African Journal of Food Science Vol. 5(6), pp. 340 - 348, June 2011
Available online http://www.academicjournals.org/ajfs
ISSN 1996-0794 ©2011 Academic Journals

Full Length Research paper

Phenotypic identification and technological properties of lactic acid bacteria isolated from selected commercial Nigerian bottled yoghurt

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Accepted 7 April, 2011

Twenty two strains of lactic acid bacteria (LAB) were isolated from 30 samples of commercial Nigerian bottled yoghurt (produced on a small scale). Isolates were phenotypically characterized and their technological properties such as acidification, exopolysaccharides (EPS) production, enzymatic activities, biogenic amine production and bacteriocin production were studied following standard procedures. The population of LAB in the yoghurt samples varied between 0.00 and 6.63 log cfu ml^{-1}. The pH and titratable acidity ranged from 3.80 to 4.48 and 0.73 to 1.78% lactic acid respectively. The isolated LAB strains belonged to *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus* and *Enterococcus* genera. Thirteen strains showed proteolytic activity while four strains showed high lipolytic activity. The LAB strains exhibited medium to low acidification activities. About 77% of the LAB strains produced EPS. Six strains produced biogenic amines. Seven strains showed antagonistic properties due to organic acid against indicator organisms (*Staphylococcus aureus, Klebsiella pneumoniae*, *Escherichia coli* and *Proteus vulgaris*) while two strains (*Enterococcus faecalis* and *Lactococcus cremoris*) produced additional inhibitory substances against *K. pneumoniae*. The results revealed that the assayed commercial Nigerian bottled yoghurts contain several LAB species and genera different from *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. The production of biogenic amine by some of the LAB strains is undesirable in yoghurt. The technological properties of the LAB associated with the fermented milk produced on a small scale will help in the selection and development of beneficial strains as starter or adjunct cultures.

Key words: Yoghurt, lactic acid bacteria, technological properties, strain selection.

INTRODUCTION

Milk from goat, ewe, buffaloes, cows and mares has been fermented by different people in different parts of the world since ancient times. Fermented milk products include: buttermilk, sour cream, yoghurt and cheese. The popularity of fermented milk is attributed to their attractive taste as well as their extended shelf life at low temperature and pH, which diminish the survival of pathogens. Yoghurt is perhaps the oldest fermented milk product known and consumed by a large segment of the population either as a part of diet or a refreshing beverage (Younus et al., 2002). The production of yoghurt involves the use of specific symbiotic / mixed culture of *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* (Masud and Sultana, 2002). When the yoghurt culture is undefined, it contains mixtures of various strains of lactic acid bacteria leading to variation in the quality of the product. Yoghurt is well known for its taste quenching and nutritive value in Nigeria today. The popularity of yoghurt has lead to the increased production of affordable bottled yoghurt on small scale level by individuals in recent times in Nigeria. This is partly due to the economy, with people trying to find a means of survival. As a result, poor quality milk, unhygienic practices associated with the process and the use of 'wild type' starter culture give rise to yoghurt with

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highly variable quality and short shelf life. Earlier studies on Nigerian fermented milk products have concentrated on the microorganisms associated with the products (Adebesin et al., 2001; Obi and Ikenebomeh, 2007; Oyeleke, 2009), and antagonistic activities of the associated lactic acid bacteria against pathogenic organisms (Adeniyi et al., 2006) without studying the technological properties which is still a basic requirement for strain selection (Ayad et al., 2004).

The objectives of this study was therefore to isolate and phenotypically identify the predominant LAB associated with bottled yoghurt (produced on small scale) in Nigeria, and provide information on the technological properties of the LAB for possible strain selection and development of starter culture for enhanced quality and safety of yoghurt produced on a small scale level.

MATERIALS AND METHODS

Studies on the phenotypic identification and technological properties of lactic acid bacteria associated with bottled yoghurt samples obtained from major towns in Osun State , Nigeria was carried out in the Food Microbiology Laboratory , Department of Microbiology, Obafemi Awolowo University, Ile – Ife, Nigeria between 5th October, 2009 and 30th September, 2010.

