Scientific Research and Essays Vol. 7(47), pp. 4037-4046, 3 December, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE12.252
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

Microbiota associated with commercially produced traditional banana beer in Rwanda

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Accepted 16 July, 2012

Brewing and consumption of traditional beers have socio-economic importance in most African countries, including Rwanda. In this study, the microbiological quality (of selected microbes) of commercially produced traditional banana beer was investigated, using both traditional culture-dependent and culture-independent methods. Two brands of commercially produced traditional banana beer (brands A and B) were obtained from a local market in Kigali, Rwanda. Brands A and B recorded the total viable counts (TVC) were 5 and 124 cfu.ml^{-1} respectively, whereas the mean count for lactic acid bacteria were found to be 30 and 80 cfu.ml^{-1}. Yeasts and moulds were 17 cfu.ml^{-1} in brand A, and none detected in brand B Coliforms, *Salmonella* spp, and *Staphylococcus* spp were not detected in either brand of traditional banana beer, through the culture-dependent and culture-independent techniques. Denaturing gradient gel electrophoresis (DGGE) analysis of the 16S rRNA genes detected the presence of *Lactobacillus farraginis*, *Lactobacillus diolivorans* and *Lactobacillus hilgardii* as dominant organisms only in brand A. The absence of pathogenic micro-organisms suggests proper hygiene and sanitation, during processing and after production of the banana beer.

Key words: Traditional banana beer 'urwagwa', Rwanda, *Lactobacillus*, microbial quality, denaturing gradient gel electrophoresis (DGGE), food safety.

INTRODUCTION

Indigenous traditional beers play a prominent role in the daily social, economic, nutritional and cultural life of the people especially in developing countries (Almeida et al., 2007; Bvochora and Zvauya, 2001; Jespersen, 2003; Naumova et al., 2003). The social and economic aspects of traditional beer consumption have also been reported by Haggblade and Holzapfel (1989), and Novellie and de Schaepdrijver (1986) during the late 80s. Banana beer 'urwagwa', produced in Rwanda, is the most popular traditional alcoholic beverage produced from juice obtained from special varieties of bananas, such as 'indege', 'inkati', 'intutu', 'kayuku', 'gisukali' and 'intokatoke (Nsabimana and van Staden, 2007). The raw materials used in the production of banana beer are banana fruits, water and sorghum. In Rwanda, banana beer is made in different ways, varying from region to region, and this leads to the production of different kinds of beer in terms of quality, alcohol content, colour, taste and shelf life which is normally dependent on raw material used and dilution ratios (Mukantwali et al., 2008).

Mostly, more than two types of banana varieties are mixed in the production of beer, but some banana beer makers prefer to use a single type of banana. In processing plants, commercially produced traditional banana beers are produced, following the traditional methods, except that conditions are closely monitored

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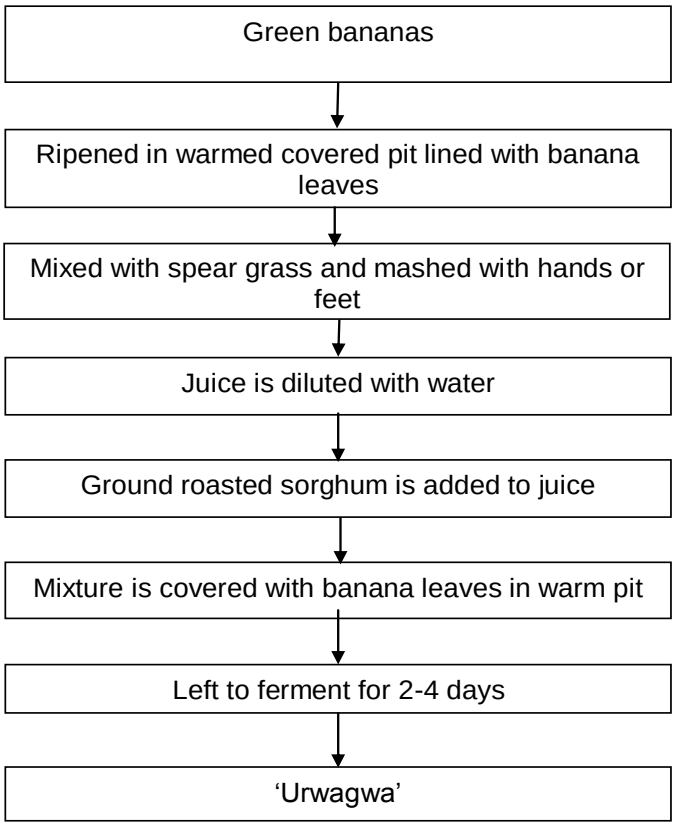

Figure 1. Major steps in traditional processing of banana beer 'urwagwa'.

Figure 2. A traditional way of squeezing juice out of ripe banana using feet and/or hands full of spear grass.

and the environment is more hygienic, compared to household level. Major steps involved in production of banana beer 'urwagwa' are summarized in Figure 1. Green bananas are ripened for 3 to 5 days in a covered, previously warmed pit, lined with banana leaves, to ensure uniform temperature. The ripe bananas are mixed with spear grass, followed by the extraction of juice by men squeezing the mixture with their feet or hands. The process of squeezing is continued until all the pulp is exhausted of juice (Figure 2). This juice is filtered through grass, held in a calabash funnel, and an amount of water added. The dilution ratios depend on the quality of banana beer needed.

The fermentation process requires the addition of coarsely-ground, roasted sorghum to the diluted banana juice, in a canoe-shaped wooden container known as 'umuvure' or in a clay pot. The fermentation broth is then covered with banana leaves and split banana stems, in warmed pit and incubated for 2 to 4 days. Towards the end of the incubation process, regular tasting is carried out by an experienced elder, until it is declared ready for consumption. This sampling procedure is carried on by piercing the coverings with a straw and taking a sip. The quality of 'urwagwa' depends on the production procedure, species of banana used and the dilution ratio and, in many cases, the weather, since there is no proper mechanism to control the fermentation temperature (Mukantwali et al., 2008; Mwesige and Okurut, 1995). 'Urwagwa' is consumed from small gourds using straws, bottles and other plastic containers. If the fermentation of banana juice is carried out under controlled conditions, a good yield of 'urwagwa' can be obtained (Nzigamasabo and Nimpagaritse, 2009). The processing of indigenous traditional beer has become the main activity in rural areas and urban centres. Its processing is mainly done by women and unemployed school leavers using simple household equipment (Amusa and Odunbaku, 2009; Muyanja et al., 2003).

