrobial screening of LAB against selected foodborne pathogens

Screening for antagonistic activity was carried out using the agar spot test. Five indicator strains were used to determine the spectrum of antimicrobial activity: two Gram-positive bacteria; *Staphylococcus aureus* and *Bacillus cereus* and three Gram-negative bacteria; *Escherichia coli*, *Salmonella typhosa*, and *Pseudomonas aeruginosa*. This was performed as described by Khay *et al*. (2011). LAB that demonstrated high antimicrobial activity against selected foodborne pathogens (zone of inhibition ≥ 10 mm) were selected for fermentation of milk blends 1:1:1 and 2:1:1 (AYBM:CM:SM) in a combination of bacilli and cocci.

Preparation of fermented milk from blends of AYB, coconut, and soybean milk

AYB milk (AYBM), coconut milk (CM), and soymilk (SM) were produced as described by Aminigo *et al*. (2007), Sanful (2009), and Udeozor *et al*. (2012), respectively. Milk from the plant produces were proportioned into 1:1:1 and 2:1:1 (AYBM:CM:SM), and then sterilised. Culture suspensions of selected LAB isolates were prepared and absorbance adjusted to 1.0 at 540 nm using a spectrophotometer (Omafuvbe *et al*., 2002) under aseptic condition. LAB strains were reactivated in skim milk (12% w/v) and inoculated into the sterilised milk as a combination of two strains at a ratio of 1:2 *Pediococcus:Lactobacillus*. Samples were fermented in an incubator at 45°C for 18 h.

Determination of the inhibitory effect of lactic acid

bacteria strains on food pathogens in fermented milk samples

Sterile sample bottles were filled with 27 mL of different fermented milk samples. Fermented milk samples were divided into four groups of 35 bottles each. The cell suspension of the pathogens was adjusted to an optical density of 1.0 (equivalent to 5 log CFU/mL) using a spectrophotometer.

Set A of the fermented milk samples were inoculated with 1 mL of 24 h old *Bacillus cereus* broth culture and homogenised for 10 s. These samples were stored at ambient temperature for 28 d and examined for counts of *B. cereus* (Ebhodaghe *et al*., 2012). At each sampling period, 1 mL of fermented milk sample was pipetted into 9 mL of 0.1% peptone diluent, mixed thoroughly and further serially diluted. *B. cereus* was enumerated using the pour plate method on egg yolk agar (EYA), incubated at 35 ± 2°C for 24 – 48 h. Colonies with clear zones were regarded as *B. cereus* and counted using a colony counter (Gallenkamp, UK).

Set B of the fermented milk samples were inoculated with 1 mL of 24 h old *Pseudomonas aeruginosa* broth culture and kept at ambient temperature for 28 d. These were observed weekly for count of *P. aeruginosa*. The dilution of each inoculated fermented milk samples were carried out, plated on *Pseudomonas* agar (PA) and incubated at 35 ± 2°C for 24 – 48 h. Colonies of *P. aeruginosa* were counted using a colony counter (Gallenkamp, UK).

The other two sets of fermented milk samples were sterilised at 121°C for 15 min at 15 psi in an autoclave and used as control. The third set was then inoculated with 1 mL of 24 h old broth culture of *B. cereus* and shaken thoroughly for 10 s. Samples were also kept at room temperature for a period of 28 d while they were examined every week for counts of *B. cereus*. The dilution of samples were done, plated on EYA and incubated at 35 ± 2°C for 24 – 48 h. Colonies of *B. cereus* were counted using a colony counter (Gallenkamp, UK).

The second set of sterilised fermented milk samples were inoculated with 1 mL of 24 h old broth culture of *P. aeruginosa*, homogenised for 10 s and kept at ambient temperature for a period of 28 d. Samples were examined for *P. aeruginosa* count every week. The dilution of each sample were carried out and plated on PA, incubated at 35 ± 2°C for 24 – 48 h. Colonies of *P. aeruginosa* were counted using a colony counter (Gallenkamp, UK).

Table 1. Morphological and biochemical characteristics of LAB isolates.