Yoghurt samples

Thirty bottled yoghurt samples comprising of ten brands were purchased from retail outlets in major towns in Osun State, Nigeria. The samples were transported within two hours after purchase in an ice box to the Food Microbiology Laboratory of the Department of Microbiology, Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria for physico - chemical and microbiological analysis.

Physico - chemical analysis

The pH of the samples were measured using an electronic digital pH meter (Hanna Instrument 8021) while the titratable acidity (TTA) was determined titrimetically (AOAC, 1990). The acidity was reported as % lactic acid by weight (1 ml of NaOH = 0.0090 g lactic acid).

Isolation and identification of LAB

Ten milliliter of sample were homogenized with 90 ml of maximum recovery diluent (MRD, Oxiod) and serially diluted in the same diluent. One milliliter of appropriately diluted sample was pour – plated in MRS agar (de Man, Rogosa, and Sharpe agar) supplemented with 1% $CaCO_3$. Plates were then incubated in anaerobic jar provided with disposable BBL gas generating pack (CO_2 system envelopes, Oxiod) at 30°C for 72 h and the viable LAB counts were determined. Representative strains of LAB were obtained and purified by successive subculturing on MRS agar. Characteristics of the isolated strains, such as catalase test, Gram staining, morphology, and motility test were studied. If the strain were catalase negative, Gram positive, non- motile, and with bacillus or coccus shape, they were preliminary identified as LAB.

Further identification was performed by using the following tests: growth at different temperatures (15 and 45°C) and different pH (3.9 and 9.6), as well as the ability to grow in different concentrations of NaCl (4.0 and 6.5%) in MRS broth, as described by Schlinger and Lucke (1989) and Dykes et al. (1994); gas production from glucose, determined in MRS broth containing inverted Durham tube; hydrolysis of arginine tested on MRS – Arginine broth (Harrigan and McCance, 1976); citrate utilization; and production of acetoin from glucose, determined by using the Voges- Proskauer test (Zourari et al., 1991). The fermentation of carbohydrates was determined in modified MRS broth (in which meat extract and glucose were omitted) containing bromocresol purple (0.04 gL^{-1}) as a pH indicator, and supplemented with 1% of the following carbohydrates; lactose, sucrose, arabinaose, xylose, sorbitol, galactose, mannitol, salicin, ribose, maltose, trehalose, cellobiose, raffinose, melezitose,and meliobiose. To ensure anaerobic conditions, each tube was topped up with two drops of sterile liquid paraffin after inoculation (Samelis et al., 1994).

Technological properties of LAB

Acidification activity

Acidifying activity of the LAB isolates was measured by change in pH during time (Ayad et al., 2004). Skimmed - milk medium was prepared from reconstituted skimmed milk powder 10% (w/v) and sterilized by autoclaving at 115°C for 10 min. The sterilized milk was inoculated with each strain (2%) (precultivated in MRS broth at 35°C for 18 h), and then incubated at 35°C. The pH of the inoculated skimmed milk medium was measured at 0, 2, 4, and 6 h using a pH meter (Hanna Instrument 8021). The acidification rate was expressed as ΔpH (Ayad et al., 2004).

Exopolysaccharides (EPSs) production

EPSs production by the isolates was carried out following the method described by Guiraud (1998). The LAB strains precultivated on MRS agar were streaked onto LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) KNO_3, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80 (Merck), 1.7% (w/v) agar, pH 7.1 ± 0.2,(Sawadogo- Lingani et al., 2007)) and incubated at 30°C for 48 h. The sticky aspect of the colonies was determined by testing them for slime formation using the inoculation loop method (Knoshaug et al., 2000). Positive results were confirmed using MRS-sucrose broth without glucose and peptone as previously described (Pidoux et al., 1990).