Due to the nature and lack of adequate skills in food handling practices, by people involved in the production of indigenous traditional beer, its processing is highly prone to microbial contamination. Ikalafeng (2008) reported the presence of *Staphylococci* species in 'maiza' and 'umqombothi', the two South African traditional beers

produced in the Northern Cape region. Roy et al. (2007) further emphasised that the presence of staphylococci was an indication of poor hygiene and sanitation practices by food handlers and lack of cleaning of the utensils. It is known that the producers of traditional beer and other indigenous foods are mostly uninformed about good hygiene practices, such as washing hands, wearing gloves, covering hair and proper food handling.

Lues et al. (2011) and Mensah et al. (2002), both stated that the practice, for example, of not washing hands has been shown to result in up to 10^6 cfu ml^{-1} growth of pathogenic micro-organisms under the fingernails of food handlers. Members of the bacteria genera *Staphylococcus*, *Escherichia*, *Salmonella* are micro-organisms closely associated with food-borne illnesses related to poor hygiene, sanitation and improper food handling (Roy et al., 2007). Microbiological safety of indigenous foods and beverages is an important issue in developing countries, including Rwanda. Isolation, identification and understanding the microbiology of traditional beer is a key factor to improving its quality and safety.

Most studies of traditional beers have focused on the application of culture techniques to screen microbial population. However, this study employs both culture and molecular-based techniques, denaturing gradient gel electrophoresis (DGGE) to study microbial diversity of commercial traditional banana beer samples produced in Rwanda. The latter method is the one of the most suitable and widely applied to study microbial communities of samples, originating from various environments (Muyzer et al., 1999).

The aim of this study was therefore to determine the prevalence of selected micro-organisms, including indicator microbes, in two brands of commercially produced traditional banana beer, 'urwagwa'; using both culture-plating and molecular-based techniques. Information gathered will shed light on how to improve the microbial quality and safety of banana beer, with the aim of assessing regional and international markets, to increase revenues as well as to improve the standard of living and safety of the product.

MATERIALS AND METHODS

Sample collection

For the purpose of this study, two brands of commercially traditional banana beer samples were purchased from a local retail market in Kigali, Rwanda. Samples were transported to the microbiology laboratory at low temperatures for analysis. Immediately after opening, beer samples were prepared for microbiological analysis, (5 ml) of each sample were kept in a freezer at -80°C for molecular. Samples of banana beer (A and B) were serial diluted in buffer peptone water (Biolab, SA) where 0.1 ml of each dilution was plated on different selective media, using the spread plate method (Herbert, 1990). All media used was obtained from Merck South Africa, unless stated otherwise. All samples were done at least in duplicate, for statistical purposes.

Microbiological analysis

Total Viable Counts (TVC)

Enumeration of Total Viable Counts was done on Plate Count Agar (PCA) after incubation at 25°C for 72 h (Houghtby et al., 1993; Vorster et al., 1994). Plates were incubated for the separate enumeration of both aerobic and anaerobic microbes with clean plates used for quality control.

Total coliforms

Selective enumeration of *Escherichia coli* and coliforms was done on Violet Red Bile Mug Agar (VRBM) and plates were incubated for 48 h at 37°C. *E. coli* (ATCC 25922) strain was used as a positive control during the study and, for negative control, a blank VRBM Agar plate was incubated at 37°C for 48 h.

Staphylococcus spp

Baird Parker agar (BPA) with 50ml egg-yolk tellurite emulsion (Merck, SA) was used to isolate *Staphylococcus* spp, by incubating plates for 48 h at 35°C. *S. aureus* (ATCC 25923) was used as a positive control and a blank Baird-Parker agar plate was used as a negative control, incubated under the same conditions.

Salmonella spp

Selective enumeration and detection of *Salmonella* spp was done using *Salmonella Shigella* agar (SSA) plates which were incubated at 37°C for 18 to 24 h. For positive control, *Salmonella enteriditis* (ATCC 13076) was used and a blank SSA plate, incubated at 37°C for 18-24 h, was used as a negative control for the study.

Total fungi (yeasts and moulds)

Potato dextrose agar (PDA) was used for enumeration and isolation of yeasts and moulds. The medium was acidified to pH 3.5 with tartaric acid (Christen et al., 1993; Frank et al., 1993). Plates were incubated at 25°C for 3 days for both anaerobic and aerobic growth.

Lactobacillus spp

Quantification of *Lactobacilli spp* was done on de Man Rogosa Sharpe Agar (MRSA), with plates incubated at 37°C for 3 days. *Lactobacillus fermentum* ATCC 9338 was used as positive control. For negative control, a blank MRSA plate incubated at 37°C for 3 days was used for this study.

pH analysis

The pH of beer samples was determined using pH meter (Eutech instruments, Singapore). The pH meter was calibrated using buffers of pH 4 and 7 as specified by the manufacturer.

Molecular analysis

DNA extraction

Two methods were used to extract genomic DNA from beer samples, with the first performed using the method described by

Labuschagne and Albertyn (2007). One millilitre of each beer sample was centrifuged briefly and 500 µl lysis solution (100 mM Tris-HCl, 50 mM EDTA, 1% SDS, pH 8.0) and 200 µl glass beads (425 to 600 µm diameter) were added to the pellet. Samples were vortexed for 4 min, cooled on ice for 5 min and 275 µl ammonium acetate (7 M, pH 7.0) was added. Samples were incubated at 65°C for 5 min, placed on ice for another 5 min, 500 µl chloroform was added. Samples were vortexed and centrifuged at 20 000 rpm for 2 min at 4°C.

The supernatant was recovered and the DNA precipitated using 1 volume isopropanol and centrifuged at 20 000 rpm for 2 min at 4°C. Pellets were washed with 70% (v/v) ethanol, dried and re-dissolved in 100 µl TE buffer (10 mM Tris-HCl; 1 mM EDTA, pH 8.0). The second method was the commercially available FastDNA® Spin Kit for Soil (MP Biomedicals); this method was used according to the manufacturers' instructions. Genomic DNA, extracted from both beer samples with each of the mentioned methods, were used as template to amplify portions of the 16S rRNA (prokarial diversity) and the 18S rRNA genes (eukaryal).