Test / Isolate	ML4	ML5	YM6	WM7	WM8	WS12	RS15	NN5	NN6	L3	L12	L27	L29
Gram's Reaction	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase Reaction	–	–	–	–	–	–	–	–	–	–	–	–	–
Nitrate Reduction	–	–	–	–	–	–	–	–	–	–	–	–	–
Growth at 15°C	+	+	+	–	+	–	–	+	–	+	+	+	+
Growth at 45°C	+	+	–	+	+	+	–	–	+	–	+	+	+
Growth in 4% NaCl	–	+	–	+	+	+	+	–	+	+	+	+	+
Growth in 6.5% Nacl	–	+	–	+	–	–	+	–	+	+	–	+	+
NH3 from Arginine Broth	+	+	–	+	+	+	+	–	+	–	+	–	+
Starch Hydrolysis	–	–	–	–	–	–	–	–	–	–	–	–	–
Citrate Utilisation	–	–	+	–	–	–	–	–	–	–	–	–	–
Methyl Red Test	+	+	–	+	+	+	+	–	–	–	+	+	+
Voges-Proskaeur Test	–	+	–	+	–	–	+	–	–	+	–	+	+
Acid from Glucose	+	+	-	+	+	+	+	+	+	-	+	+	+
Acid from Lactose	+	+	-	+	+	-	+	+	+	-	+	+	+
Acid from Sucrose	-	+	+	+	-	-	+	+	+	-	-	+	+
Acid from Maltose	+	+	-	+	+	+	+	-	-	-	+	+	+
Acid from Galactose	+	+	-	+	+	+	+	+	+	-	+	+	+
Acid from Arabinose	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from Mannitol	-	-	-	-	-	+	-	-	-	-	-	+	-
Acid from Salicin	-	-	-	+	+	+	+	-	-	-	+	-	+
Acid from Sorbitol	-	-	-	-	-	-	-	-	-	-	-	+	-
Acid from Trehalose	+	+	-	+	+	+	+	-	-	-	+	+	-
Acid from Xylose	-	-	-	-	-	-	-	-	-	-	+	+	+
Acid from Melibiose	-	-	-	-	-	-	-	-	+	-	-	+	-

+ = positive; - = negative. Probable identity of organisms: ML4: *Leuconostoc mesenteroides;* ML5: *Streptococcus faecalis*; YM6: *Lactococcus lactis*; WM7: *Pediococcus acidilactici*; WM8: *Leuconostoc paramesenteroides*; WS12: *Streptococcus* sp; RS15: *Pediococcus pentosaceus*; NN5: *Lactobacillus acidophilus*; NN6: *Lactobacillus delbrueckii;* L3: *Lactobacillus paracasei*; L12: *Lactobacillus casei*; L27: *Lactobacillus paracasei* and L29: *Lactococcus lactis*.

Results

Morphological and biochemical characteristics of the isolated microorganisms are shown in Table 1. Thirteen lactic acid bacteria isolates were obtained from *ogi*, *lafun*, *nunu* and *wara*. These isolates were identified as *Leuconostoc mesenteroides, Streptococcus faecalis, Lactococcus lactis, Pediococcus acidilactici, Leuconostoc paramesenteroides, Streptococcus sp., Pediococcus pentosaceus, Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus paracasei, and Lactobacillus casei.*

The antimicrobial activity of LAB isolates from local fermented foods and drinks against selected foodborne pathogens are shown in Table 2 and Figure 1. Isolates from dairy products NN5, NN6, L12, L27, and L29 demonstrated high antimicrobial activities against all selected food pathogens compared to those from fermented foods which inhibited Gram-negative pathogens rather than Gram-positive ones. Overall, 53% of the isolates showed antimicrobial activities against *Bacillus cereus* and *Staphylococcus aureus*, 76.9% inhibited *Pseudomonas aeruginosa* and *Escherichia coli* while 46% were active against *Salmonella typhosa*. Gram-positive pathogens were

Table 2. Antimicrobial spectra of LAB isolates against selected foodborne pathogens.

Isolate	*Bacillus cereus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhosa*
ML4	+ (6 mm)	-	+ (5 mm)	+ (12 mm)	-
ML5	+ (4 mm)	-	+ (< 1 mm)	+ (10 mm)	-
YM6	-	-	-	-	-
WM7	+ (16 mm)	+ (10.5 mm)	+ (11 mm)	+ (12 mm)	+ (11.5 mm)
WM8	+ (5 mm)	+ (9 mm)	+ (14 mm)	+ (12 mm)	-
WS12	-	-	-	-	+ (17 mm)
RS15	+ (10 mm)	+ (10 mm)	+ (18 mm)	+ (11 mm)	+ (15 mm)
NN5	-	+ (11 mm)	+ (5 mm)	+ (7 mm)	+ (9 mm)
NN6	-	-	+ (6 mm)	+ (11 mm)	+ (8 mm)
L3	-	-	-	-	-
L12	+ (19 mm)	+ (16.5 mm)	+ (17 mm)	+ (15 mm)	+ (10.5 mm)
L27	+ (15 mm)	+ (10 mm)	+ (16 mm)	+ (16 mm)	+ (24 mm)
L29	+ (15 mm)	+ (11 mm)	+ (13 mm)	+ (16.5 mm)	+ (20 mm)