Biogenic amine formation

The ability of the LAB isolates to produce biogenic amine was determined qualitatively on an improved screening medium as described by Joosten and Northold (1989) using a cocktail of four precursor of amino acids (histidine, lysine, ornithine and tyrosine). The concentration of each amino acid was 1% and bromocresol purple (0.006%) was used as pH indicator. The pH of the medium was adjusted to 5.3 and then sterilized by autoclaving for 10 min at 121°C. A change in bromocresol indicator to purple following the growth of the test organism was considered as index of significant amino acid decarboxylase activity, corresponding to > 350 mg of a particular amino acid L^{-1} (Olasupo et al., 2001).

Antagonistic activity

The LAB isolates were screened for antagonistic activity using the agar well diffusion assay technique (Schlinger and Lucke, 1989). The indicator organisms used for antagonistic test included: *Staphylococcus aureus* NCIB 8588, *Bacillus subtilis* NCIB 3610, *Streptococcus faecalis* NCIB 775, *Klebsiella pneumoniae* NCIB

418, *Escherichia coli* NCIB 86, and *Proteus vulgaris* NCIB 67. Briefly, the LAB were grown in MRS broth for 24 h at 30°C. The cell free supernatant of the broth culture was obtained (by centrifugation at 15, 000 rpm for 15 min). The supernatant was adjusted to pH 6.2 using 2.5 N NaOH to rule out inhibition due to pH reduction caused by organic acids. The pH adjusted supernatant was filtered through a syringe filter with a pore size of 0.22 µm (Satorius Millipore, UK). Antagonistic activities of both pH adjusted and unadjusted cell free supernatant of LAB were tested. Inhibition occurring with the use of the pH adjusted supernatant was assumed to the presence of inhibitory substance other than pH.

Assessment of proteolytic activity

Surface dried plates of milk agar (Gordon et al., 1973) were streaked with 24 h old culture of the LAB, incubated at 30°C for 4 days and examined for any clearing of casein around and beneath the growth for assessment of proteolytic activity. Proteolytic activity of isolates which gave positive reaction on milk agar were measured by a modification of the method of Maeda et al. (1993) as described by Thapa et al. (2006). One unit of proteolytic activity was defined as the amount which produced an absorbance increase of 0.01 units under the assay conditions.

Assessment of amylolytic activity

Surface dried plates of starch agar (Gordon et al., 1973) were streaked with 24 h old culture of LAB and incubated at 30°C for 4 days. The plates were flooded with Gram`s iodine solution for 15 to 30 min and examined for clear zones around and underneath the growth for assessment of amylolytic activity.

Assessment of lipolytic activity

Surface dried plates of tributyrin agar (Leuschner et al., 1997) were streaked with 24 h old culture of LAB and incubated at 30°C for 4 days. Lipolytic activity was detected by a clear zone surrounding the growth in the turbid tributyrin agar. Lipolytic activity of strains that gave positive results was measured using the titrimetric assay method (Marcin et al., 1993). One unit of lipase activity was defined as equivalent of 1.0 ml of 0.5 N NaOH.

RESULTS

Twenty two LAB strains belonging to the genera *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, and Enterococcus* were isolated and characterized phenotypically (Table 1) The pH and TTA of the yoghurt samples ranged from 3.80 ± 0.21 to 4.48 ± 0.03 and 0.73 ± 0.08 to 1.78 ± 0.21 % lactic acid respectively (Table 2). The population of LAB in the yoghurt brands ranged from 0.00 to 6.63 ± 0.13 log cfu g^{-1}. LAB was not detected in two of the brands studied (Table 2) under the experimental conditions. The occurrence pattern of the LAB strains in the yoghurt brands is shown in Table 2.

Technological studies

The acidification activity analyses showed that the isolated LAB strains exhibited medium to low acidification activities within the range of 0.03 to 0.56 ΔpH after 6 h (Figure 1). *Lactobacillus fermentum* ($2E_2$) gave the highest acidification activity while *Lactobacillus plantarum* ($3I_2$) gave the least activity.

A total of 77% of the isolated LAB strains produced exopolysaccharides under the experimental condition described (Table 3). About 42% of these had good EPSs production ability with *Enterococcus faecalis* giving the highest in this category. The other 35% were poor producers of EPSs.