Polymerase chain reaction (PCR) amplification of target genes

Two set of primers were used to amplify the mentioned target genes of 1 300 bp and 1 700 bp, respectively; 63-F (5'-CAGGCCTAACACATGCAAGTC-3') and -1387-R (5'GGGCGGWGTGTACAAGGC-3') (Marchesi et al., 1998), to amplify bacterial 16S rRNA fragment and EukA (5'-AACCTGGTTGATCCTGCCAGT-3') and EukB (5'-TGATCCTTCTGCAGGTTCACCTAC-3') (Diez et al., 2001) for eukaryotic 18S rRNA genes. PCR was performed in a final volume of 25 µl containing, 1x PCR reaction buffer (100 mM Tris-HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3), 0.52 µM of each primer, 0.4 mM of each dNTP, 1U of Taq DNA Polymerase, 1 mg ml^{-1} BSA (Bovine serum albumin) and 60 to 80 ng $µl^{-1}$ of the extracted DNA as template. The PCR amplification was carried out under the following conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 2 min and final elongation at 72°C for 10 min. PCR products were verified by electrophoresis in 1% agarose gel, stained with 0.05% GoldView (Guagnzhou Geneshun Biotech) and visualized under UV light.

Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) prokaryotic

Gel stab (1 µl) was used as template to amplify 230 bp of 16S fragment by using primers; 341F-GC with GC clamp (5'-CGCCCGCCGCGCCCCGCGCCCGTCCCCCCGCCCCCGCCCCC TACGGGAGGCAGCAG-3') and -517-R (5'-ATTACCGCGGCTGCTGG-3') (Muyzer et al., 1993). The PCR reaction included 0.4 mM of each dNTP, 0.5 µM of each primer, 1x PCR reaction buffer (100 mM Tris-HCL, 15 mM $MgCL_2$, 500 mM KCL, pH 8.3), 1U of Taq DNA Polymerase, and 1 mg ml^{-1} BSA in the final volume of 50 µl. Amplification conditions were as follows: 94°C for 3 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min and finally, 72°C for 10 min.

DGGE was performed on the DCode ™ Universal Mutation Detection System (Bio-Rad) as described previously (Muyzer et al., 1997). Electrophoresis was performed in an 8% (w/v) polyacrylamide gel 40% acrylamide/bis solution (37.5:1) submerged in 1× TAE buffer (40 mM Tris, 20 mM acetate, 1.0 mM Na_2EDTA). A polyacrylamide gel with a denaturant-gradient ranging from 40 to 60% urea and formamide (100% denaturant contains 7 M urea and 40% (v/v) formamide) was run at 130 V at 60°C, for 4.5 h. The gel was stained with 0.05% Gelstar (Cambrex) for 15 min and visualized under UV light.

Prominent bands were excised from the gel, 50 µl water was added to each slice and incubated at 50°C for at least 6 h. Five microlitres (5 µl) was used as a template to re-amplify the 230 bp fragment using the same PCR setup and conditions as described above for PCR-DGGE. Amplified fragments were confirmed on a 2% agarose gel and 1 µl was used as template for sequencing, which was performed with an ABI Prism 3130 XL Genetic Analyser with the Big Dye® Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems). Sequence results were analyzed, using Basic Logal Alignment Search Tool (BLAST) to compare with the National Centre for Biotechnology Information (NCBI) GenBank Database for identification.

RESULTS AND DISCUSSION

Microbiological analysis

The processing of banana beer involves mainly four principal steps: ripening, peeling, juice extraction and fermentation process (Davies, 1993; Mwesigye and Okurut, 1995). Apart from roasted sorghum grains, raw materials used in banana beer production do not undergo any heating or cooking, at any stage of the process. However, for Brand B, pasteurization is carried out after bottling to stop fermentation and/or possible microbial proliferation.

Total viable counts (TVC*)*

The rapid determination of total viable cell counts, in both raw and prepared foods, is an essential and effective way of ensuring the efficient monitoring of microbiological quality (Pishawiker et al., 1992). The mean TVC for brands A and B of commercial traditional beer, were 5 $cfu.ml^{-1}$ and 124 $cfu.ml^{-1}$ respectively (Figure 3). The high counts in brand B could be attributed to a possibility of the growth of different micro-organisms present in food product on this non-selective media. Low counts in brand A could be attributed to the possibility of proper and good manufacturing practices from the production line.

Total fungi

Yeasts are significant groups of micro-organisms in the food industry, as they are well known for their beneficial role in production of bread, alcoholic beverages, cheeses and other food products (María et al., 2005). However, yeasts can cause spoilage in a wide range of food products (Loureiro, 2000; Salo and Wirtanen, 2005), including alcoholic beverages. Yeasts can generally survive extreme conditions better than bacteria, hence their ability to spoil certain foods (Salo and Wirtanen, 2005). The presence of yeasts and moulds may also result in off-flavor, sourness and cloudiness in beer. In the current study, yeast and mould counts were 17 $cfu.ml^{-1}$ for brand A and absent in brand B (Figure 3). The presence of yeasts and moulds in Brand A could be due

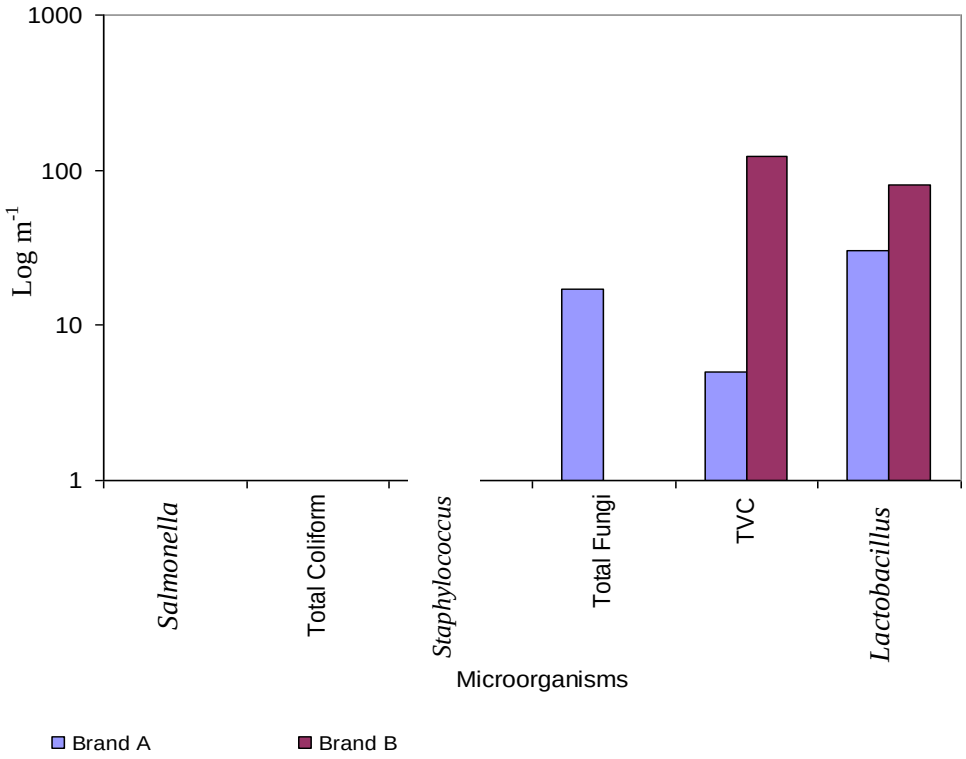

Figure 3. Micro-organisms present in the two brands of commercially produced traditional banana beers.