+ = inibition present; - = no inhibition; Values in parenthesis indicate degree of inhibition. ML4: *Leuconostoc mesenteroides;* ML5: *Streptococcus faecalis*; YM6: *Lactococcus lactis*; WM7: *Pediococcus acidilactici*; WM8: *Leuconostoc paramesenteroides*; WS12: *Streptococcus* sp; RS15: *Pediococcus pentosaceus*; NN5: *Lactobacillus acidophilus*; NN6: *Lactobacillus delbrueckii;* L3: *Lactobacillus paracasei*; L12: *Lactobacillus casei*; L27: *Lactobacillus paracasei* and L29: *Lactococcus lactis*.

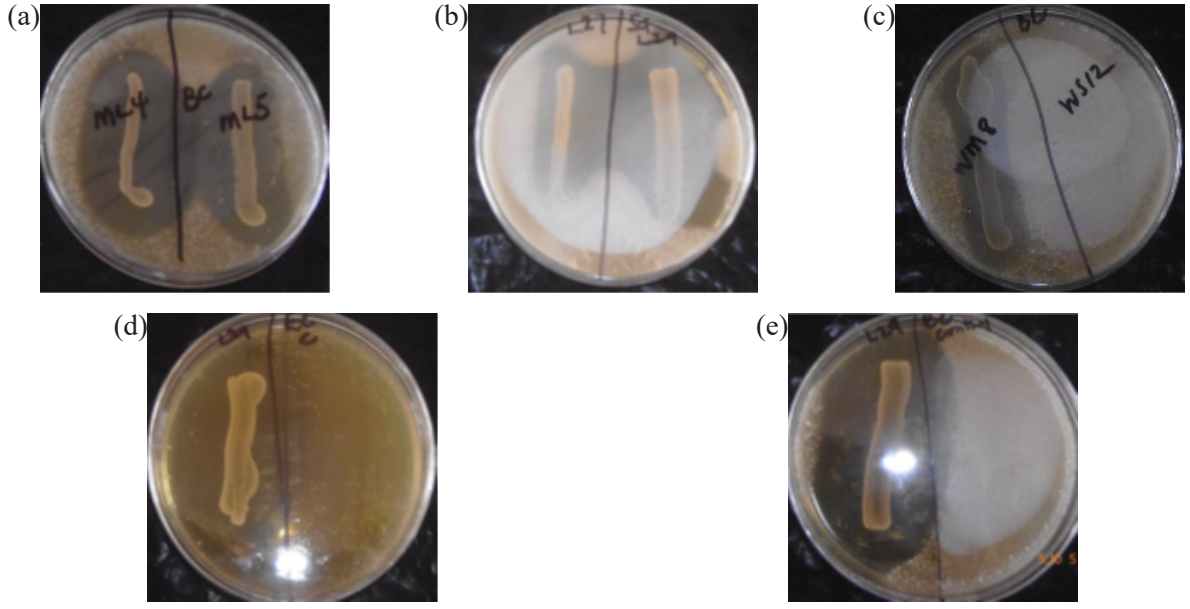

Figure 1. Antimicrobial activities of LAB against some foodborne pathogens: (a) antimicrobial activity of *Leuconostoc mesenteroides* ML4 and *Streptococcus faecalis* ML5 against *Bacillus cereus*; (b) antimicrobial activity of *Lactobacillus paracasei* L27 and *Lactococcus lactis* L29 against *Staphylococcus aureus*; (c) antimicrobial activity of *Leuconostoc paramesenteroides* WM8 and *Streptococcus* sp. WS12 against *Bacillus cereus*; (d) antimicrobial activity of *Lactococcus lactis* L29 against *Escherichia coli*; and (e) antimicrobial activity of *Lactococcus lactis* subsp. *lactis* L29 against *Bacillus cereus*.

Table 3. *Bacillus cereus* count (log CFU/mL) in fermented milk samples with and without LAB during storage.