All the LAB isolates were screened for their ability to produce biogenic amines (decarboxylation of amino acids). Six of the LAB strains representing 27% exhibited decarboxylase activity (Table 4). The most common pattern was the decarboxylation of tyrosine and lysine by 6 and 5 of the positive LAB strains respectively. The two *Enterococcus faecalis* strains (codes L_1 and L_3) encountered in the study decarboxylated the four amino acid cocktail used for the test. *Lactobacillus casei* strain $3H_5$ decarboxylated only tyrosine.

Thirteen of the LAB strains showed proteolytic activity within the range of 0.17 ± 0.03 to 18.30 ± 1.85 units ml^{-1} (Table 3). *Lactobacillus acidophilus* strain $2H_1$ gave the highest proteolytic activity. Four strains showed lipolytic activity within the range of 13.33 ± 3.33 to 20.00 ± 5.00 units ml^{-1} (Table 3). None of the LAB strains produced amylase enzyme.

Screening of the LAB strains for antagonistic activities showed that eight strains produced inhibitory activities due to organic acids against the indicator organisms (*S. aureus* NCIB 8588, *K. pneumoniae* NCIB 418, *E. coli* NCIB 86, *P. vulgaris* NCIB 67) (Table 5). After the elimination of organic acid by standardizing the cell free supernatant of the LAB strains to pH 6.2 with 2.5 M NaOH, only two of the LAB strains (*L. cremoris* ($2B_2$) and *E. faecalis* (L_1) were found to inhibit *K. pneumoniae* NCIB 418 (Table 5).

DISCUSSION

The pH range of the yoghurt brands studied fall within the values reported in registered commercial bottled yoghurt sold in Nigeria (Green and Ibe, 1987; Bamise and Bamise, 2008) and commercial yoghurts in other countries (Gyosheva et al., 1996; Yeganehzad et al., 2007) but in contrast with other reports (Younus et al., 2002; Hussain et al., 2009). The TTA range recorded in this study is similar to values reported previously (Muhammed et al., 2005; Hussain *et al*., 2009) but in contrast with others (Haddadin, 2005; Green and Ibe, 1987). The pH of the yoghurt brands were within the acceptable limit set by the Standard Organization of Nigeria (SON) for industrial production of yoghurt (NIS: 337, 1997). Eight of the yoghurt brands had a TTA which fell within the acceptable limit set by SON while two brands had higher values. The starter culture composition and fermentation temperature could influence the pH and TTA

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Table 1. Characteristics of LAB strains isolated from selected bottled yoghurt.

Identity of isolates	Isolate code	Gram stain	Morphology	Catalase test	Arginine hydrolysis	Production of gas from MRS broth	Methyl red test	Voges prokaeur test	Nitrate reduction	Growth at 15°C	Growth at 45°C	Growth at pH 3.9	Growth at pH 9.6	Growth at 4.5%NaCl	Growth at 6.5% NaCl	Xylose	Galactose	Sorbitol	Mannitol	Maltose	Melobiose	Arabinose	Ribose	Trehalose	Salicin	Lactose	Raffinose	Cellobiose	Sucrose
Lactobacillus brevis	H_2	+	B	-	+	+	-	-	-	+	-	+	+	+	-	-	+	-	-	+	+	-	-	+	-	-	+	-	+
Leuconostoc mensenteroides	F_1	+	B	-	-	+	-	-	-	-	-	+	+	+	+	-	+	-	-	+	+	-	+	-	-	+	+	+	+
Lactobacillus casei	I_1	+	B	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	N	+	N	-	+	N	N	+	+	+	+
Enterococcus faecalis	L_1	+	C	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Enterococcus faecalis	L_3	+	C	-	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Leuconostoc mensenteroides	$2E_1$	+	B	-	-	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-	+	+	+	+	+	-	+
Lactobacillus fermentum	$2E_2$	+	B	-	+	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-	+	+	-	+	+	-	+
Lactobacillus acidophilus	$2H_1$	+	B	-	+	-	+	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-	-	+	+
Lactobacillus casei	$2H_3$	+	B	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Lactococcus cremoris	$2B_2$	+	B	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Lactobacillus casei	$3H_5$	+	B	-	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Leuconostoc mensenteroides	$3F_2$	+	B	-	-	+	+	-	-	-	+	+	+	+	+	+	-	-	-	+	-	-	+	+	+	+	+	+	+
Lactobacillus plantarum	$3I_2$	+	B	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus acidophilus	$3H_1$	+	B	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+
Lactobacillus fermentum	$2E_4$	+	B	-	+	+	-	-	-	-	+	+	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	+	+
Streptococcus thermophillus	$2L_3$	+	B	-	-	-	+	+	-	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactobacillus fermentum	C_{18}	+	B	-	+	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	+
Lactobacillus cellobiosus	$2L_4$	+	B	-	-	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+
Lactobacillus cellobiosus	M_4	+	B	-	+	+	-	-	-	+	+	+	-	+	-	-	+	+	N	+	N	-	+	-	-	+	+	+	+
Lactobacillus casei	L_2	+	C	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	N	+	N	+	+	+	+	N	N	N	+
Lactococcus cremoris	A_1	+	B	-	-	-	-	-	-	+	-	+	-	-	-	+	+	+	+	+	N	+	+	+	+	+	N	+	+
Lactobacillus fermentum	$3L_4$	+	B	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	N	+