to the fact that yeasts were included in the ingredients used in the production of traditional banana beer and that no proper pasteurization was carried out. Several studies have reported the presence of yeasts in various indigenous fermented foods and beverages. The most predominant yeast is *Saccharomyces cerevisiae* and it is sometimes introduced as a starter culture (Glover et al., 2005; Kutyauripo et al., 2009). However, the absence of yeasts and moulds in brand B may be attributed to the proper pasteurization, carried out after packaging, to stop fermentation and/or possible microbial proliferation.

Total coliforms

Total coliform bacteria are organisms that are abundant in the environment such as in soil, surface water, vegetation, the intestinal tracts and faeces of warm-blooded animals and humans. Members of coliforms include genera *Citrobacter*, *Enterobacter*, *Escherichia*, *Hafnia*, *Klebsiella* and *Serratia*. Coliforms are Gram-negative bacteria; predominant facultative anaerobes in the bowel (Collins et al., 1995; Hayes et al., 2001) are members of *Enterobacteriaceae*. Some strains of *E. coli* are human pathogens and are associated with diarrhoea, gastroenteritis and urinary infection (Bell and Kyriakides, 1998; Elmahmood and Doughari, 2007). Moreover, members of the total coliform group, especially *E. coli,* are used as indicator organisms of faecal contamination (Bell and Kyriakides, 1998; Oyedeji et al., 2010) and their occurrence indicates the possible presences of pathogenic bacteria, such as *Salmonella spp, Shigella spp, Campylobacter jejuni, Campylobacter coli, Vibrio cholerae, Yersinia enterocolitica* and pathogenic *E. coli* (Lee et al., 2009; Wutor et al., 2009).

In this study, no total coliforms were isolated from either commercially available traditional banana beers (Figure 3). This is in agreement with the study by Namugumya and Muyanja (2009), who reported the decrease in the coliforms counts from 1.59 ± 0.76 log cfu.ml^{-1} to undetectable level during fermentation of ‘kwete’ and complete disappearance of coliforms after 24 h of fermentation as pH dropped to 3.58. Kunyanga et al., (2009) and Muyanja et al. (2003) also reported the decrease in coliform counts as the pH dropped, due to the fermentation effect of lactic acid bacteria present in ‘bushera’ and ‘kirario’. Antimicrobial substance produced by lactic acid bacteria, in addition to lactic acid, may have contributed to the inhibition of pathogenic microorganisms (Helander et al., 1997; Mensah et al., 1991). In addition, organic acids formed during fermentation process lower pH values of most fermented foods and traditional beverages, and are particularly effective in inhibiting pathogenic and spoilage

micro-organisms making food generally safe for human consumption.

Salmonella spp

Salmonella species are the most common pathogenic bacteria associated with food-borne illness worldwide (Cetinkaya et al., 2008). *Salmonella* species were not detected in the traditional banana beers (Figure 3) analysed in this study. As stated by Beal et al. (2002) *Salmonella* species were not able to survive in animal feed fermented by lactic acid bacteria that has pH values ranging from 3.8 to 4. This explains the absence of these organisms in the traditional banana beer with low pH levels of 4.23 and 3.35, in brand A and B, respectively as indicated in this study. Beal et al. (2002), reported that food products with low pH levels inhibit the proliferation of pathogenic microorganisms.

Staphylococcus spp

Staphylococcus species, on the other hand, are normal biota of the human skin, nose, fingernails, palms, hair, throat and mucus membranes of healthy individuals. *Staphylococcus* species were not isolated in the two commercially available traditional banana beers (Figure 3). This is an indication that these traditional banana beers were processed under hygienic conditions, or this result may be attributed to the fact that *Staphylococcus* species and other pathogenic organisms are not able to survive fermentation conditions, as several researchers have reported. According to Tadesse et al. (2005), research in Ethiopia indicated that LAB (*Lactobacillus* sp, *Leuconostoc* sp, *Pediococcus* sp, and *Streptococcus* sp) isolated from 'borde' and 'shimita', traditional fermented beverages, posses an inhibitory against test strains (*Salmonella* sp, *Staphylococcus aureus, Shigella flexneri* and *E. coli* 0157:H 7). Contrarily, Ikalafeng (2008) indicated the presence of *Staphylococci* species in 'maiza' and 'umqombothi', the two South African traditional beers produced in the Northern Cape region. Therefore, presence of *Staphylococcii* bacteria in a food item is a good indicator of the poor hygienic practices of food handlers and improper food handling.

Lactobacillus spp

Lactobacillus is a genus of Gram-positive, facultative anaerobic or microaerophilic bacteria, rod-shaped bacilli or coccoid, non-sporulating, catalase-negative, aerotolerant, acid tolerant and produce lactic acid as end product from the fermentation of carbohydrates (Kao et al., 2007; Sakamoto and Konings, 2003). Lactic acid bacteria and yeasts have been reported to be involved in the fermentation of various indigenous fermented foods and beverages, such as 'mawe' in Benin (Stringini et al., 2009), 'pito' and 'burukuta' in Ghana, Togo and Nigeria (Glover et al., 2005; Sawadogo-Lingani et al., 2010), 'dolo' in Togo and Burkina Faso, 'ogi,' 'palm wine' in West Africa (Olawale et al., 2010; Stringini et al., 2009), and several others. These genera have great significant impact on food quality, such as taste, texture, odour and nutritional value (Aidoo et al., 2005).

Lactic acid bacteria (*Lactobacilli*) in commercially produced traditional banana beer, were 30 and 80 cfu.ml^{-1}, for brand A and brand B respectively (Figure 3). Lactic acid bacteria are widely known for their ability to produce a variety of bacteriocins that possess antimicrobial effects, against a broad range of micro-organisms, including spoilage and food-borne pathogenic bacteria (Basanta et al., 2009; Shizuka et al., 2007). Most of the inhibitory activity, showed by the lactic acid bacteria (LAB) strains, was also attributed to pH reduction by organic acids, lactic acid, and may enhance the safety of food products as well. But some LAB, such as *Lactobacillus brevis, Lactobacillus lindneri*, *Lactobacillus casei* including *Pediococcus damnosus*, are able to spoil beer and are recognized as the most hazardous bacteria for breweries, being responsible for approximately 70% of microbial beer spoilage incidents (Garry et al., 2010; Sakamoto and Konings, 2003; Shizuka et al., 2009; Vaughan et al., 2005).

Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) Analysis

Samples were subjected to genomic DNA extracted directly from the beer samples. Two methods were used to extract genomic DNA which was used as a template to amplify 16S rRNA and 18S rRNA gene fragments. The first extraction method (Labuschagne and Albertyn, 2007) was not successful in producing adequate/intact gDNA for PCR amplification (Figure 3). From the gDNA extracted with the second method (commercial available FastDNA® Spin Kit for Soil) only the 16S rRNA gene region could be amplified from beer sample/brand A (Figure 4, lane 3).

The 1 300 bp 16S amplicon obtained from beer brand A (Figure 4, lane 3) was used as a template in a nested PCR to re-amplify a shorter fragment (~230 bp), modified with a GC clamp (Figure 5), for DGGE analysis. The DGGE analysis of short targeted fragment of the 16S rRNA (~230 bp) revealed limited microbial diversity in beer sample A (Figure 6), where sequencing results of the prominent bands indicated the presence of a Lactic acid bacterial community composed of *Lactobacillus farraginis*, *Lactobacillus diolivorans* and *Lactobacillus hilgardii*.

Lactic acid bacteria have been isolated from various indigenous traditional beer and other beverages. The

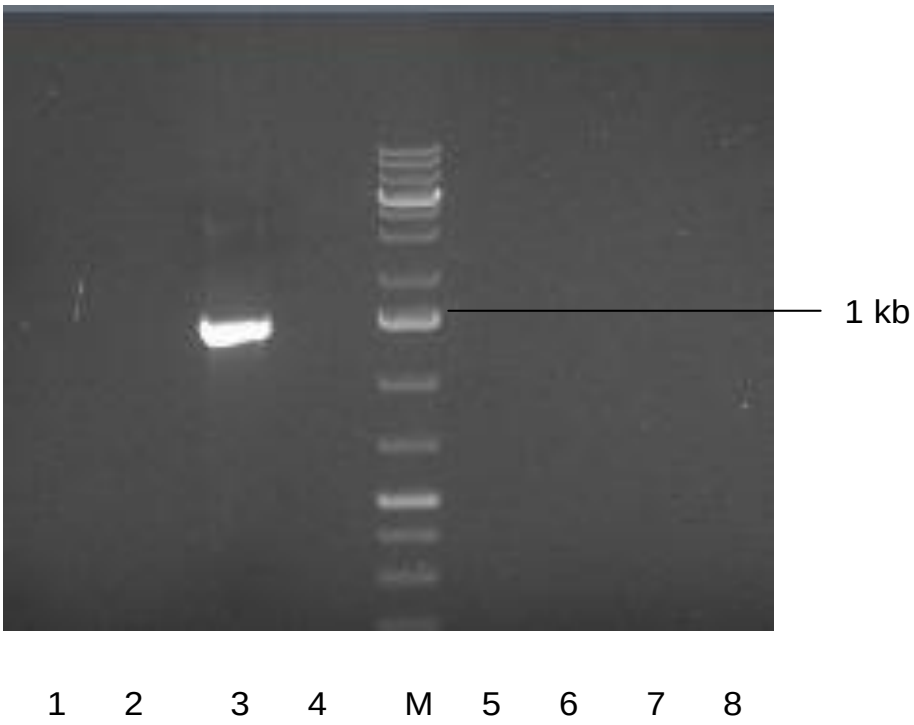

Figure 4. Agarose gel electrophoresis of amplified target genes 16S rRNA (~1300 bp; lanes 1 – 4) and 18S rRNA (~1700 bp; lanes 5 - 8) using primer pairs 63-F and 1387-R and EukA and EukB, respectively. Lanes: 1, 2, 5 and 6 represent amplification attempts using gDNA extracted from both samples A and B with the harsh lysis method. Lanes 3, 4, 7 and 8 represent amplification attempts using gDNA extracted from both brands A and B using the FastDNA® Spin Kit for Soil. Lane M represents the GeneRuler ™ 1 kb DNA Ladder Plus (Fermentas).

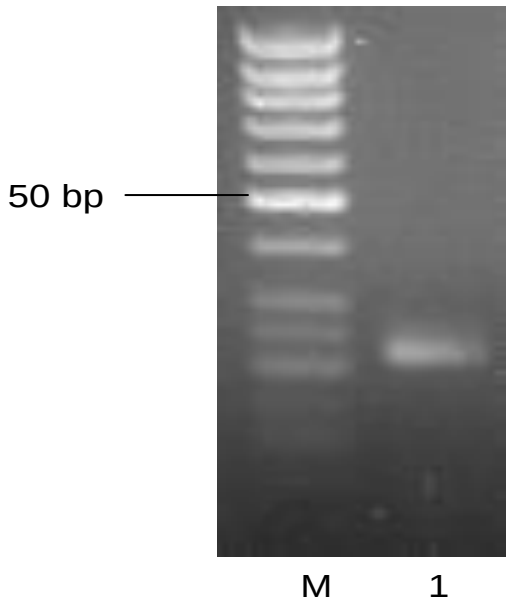

Figure 5. Agarose gel electrophoresis representing the shorter ~230 bp PCR fragment of the 16S rRNA gene amplified using primers 341-F GC and -517-R (lane 1). Lane M represents the GeneRuler ™ 50 bp DNA Ladder plus (Fermentas).