Day of storage	AC	AR	ARC	BC	BR	BRC	AYBF	ACS	ARS	ARCS	BCS	BRS	BRCS	AYBFS
0	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.77 ± 0.07^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}	5.96 ± 0.05^{a}
7	6.40 ± 0.02^{a}	4.81 ± 0.01^{f}	5.91 ± 0.02^{b}	5.69 ± 0.03^{c}	5.48 ± 0.06^{d}	5.74 ± 0.02^{c}	5.18 ± 0.03^{e}	6.19 ± 0.13^{ab}	5.98 ± 0.20^{b}	5.96 ± 0.06^{b}	6.39 ± 0.14^{a}	6.02 ± 0.02^{ab}	5.98 ± 0.02^{b}	6.12 ± 0.01^{ab}
14	4.51 ± 0.03^{c}	4.58 ± 0.19^{bc}	3.98 ± 0.02^{d}	4.80 ± 0.02^{abc}	4.98 ± 0.03^{ab}	4.72 ± 0.02^{bc}	5.18 ± 0.25^{a}	6.60 ± 0.09^{a}	6.39 ± 0.01^{ab}	6.23 ± 0.01^{b}	6.40 ± 0.12^{ab}	6.23 ± 0.05^{b}	6.46 ± 0.04^{ab}	6.60 ± 0.06^{a}
21	3.76 ± 0.03^{ab}	4.27 ± 0.33^{a}	3.99 ± 0.01^{ab}	4.31 ± 0.01^{a}	4.45 ± 0.51^{a}	3.73 ± 0.03^{ab}	4.24 ± 0.02^{a}	6.90 ± 0.09^{a}	5.71 ± 0.05^{bc}	5.49 ± 0.04^{c}	5.85 ± 0.03^{b}	5.95 ± 0.05^{b}	5.89 ± 0.09^{b}	7.04 ± 0.10^{a}
28	3.18 ± 0.01^{c}	3.11 ± 0.07^{c}	3.14 ± 0.04^{c}	3.11 ± 0.05^{c}	3.52 ± 0.10^{b}	3.08 ± 0.05^{c}	3.60 ± 0.06^{a}	6.92 ± 0.02^{b}	6.17 ± 0.05^{de}	6.30 ± 0.03^{d}	6.20 ± 0.04^{de}	6.11 ± 0.01^{e}	6.57 ± 0.07^{c}	7.22 ± 0.02^{a}

Means with the same superscript within rows are not significantly different ($p > 0.05$). AC: equal proportion of milk blends fermented with *P. pentosaceus*; AR: equal proportion of milk blends fermented with *L. paracasei;* ARC: equal proportion of milk blends fermented with the combination of *L. paracasei* and *P. pentosaceus*; BC: milk blend proportion 2:1:1 fermented with *P. pentosaceus*; BR: milk blend proportion 2:1:1 fermented with *L. paracasei;* BRC: milk blend proportion 2:1:1 fermented with the combination of *L. paracasei* and *P. pentosaceus*; AYBF: 100% AYB milk fermented with commercial starter culture; ACS: equal proportion of milk blends fermented with *P. pentosaceus* without LAB; ARS: equal proportion of milk blends fermented with *L. paracasei* without LAB; ARCS: equal proportion of milk blends fermented with the combination of *L. paracasei* and *P. pentosaceus* without LAB; BCS: milk blend proportion 2:1:1 fermented with *P. pentosaceus* without LAB; BRS: milk blend proportion 2:1:1 fermented with *L. paracasei* without LAB; BRCS: milk blend proportion 2:1:1 fermented with the combination of *L. paracasei* and *P. pentosaceus* without LAB; AYBFS: 100% AYB milk fermented with commercial starter culture without LAB. Samples AC, AR, ARC, BC, BR, BCR, and AYBF contained live cultures of lactic acid bacteria and *Bacillus cereus* while ACS, ARS, ARCS, BCS, BRS, BCRS and AYBFS were sterilised after fermentation and then inoculated with *Bacillus cereus*.

inhibited by 61.5% of the LAB isolates while 81.6% of LAB isolates had antimicrobial effects against Gram-negative pathogens. Two isolates, YM6 and L3, had no antimicrobial effect on all the selected pathogens. Isolates WM7, RS15, L12, L27, and L29 exhibited high antimicrobial activities against both Gram-positive and Gram-negative pathogens.