Key: + = Positive reaction, - = negative reaction, N = not determined, B = bacillus, C = coccus.

Table 2. pH, titratable acidity, lactic acid bacteria population and occurrence in yoghurt brands.

Yoghurt brand	pH	TTA (% Lactic acid)	LAB (Cfu/ ml)	Species of LAB
B	3.84 ± 0.41[a]	1.28 ± 0.02[c]	5.09 ± 0.50[d]	*Leuconostoc mensenteroides, Lt. acidophilus, L. casei, L. cremoris*
C	3.98 ± 0.27[a]	0.73 ± 0.08[b]	5.31 ± 0.62[d]	*L. fermentum, L. casei, L. plantarum, S. thermophilus*
D	3.98 ± 0.36[a]	1.78 ± 0.21[c]	NG[e]	None*
E	4.05 ± 0.30[a]	1.25 ± 0.17[c]	6.63 ± 0.13[d]	*L. mensenteroides, L. fermentum*
F	3.89 ± 0.38[a]	0.95 ± 0.19[b]	6.07 ± 0.52[d]	*Lt. brevis, c. mensenteroides, L. cremoris*
H	3.98 ± 0.27[a]	1.56 ± 0.22[c]	4.86 ± 0.79[d]	*L. brevis, L. fermentum, L. acidophilus, L. casei, L. plantarum, S. thermophilus*
I	3.80 ± 0.21[a]	1.33 ± 0.13[c]	4.63 ± 0.94[d]	*Lact. casei, Lact. plantarum, Strep. thermophilus*
L	4.43 ± 0.09[a]	1.18 ± 0.09[c]	5.15 ± 0.91[d]	*E. faecalis, L. fermentum, L. cremoris, L. casei, L. plantarum, S. thermophilus, L. cellobiosus*
M	4.12 ± 0.05[a]	1.75 ± 0.20[c]	5.28 ± 0.41[d]	*L. casei, L. cellobiosus*
N	4.48 ± 0.03[a]	1.16 ± 0.15[c]	NG[e]	None*

TTA, Titratable acidity; LAB, Lactic acid bacteria.*LAB was not detected under the experimental condition of isolation (incubation at 30°C in anaerobic jar equip with CO_2 system envelopes, Oxiod). Values represent the mean of three determinations ± standard deviation.Means having the same superscript within the same column do not differ significantly ($p>0.05$).