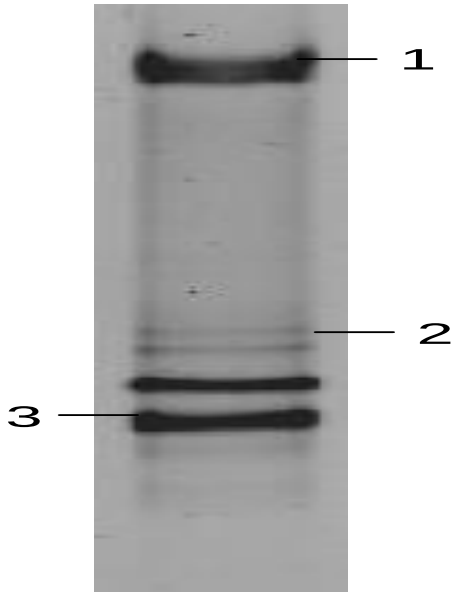

Figure 6. DGGE diversity profile of the bacterial community of beer brand A. Bands labelled 1 to 3 were excised, sequenced and identified as *Lactobacillus farraginis*, *Lactobacillus hilgardii* and *Lactobacillus diolivorans*, respectively.

identified species in this study can impact greatly on the quality of traditional beer in various ways. *L. farraginis* has been recently isolated as novel species from Japanese compost distilled spirit (Shochu) residue (Endo and Okada, 2007). *L. farraginis* strains are heterofermentive bacteria that produce lactic acid, carbondioxide, ethanol or acetic acid from D-glucose (Endo and Okada, 2007), and the characteristic effect on the quality of Japanese Shochu has not yet been reported (Endo and Okada, 2007). *L. diolivorans* species are capable of converting 1,2-propanediol and acetate produced by *L. buchneri* into 1-propanol and propionate (Sriramulu et al., 2008; Zhang et al., 2010). The maize silage inoculated with *L. diolivorans* produced the highest level of propionate in the presence of 1,2-propanediol as sole carbon source (Krooneman et al., 2002). Propionate formation by *L*. *diolivorans* and *L. buchneri* during growth in silage was suggested to prevent fungal formation (Krooneman et al., 2002). The sourdough, co-fermented with *L*. *diolivorans* and *L. Buchneri,* also exhibited increased antifungal properties (Zhang et al., 2010). Use of propionate-producing cultures as a new approach, to replace the addition of propionate as a preservative, has been demonstrated by Zhang et al. (2010).

L. hilgardii have been isolated during the fermentation of *A*. *salmiana* (a Mexican alcoholic beverage obtained from distillation of fermented juices of cooked *Agave* ssp) and Italian red wine (Liu, 2003; Mazzoli et al., 2009). *L*. *hilgardii* strains have been identified as common wine spoilage bacteria (Bossi et al., 2006; García-Ruiz et al., 2009), and even though they are lactic acid-producing micro-organisms, they are also capable of producing acetic acid (Liu, 2003). Excess acetic acid impacts negatively on the quality of the final product, leading to an off-flavour smell (Bvochora and Zvauya, 2001). *L*. *hilgardii* strains are used as indictors for quality control in winemaking and other food processes (Sohier et al., 1999).

Conclusion

Based on culture-dependent analysis the microbiological status of the commercially available traditional banana beers investigated in this study, the results indicated that the processing was carried out under proper good manufacturing, hygienic and sanitary conditions. This is due to the absence of total coliforms, *Salmonella* and *Staphylococcus,* species that are regarded as indicators of poor hygiene and sanitation. This was not entirely unexpected, since the presence of high counts of lactic acid bacteria in commercially traditional banana beers suppresses the growth of pathogenic bacteria, such as enterotoxigenic *E. coli*, *Shigella flexneri*, *Salmonella typhimurium*, *Bacillus cereus*, and *Campylobacter jejuni* (Adebayo and Aderiye, 2007; Erdoğrul and Erbilir, 2006; Tetteh et al., 2004). The inability to extract useable gDNA for 16S and 18S rDNA amplification and limited diversity, was somewhat disappointing. The possible reason for the difference in the results obtained between the culture PCR methods may be the difference in the preparation regimes of the two products, which could introduce compounds such as PCR inhibitors into the products. Reports of PCR amplifications, in previous studies, have indicated discrepancies in findings originating from such inhibitors (Booysen, 2007; Oikarinen et al., 2009). The introduction of foreign DNA into the product during processing, that may influence molecular methods, is also not excluded.

There is clearly a need to investigate the applicability of available extraction methods for this traditional product, to maximize diversity detection. Even though the result obtained provides an incomplete interpretation of the microbial diversity of traditional banana beer, it is the first report on microbial communities present in this product. The results are, however, in agreement with culture-based methods, where lactobacillus was identified also with molecular methods.

REFERENCES

Adebayo CO, Aderiye BI (2007). Ecology and antibacterial potential of lactic acid bacteria associated with fermented cereals and cassava. Res. J. Microbiol. 2(5):426-435.

Aidoo KE, Nout MJR, Sarkar PK (2005). Occurrence and function of yeasts in African indigenous fermented foods. FEMS Yeast Res. 6:30-39.

Almeida EG, Rachid CCTC, Schwan RF (2007). Microbial population present in fermented beverage 'cauim' produced by Brazilian Amerindians. Int. J. Food Microbiol. 120(1-2):146-151.

Amusa NA, Odunbaku OA (2009). Microbiological and nutritional quality of hawked kunun (a sorghum based non-alcoholic beverage) widely consumed in Nigeria. Pak. J. Nut. 8(1):20-25.

Basanta A, Herranz C, Gutiérrez J, Criado R, Hernández PE, Cintas LM (2009). Development of bacteriocinogenic strains of *Saccharomyces cerevisiae* heterologously expressing and secreting the leaderless enterocin L50 peptides L50A and L50B from *Enterococcus faecium* L50. App. Environ. Microbiol. 75(8):2382-2392.

Beal JD, Niven SJ, Campbell A, Brooks PH (2002). The effect of temperature on the growth and persistence of salmonella in fermented pig feed. Int. J. Food Microbiol. 79:99-104.

Bell C, Kyriakides A (1998). *E. coli*, A practical approach to the organism and its control in foods. An Imprint of Chapman & Hall. London, UK.

Booysen E (2007). A qualitative and quantitative assessment of current methodologies for the identification of *Escherichia coli* in foodstuffs in South Africa. Doctor of Technologiae: Environmental Health. Central University of Technology, Free State, Bloemfontein, South Africa.

Bossi A, Rinalducci S, Zolla L, Antonioli P, Righetti PG, Zapparoli G (2006). Effect of tannic on *Lactobacillus hilgardii* analysed by a proteomic approach. J. App. Microbiol. 102(3):787-795.

Bvochora JM, Zvauya R (2001). Biochemical changes occurring during the application of high-gravity fermentation technology to the brewing of Zimbabwean traditional opaque beer. Proc. Biochem. 37(4):365-370.

Cetinkaya F, Cibik R, Soyutemiz GE, Ozakin C, Kayali R, Levent R (2008). *Shigella* and *Salmonella* contamination in various foodstuffs in Turkey. Food Con. 19:1059-1063.

Christen GL, Davidson PM, McAllister JS, Roth LA (1993). Coliform and other indicator bacteria. In: R.T. Marshall (Ed.). Standard methods for the examination of dairy products. Washington DC: American Public Health Association pp.247-269.