Viable counts of *Bacillus cereus* in fermented milk samples with or without LAB strains and commercial starter culture, and stored at ambient temperature, are shown in Table 3. Fermented milk samples had an initial *B. cereus* population of 5.77 log CFU/mL. A reduction of 2.17 to 2.69 log CFU/mL was observed in all the samples after 28 d of storage as compared to an increment of 0.15 to 1.26 log CFU/mL observed in samples containing only the pathogen. Likewise, Table 4 shows the counts of *P. aeruginosa* with or without LAB in fermented milk samples and the growth pattern of LAB cultured with *P. aeruginosa* sequentially. Fermented milk samples had an initial viable count of 7.72 log CFU/mL of *P. aeruginosa*. Following 28 d storage, a range of 0.00 (ARC) to 4.17 (AC) (log CFU/mL) was observed.

Table 4. *Pseudomonas aeruginosa* count (log CFU/mL) in fermented milk samples with and without LAB during storage.

Day of storage	AC	AR	ARC	BC	BR	BRC	AYBF	ACS	ARS	ARCS	BCS	BRS	BRCS	AYBFS
0	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	7.72 ± 0.05^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}	6.36 ± 0.12^{a}
7	6.02 ± 0.02^{c}	5.59 ± 0.01^{e}	6.84 ± 0.02^{a}	6.39 ± 0.03^{b}	4.94 ± 0.04^{f}	5.72 ± 0.05^{d}	5.59 ± 0.01^{e}	6.85 ± 0.03^{a}	6.31 ± 0.01^{c}	5.73 ± 0.04^{e}	6.02 ± 0.02^{d}	6.68 ± 0.04^{b}	5.68 ± 0.02^{e}	6.65 ± 0.01^{b}
14	4.68 ± 0.03^{c}	4.32 ± 0.02^{d}	4.99 ± 0.01^{b}	4.30 ± 0.15^{d}	5.46 ± 0.06^{a}	4.02 ± 0.02^{e}	5.06 ± 0.01^{b}	6.98 ± 0.01^{b}	6.24 ± 0.00^{d}	6.06 ± 0.02^{e}	6.06 ± 0.01^{e}	7.64 ± 0.04^{a}	6.67 ± 0.03^{c}	6.12 ± 0.01^{e}
21	4.22 ± 0.01^{a}	4.04 ± 0.01^{b}	4.25 ± 0.01^{a}	4.05 ± 0.03^{b}	4.13 ± 0.10^{ab}	3.00 ± 0.01^{d}	3.57 ± 0.03^{c}	6.22 ± 0.04^{c}	6.54 ± 0.02^{b}	6.81 ± 0.05^{a}	6.52 ± 0.04^{b}	6.04 ± 0.03^{d}	6.02 ± 0.01^{d}	6.56 ± 0.10^{b}
28	4.17 ± 0.01^{a}	4.05 ± 0.01^{b}	0.00 ± 0.00^{g}	3.56 ± 0.04^{d}	3.78 ± 0.03^{c}	3.09 ± 0.04^{f}	3.38 ± 0.02^{e}	6.74 ± 0.06^{bc}	6.59 ± 0.02^{bc}	6.85 ± 0.01^{b}	6.68 ± 0.02^{bc}	9.35 ± 0.20^{a}	6.45 ± 0.10^{c}	6.62 ± 0.08^{bc}

Means with the same superscript within rows are not significantly different ($p > 0.05$). AC: equal proportion of milk blends fermented with *P. pentosaceus*; AR: equal proportion of milk blends fermented with *L. paracasei;* ARC: equal proportion of milk blends fermented with the combination of *L. paracasei* and *P. pentosaceus*; BC: milk blend proportion 2:1:1 fermented with *P. pentosaceus*; BR: milk blend proportion 2:1:1 fermented with *L. paracasei;* BRC: milk blend proportion 2:1:1 fermented with the combination of *L. paracasei* and *P. pentosaceus*; AYBF: 100% AYB milk fermented with commercial starter culture; ACS: equal proportion of milk blends fermented with *P. pentosaceus* without LAB; ARS: equal proportion of milk blends fermented with *L. paracasei* without LAB; ARCS: equal proportion of milk blends fermented with the combination of *L. paracasei* and *P. pentosaceus* without LAB; BCS: milk blend proportion 2:1:1 fermented with *P. pentosaceus* without LAB; BRS: milk blend proportion 2:1:1 fermented with *L. paracasei* without LAB; BRCS: milk blend proportion 2:1:1 fermented with the combination of *L. paracasei* and *P. pentosaceus* without LAB; AYBFS: 100% AYB milk fermented with commercial starter culture without LAB. Samples AC, AR, ARC, BC, BR, BCR, and AYBF contained live cultures of lactic acid bacteria and *Pseudomonas aeruginosa* while ACS, ARS, ARCS, BCS, BRS, BCRS and AYBFS were sterilised after fermentation and then inoculated with *Pseudomonas aeruginosa*.