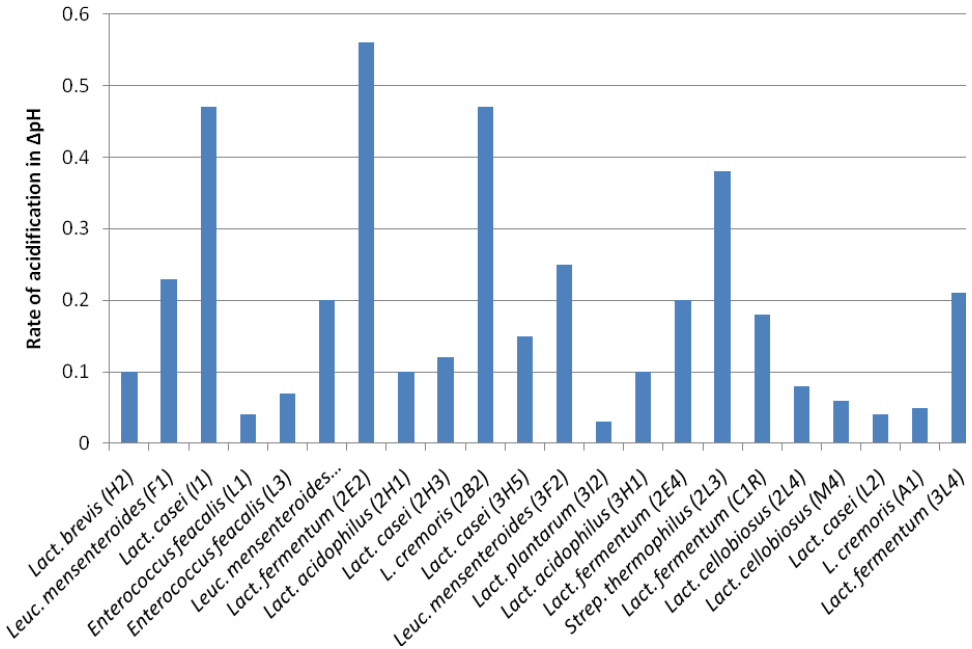

Figure 1. Acidification activity (ΔpH) after 6 h of incubation (in skimmed milk medium) of lactic acid bacteria isolated from bottled yoghurt.

Table 3. Enzymatic activities* and exopolysaccharide production by LAB strains isolated from yoghurt.

LAB isolate	Proteolytic activity	Lipolytic activity	EPS Production
L. brevis (H_2)	-	-	-
L. mensenteroides (F_1)	2.07 ± 0.15^b	-	-
L. casei (I_1)	2.97 ± 0.03^b	-	-
E. faecalis (L_1)	0.40 ± 0.06^b	13.33 ± 3.33^a	2+
E. faecalis (L_3)	-	-	4+
L. mensenteroides ($2E_1$)	-	-	2+
L. fermentum ($2E_2$)	8.90 ± 0.10^a	-	3+
L. acidophilus ($2H_1$)	18.30 ± 1.85^a	-	2+
L. casei ($2H_3$)	-	-	3+
L. cremoris ($2B_2$)	-	-	3+
L. casei ($3H_5$)	1.00 ± 0.06^b	-	2+
L. mensenteroides ($3F_2$)	0.17 ± 0.03^b	-	2+
L. plantarum ($3I_2$)	1.87 ± 0.26^b	-	2+
L. acidophilus ($3H_1$)	-	-	-
L. fermentum ($2E_4$)	-	-	-
S. thermophillus ($2L_3$)	2.37 ± 0.20^b	-	2+
L. fermentum (C_{1R})	0.43 ± 0.09^b	18.33 ± 1.67^a	3+
Lactobacillus cellobiosus ($2L_4$)	-	-	2+
L. cellobiosus (M_4)	-	-	2+
L. casei (L_2)	2.37 ± 0.55^b	16.67 ± 4.41^a	3+
L. cremoris (A_1)	2.77 ± 0.09^b	20.00 ± 5.00^a	2+
L. fermentum ($3L_4$)	2.67 ± 0.03^b	-	2+

*Enzyme activities expressed as Unit mL^{-1} and data represent the means of three determinations ± standard error. EPS, Exopolysaccharide; 2+, 3+, 4+ (degree of opacity, EPS production); 2+, slightly opaque; 3+, opaque; 4+, very opaque. Means having different superscript within the same column differ significantly ($p < 0.05$).None of the LAB isolates produced amylase.

influence the pH and TTA hence the contrast or variations in the values of pH and TTA compared to other studies.