Collins CH, Lyne PM, Grange J (1995). Collins and Lyne's microbiological methods. London: Butterworth-Heinemann.
Davies G (1993). Domestic banana beer production in Mpigi District, Uganda. Infomusa 2:12-15.
Díez M, Pedrós-Alió C, Marsh TL, Massana R (2001). Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparision of DGGE with other molecular techniques. App. Environ. Microbiol. pp. 2942-2951.
Elmahmood AM, Doughari JH (2007). Microbial quality of 'kunun-zaki' beverage sold in Girei town of Adamawa State, Nigeria. Afr. J. Food Sci. pp. 011-015.
Endo A, Okada S (2007). *Lactobacillus farraginis* sp. Nov. and *Lactobacillus parafarraginis* sp.Nov., heterofermentative lactobacilli isolated from a compost of distilled shochu residue. Int. J. Syst. Evol. Microbiol. 57:708-712.
Erdoğrul Ö, Erbilir F (2006). Isolation and characterization of *Lactobacillus burgaricus* and *Lactobacillus casei* from various foods. Turk J. Biol. 30:39-44.
Frank JF, Christen GL, Bullerman LB (1993). Tests for groups of micro-organisms. In: R.T. Marshall (Ed.). Standard methods for the examination of dairy products. Washington DC: American Public Health Association pp.271-286.
García-Ruiz A, Bartolomé B, Cueva C, Martín-Álvarez PJ, Moreno-Arribas MV (2009). Inactivation of oenological lactic acid bacteria (*Lactobacillus hilgardii and pediococcus pentosaceus*) by wine phenolic compounds. Int. J. Food Microbiol. 107:1042-1053.
Garry M, Andrighetto C, Lombardi A, Corich V, Aldred P, Vriesekoop F (2010). Isolation, identification and characterisation of beer-spoilage lactic acid bacteria from micro-brewed beer from Victoria, Australia. J. Ins. Brew. 116:14-22.
Glover LKR, Abaidoo RC, Jakobsen M, Jespersen L (2005). Biodiversity of *Saccharomyces cerevisiae* isolated from a survey of 'pito' production sites in various parts of Ghana. Syst. App. Microbiol. 28:755-761.
Haggblade S, Holzopfel WH (1989). Industrialization of Africa's indigenous beer brewing. In: Steinkraus K H, ed. Industrialization of indigenous fermented foods, Marcel Dekker Inc, New York pp.191-284.
Hayes MC, Ralyea RD, Murphy SC, Carey NR, Scarlett JM, Boor KJ (2001). Identification and characterization of elevated microbial counts in bulk tank raw milk. J. Dairy Sci. 84(1):292-298.
Helander LM, Von Wright A, Mattlia-Sandholm TM (1997). Potential of lactic acid bacteria and novel antimicrobial against Gram-negative bacteria. Trends Food Sci. Technol. 8:146-150.
Herbert RA (1990). Methods for enumerating micro-organisms and determining biomass in natural environments. In: R. Grigorova & J.R. Norris (Eds.). Methods of microbiology. New York: Academic Press pp. 1-39.
Houghtby GA, Maturin LJ, Koenig EK (1993). Microbial count methods. In: R.T. Marshall (Ed.). Standard methods for the examination of dairy products. Washington DC: American Public Health Association pp. 213-246.
Ikalafeng BK (2008). Microbiota and mycotoxins in traditional beer of the greater Kimberley area and associated brewing and consumption practices. Doctor of Technologiae: Environmental Health. Central University of Technology, Free State, Bloemfontein, South Africa.
Jespersen L (2003). Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages. FEMS Yeast Res. 3(2):191-200.
Kao Y, Liv Y, Shyu Y (2007). Identification of *Lactobacillus* spp in probiotic products by real-time PCR and melting curve analysis. Food Res. Int. 40:71-79.
Kebede A, Beyene F, Langsrud T, Narvhus JA (2002). Indigenous processing methods and raw materials of borde an Ethiopian traditional fermented beverage. J. Food Technol. Afr. 7:59-64.
Krooneman J, Faber F, Alderkamp AC, Oude-Elferink SJHW, Drieuhuis F, Cleenwerck Swings J, Gottschal JC, Vancanneyt M (2002). *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. Int. J. Syst. Evol. Microbiol. 52:639-646.
Kuene NF, Hastings JW, Von Holy A (1999). Bacterial population associated with a sorghum–based fermented weaning cereal. Int. J. Food Microbiol. 49:75-83.
Kutyauripo J, Parawira W, Tinofa S, Kudita I, Ndengu C (2009). Investigation of shelf-life extension of sorghum beer (Chibuku) by removing the second conversion of malt. Int. J. Food Microbiol. 129:271-276.
Labuschagne M, Albertyn Jl (2007). Cloning of an epoxide hydrolase-encoding gene from *Rhodotorula mucilaginosa* and functional expression in *Yarrowia lipolytica*. Yeast 24(2):69-78.
Lee YG, Wu HY, Hsu CL, Liang HJ, Yuan CJ, Jang HD (2009). A rapid and selective method for monitoring the growth of coliforms in milk using the combination of amperometric sensor and reducing of methylene blue. Sensors Actuators B 141:575-580.
Liu SQ (2003). Practical implication of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentation. Int. J. Food Microbiol. 83:115-131.
Loureiro V (2000). Spoilage yeasts in foods and beverages: Characterisation and ecology for improved diagnosis and control. Food Res. Int. 3:247-256.
Lues JFR, Ikalafeng BK, Maharasoa M, Shale K, Malebo NJ, Pool E (2011). *Staphylococci* and other selected microbiota associated with indigenous traditional beer. Afr. J. Microbiol. Res. 5(13):1691-1696.
Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SC, Wade WG (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. App. Environ. Microbiol. 64(2):795-799.
María BM, Martín R, Sanz A, Hernández PE, González I, García T (2005). Detection of *Kluyveromyces marxianus* and other spoilage yeasts in yoghurt using a PCR-culture technique. Int. J. Food Microbiol. 105:27-34.
Mazzoli R, Lamberti C, Ciosson JD, Purrotti M, Castillo M, Giuffrida MG, Giunta C, Pessione E (2009). Influence of ethanol, malate and arginine on histamine production of *Lactobacillus hilgardii* isolated from Italian red wine. Amino Acids 36:81-89.
Mensah P, Tomkins AM, Drasar BS, Harrison TJ (1991). Antimicrobial effect of fermented Ghanaian maize dough. J. Appl. Microbiol. Biotechnol. 70:203-210.
Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A (2002). Street foods in Accra, Ghana: How safe are they? Bul. World Health Org. 80(7):546-334.
Mugula JK (2001). Microbiology, fermentation and shelf life extension of *togwa*, a Tanzanian indigenous food. *Ph.D. Thesis*. Department of Food Science, Agricultural University of Norway, Ås, Norway.
Mukantwali C, Shingiro JB, Dusengemungu L (2008). Banana production, post harvest and marketing in Rwanda. Traditional banana juice extraction method in Eastern province in Rwanda. Kigali Institute of Science and Technology, Technical Report.
Muyanja CMBK, Narvhus JA, Treimo J, Langsrud T (2003). Isolation, characterization and identification of lactic acid bacteria from bushera: A Ugandan traditional fermented beverage. Int. J. Food Microbiol. 80:201-210.
Muyzer G (1999). DGGE/TGGE a method for identifying genes from natural ecosystems. Curr. Opin. Microbiol. 2:317-322.
Muyzer G, Brinkhoff T, Nubel U, Santegoeds C, Schafer H, Wawer C (1997). Denaturing gradient gel electrophoresis (DGGE) in microbial ecology, pp. 1–27. *In* A. D. L. Akkermans, J. D. van Elsas, and F. J. de Bruijn(ed.) Molecular microbial ecology manual, vol. 3.4.4. Kluwer Academic Publishers, Dordrecht, The Netherlands.
Muyzer G, Waal ECD, Uitterlinden AG (1993). Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59(3):695-700.
Mwesige PK, Okurut TO (1995). A survey of the production and consumption of traditional alcoholic beverages in Uganda. Proc. Biochem. 30(6):497-501.
Namugumya BS, Muyanja CMBK (2009). Traditional processing, microbiological, physiochemical and sensory characteristics of 'kwete', Ugandan fermented maize based beverage. AJFAND pp. 1684-5374.
Naumova ES, Korshunova IV, Jespersen L, Naumov GI (2003). Molecular genetic identification of *Saccharomyces* sensu stricto