Discussion

Species of *Lactobacillus* were observed to be predominant in dairy products while cocci were predominant in fermented foods. Four bacilli and one coccus strain were isolated from *wara* and *nunu*, while seven cocci and one bacillus strain were identified from fermented foods. The genera *Lactobacillus, Streptococcus, Pediococcus, Lactococcus,* and *Leuconostoc* have been reported to be associated with most local fermented foods from different varieties of maize, sorghum, millet, cassava, and also dairy products. The microorganisms identified in the present work have also been reported by Mohammed and Ijah (2013) and Akabanda *et al*. (2010), as present in locally fermented foods like *nunu*, *ogi*, *lafun*, and fermented milk samples. Some isolates yet to be reported were also identified in the present work. These included *Lactococcus lactis* which fermented only sucrose. This microorganism was said to be isolated from leafhopper and does not produce acid from galactose, lactose, maltose, or ribose, and *Lactobacillus paracasei* did not utilise any of the sugars while sugar alcohols are normally found in dairy products (Teuber, 1995).

The zones of inhibition found in the present work were higher as compared to the findings of Adebayo *et al*. (2013) for activities of *Lactobacillus casei* against *Bacillus* sp. and *E. coli*. However, the antimicrobial activities of both *Leuconostoc mesenteroides* and *Pediococcus acidilactici* (no zone of inhibition) contrasted with Adebayo *et al*. (2013). The phase at which these microorganisms were isolated and the difference in strains of isolates might be responsible for the observed ineffectiveness. Growth phase affects bacteriocin production (Lejeune *et al*., 1998; Soomro *et al*., 2002). Milk blends were cultured with LAB isolates which showed high inhibitory effect against selected pathogens (zone of inhibition ≥ 10 mm).

The highest inhibitory effect was observed in sample BRC (2.69 log CFU/mL) containing both LAB strains while the pathogen was least inhibited in sample AYBF (commercial starter culture) (2.17 log CFU/mL). The production of organic acids and low molecular metabolites by LAB which displayed antibacterial activity against closely related species especially foodborne pathogens such as *Listeria monocytogenes* and *S. aureus* (De Vuyst and Leroy, 2007) has been reported. Anas *et al*. (2008) reported a reduction of 1.60 log CFU/mL of *S. aureus* in mixed culture with *L. plantarum* after 24 h of incubation. After 7 d of storage, counts of *B. cereus* were observed to increase in samples AC and ARC containing LAB by 10.9% and 2.4%, respectively, as compared with the decrease observed generally in all other samples, though this increment was not significantly different from an initial population of the pathogen. *B. cereus* was only suppressed during the storage period but extinction was not observed as reported by Ebhodaghe *et al*. (2012). According to Adams and Moss (1995), for an outbreak of *B. cereus* food poisoning to occur, the implicated foods must contain a viable count of 5 log CFU/g of the microbial cells. *Bacillus cereus* population had been reduced to less than 5 log CFU/mL after 14 d of treatment which implies that the pathogen's ability to poison the food has been drastically impaired. This result is in agreement with the findings of Aramide *et al*. (2009) who observed a reduction in the population of *S. aureus* in roselle juice containing *Lactobacillus* sp. after three weeks of storage.

Milk samples fermented with a combination of the LAB strains exhibited 100% (ARC) and 61% (BRC) decrease after 28 d, which is in agreement with the findings of Aramide *et al*. (2009) who remarked a decrease in *E. coli* population in roselle juice containing LAB until the third week when the pathogen finally disappeared. After 7 d treatment, significant difference (≤ 0.05) was observed in the population of the pathogen and thus, a gradual reduction throughout storage. The two LAB strains had no difference in inhibitory effect over storage period with *L. paracasei* possessing an average of 51.5% and *P. pentosaceus* 54%, while the combination of both strains was highly effective in inhibiting the growth of the pathogen. Antimicrobial substances produced by LAB are believed to possess a narrow spectrum and less active against Gram-negative pathogens (Gillor *et al*., 2005). Contrary to this, the findings of Aramide *et al*. (2009) and Ebhodaghe *et al*. (2012) have indicated that foodborne illnesses caused by Gram-negative pathogens could be combated by LAB strains which was also observed in the present work.

Conclusion

In conclusion, locally fermented foods are rich sources of lactic acid bacteria which can be sourced for commercialisation and development of health-promoting beverages to eradicate foodborne illnesses.

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