Two of the yoghurt brands had no LAB, probably resulting from the use of synthetic analogue in their preparation or chemical preservatives and high temperature to extend their shelf life. The population of LAB observed in the other brands corresponds with the findings of Al-Tahiri (2005) and Hussain et al. (2009) that reported a mean of 6.0×10^6 cfu/ml for yoghurts produced by modern dairies. The LAB load observed in this study is in line with the International Regulatory Body for dairy products which recommended a minimum of 10^6 cfu/g. of viable lactic acid bacteria for effective probiotic functions in yoghurt throughout their shelf life (Adolfsson et al., 2004). The observed LAB load of six of the yoghurt brands would undoubtedly offer some advantages to the products if they contain probiotic strains of LAB. The observed wide variety of LAB strains in the yoghurt brands are in agreement with reports from other studies on traditional fermented milk products (Feresu and Muzondo, 1990; Isono et al., 1994; Savadogo *et al.*, 2004). The yoghurt brands lacked the defined starter culture – *Lactobacillus bulgaricus* and S*. thermophilus.* The diversity of LAB strains in the yoghurt brands may have resulted from contamination from dairy utensils or production line and the use of artisanal cultures as starter culture for a new batch of production.

None of the LAB strains isolated in this study can be characterized as fast acid producer since they did not produce a ΔpH of 0.4 U after 3 h. *L. casei* (I_1), *L. fermentum* ($2E_2$), and *Lactobacillus cremoris* ($2B_2$) showed medium acidification activity. The low acidification activity of the LAB strains encountered in this study is in line with previous reports (Sarantinopoulous et al., 2001; Badis et al., 2004; Dagdemir and Ozdemir, 2008). A rapid decrease in pH is essential for coagulation and prevention or reduction of growth of adventitious microflora in yoghurt production. The fast acidifying strains are therefore good candidates for dairy fermentation process as primary starter culture while poor acidification strains can be used as adjunct cultures depending on other properties (Ayad et al., 2004).

About 59% of the encountered LAB strains in this study showed low proteolytic activity except *L. acidophilus* ($2H_1$). This is an indication that the LAB strains have very low proteolytic activities in Milk, in line with the findings of other investigators on LAB from other sources (Thapa et al., 2006; Ayan et al., 2005; Hassaine et al., 2007). Our result is however in contrast with those of Durlu–Ozkaya et al. (2001) and Dagdemir and Ozdemir (2008) who reported high proteolytic activity for LAB isolated from

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Table 4. Biogenic amine production (amino acid decarboxylation) pattern of LAB strains isolated from yoghurt.

LAB isolate	Amino acid				Total amino acids decarboxylated
	Lysine	Tyrosine	Histidine	Ornithine	
E. faecalis (L_1)	+	+	+	+	4
E. faecalis (L_3)	+	+	+	+	4
L. mensenteroides ($2E_1$)	+	+	-	+	3
L. fermentum ($2E_2$)	+	+	-	-	2
L. acidophilus ($2H_1$)	+	+	-	+	3
L. casei ($3H_5$)	-	+	-	-	1

Each amino acid was tested separately in a decarboxylase assay medium. +; Positive; Negative.

Table 5. Antagonistic effect before and after neutralizing the pH of cell free supernatant of LAB isolated from yoghurt against selected bacteria.

LAB isolate	Indicator microorganisms/ Zone of inhibition (diameter in mm)			
	S. aureus **NCIB 8588**	***K. pneumonia*** **NCIB 418**	***E. coli*** **NCIB 86**	***P. vulgaris*** **NCIB 67**
L. casei (I_1)	-	+4	-	-
E. faecalis (L_1)	-	+3 +2*	-	+6
E. faecalis (L_3)	+2	+3	-	+2
L. mensenteroides ($2E_1$)	+4	-	-	-
L. casei ($3H_5$)	-	-	+2	-
L. mensenteroides ($3F_2$)	+2	-	-	-
L. cellobiosus (M_4)	+2	+2	-	-
L. cremoris ($2B_2$)		+2*		

*Antagonistic effect of cell free supernatant after neutralizing the pH; diameter of zone of inhibition in parenthesis.- ,No inhibition activity; +,antimicrobial activity.

cheese. The contrast in proteolytic activities may be due to the strains associated with the products. The proteolytic activity of dairy LAB is essential for the growth of the organisms in milk and it is involved in the development of organoleptic properties of different fermented products (Christensen et al., 1999; Hassaine et al., 2007). Only 18% of the isolated LAB strains showed lipolytic activity in this study, thus confirming the report of Montel et al. (1999). The ability of LAB to show lipolytic activity *in vitro* lipolytic activity *in vitro* is very promising. It is assumed that such activity can be manifested by the isolates *in vivo* which will lead to the reduction of cholesterol level in humans if used as a starter or an adjunct culture. None of the isolated LAB strains produced amylase enzyme. Although no amylase producing strain of LAB has been reported from dairy products, very few amylase producing strains have been reported in LAB isolated from African fermented maize and cassava products (Olasupo et al., 1996; Agati et al., 1998; Sanni et al., 2002).

Twenty seven percent of the isolated LAB strains produced biogenic amine. The enterococci strains isolated in this study decarboxylated all the amino acids (lysine, tyrosine, histidine, and ornithine) used. This is in line with the reports of Bover – Cid et al. (2001) on enterococci as an important tyramine producer in association with fermented foods. It is worthy of note that the strains producing biogenic amines in this study are not the usual strains involve in the production of yoghurt. Production of biogenic amines by LAB to be selected as starter culture is not a desirable property (Buchenhuskes, 1993). The inability of seventy three percent of the LAB strains in this study to produce biogenic amines is a good indication of their acceptability in the possible development of starter cultures.

About 77% of the encountered LAB in this study produced exopolysaccharides, which was however not a surprise since many strains of LAB have been reported to produce expolysaccharides (De Vuyst et al., 2001; Ayad et al., 2004; Sawadogo et al., 2007). The formation of exopolysaccharide in food has been reported to function as viscosifying agents, stabilizers, emulsifiers, gelling agents or water binding agents (Verdern Berg et al.,

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1995; De Vuyst et al., 2001) and may play important role in the texture of the product (De Vuyst and Degeest, 1999). Exopolysaccharide production is therefore an important property of LAB in yoghurts.

Seven strains belonging to the species of *Lactococcus, Lactobacillus, Enterococcus, and Leuconostoc* showed antagonistic activities due to organic acid against the indicator organisms. In addition, only *E. faecalis* (L_1) and *L. cremoris* ($2B_2$) inhibited *K. pneumoniae* due to the production of other inhibitory substances not related to organic acid. Although the production of bacteriocin - like substance was not tested in this study, the production of bacteriocin by *Lactococcus* spp associated with milk and dairy products has been reported (Olasupo et al., 1999; Kumari and Garg, 2007). Production of bacteriocin by LAB is frequent and may contribute to their colonizing and competitive ability. In addition, bacteriocin producing strains have been used as starter cultures or adjunct to improve safety and quality of fermented products (Einarsson and Lauzon, 1995; Delves–Broughton et al., 1996). However, selection of suitable microorganisms as a mixed starter should ensure that the organisms do not inhibit themselves.

Conclusion

The results obtained in this study revealed that the assayed commercial Nigerian bottled yoghurt contain several LAB species and genera within the limit of the traditional phenotypic method of identification used. Further characterization of the isolated LAB strains using molecular methods would be necessary. However, the results suggest that *S. thermophilus* ($2L_3$), *L. casei* (I_1), *L. fermentum* (C_{1R}, $3L_4$) and *L. cremoris* ($2B_2$) can be selected based on their technological properties as good starter culture candidates for producing yoghurt. Further study is however required to evaluate the selected strains on a pilot scale individually and in mixed cultures.

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