strains from African sorghum beer. FEMS Yeast Res. 3(2):177-184.
Novellie L, de Schaepdrijver P (1986). Modern developments in the traditional African beers. In: Admas M.R., Ed. Progress in Industry Microbiology, Science Amsterdam 23:73-157.
Nsabimana A, Vas Staden J (2007). Assessment of genetic diversity of highland bananas from the National Banana Germplasma collection at Rubona, Rwanda using RAPD markers. Sci. Hortic. 113:293-299.
Nzagamasabo A, Nimpagaritse A (2009). Traditional fermented foods and beverages in Burundi. Food Res. Int. 42:588-594.
Oikarinen S, Taurianen S, Viskari H, Simell O, Knip M, Virtanen S, Hyöty H (2009). PCR inhibitors in stool samples in relation to age of infants. J. Clin. Virol. 44:211-214.
Olawale AK, Akinbiyi AO, David Oluwole DM (2010). Evaluation of microbial quality and alcoholic improvement of natural and fermented Raphia Palmwine ('ogoro'). New York Sci. J. 3(2):35-39.
Oyedeji O, Olutiola PO, Moninuola A (2010). Microbiological quality of packaged drinking water brands marketed in Ibadan metropolis and Ile-Ife city in South Western Nigeria. Afr. J. Microbiol. Res. 4(3):169-179.
Pishawikar MS, Singhal RS, Kulkarni PR (1992). Rapid non-microbiological methods for detecting microorganisms in foods. Trends Food Sci. Technol. 3:165-169.
Roy A, Moktan B, Sarkar KP (2007). Microbiological quality of legume-based traditional fermented foods marketed in West Bengal, India. Food Control 18(11):1405-1411.
Sakamoto K, Konings WN (2003). Beer spoilage bacteria and hop resistance. Int. J. Food Microbiol. 89: 105-124.
Salo S, Wirtanen G (2005). Disinfectant efficacy on food-borne spoilage yeast strains. Food Bio-prod. Proc. 83(C4):288-296.
Sawadogo-Lingani H, Diawara B, Glover RK, Tano-Debrah K, Traoré AS, Jakobsen M (2010). Predominant lactic acid bacteria associated with the traditional malting of sorghum grains. Afr. J. Microbiol. Res. 4:169-179.
Shizuka A, Kazumaru I, Suzuki K, Motoyama Y, Ogata Y, Kitagawa Y (2009). Rapid detection and identification of beer spoilage lactic acid bacteria by micro-colony method. J. Biosci. Bioeng. 108(2):124-129.
Shizuka A, Suzuki K, Kazumaru I, Motoyama Y, Kuriyama H, Kitagawa Y (2007). Effects morphological changes in beer-spoilage lactic acid bacteria on membrane filtration in Breweries. J. Biosci. Bioeng. 104(4):334-338.
Sohier D, Coulon J, Lonvaud-Funel A (1999). Molecular identification of *Lactobacillus hilgardii and* genetic relatedness with *Lactobacillus brevis*. Int. J. Syst. Bacteriol. 49:1075-1081.
Sriramulu DD, Liang M, Hernandez-Romero D, Raux-Deery E, Lnsdorf H, Parsons JB, Warren MJ, Prentice MB (2008). *Lactobacillus reuteri* DSM 20016 produces cobalamin-dependent diol dehydratase in metabolosomes and metabolizes 1,2-propanediol by disproportionation. J. Bacteriol. 190:4559-4567.
Stringini M, Comitini F, Taccari M, Ciani M (2009). Yeast diversity during tapping and fermentation of palm wine from Cameroon. Food Microbiol. 26:415-420.
Tadesse G, Ashenafi M, Ephraim E (2005). Survival of *E. coli* 015:H7, *Staphylococcus aureus, Shigella Flexneri* and *Salmonella* spp. in fermenting 'borde', a traditional Ethiopian beverage. Food Control 16:189-196.
Tetteh LG, Sefa-Dedeh SK, Dixon RP, Beuchat LR (2004). Survival and growth of acid-adapted and un-adapted *Shigella flexneri* in traditional fermented Ghanaian weaning food as affected by fortification with cowpea. Int. J. Food Microbiol. 90:189-195.
Vaughan A, O'Sullivan T, Sinderen D (2005). Enhancing the microbiological stability of malt and beer - A Review. J. Inst. Brew. 111:355-375.
Vorster SM, Greebe RP, Nortje GL (1994). Incidence of *Staphylococcus aureus* and *Escherichia coli* in ground beef, broilers and processed meats in Pretoria, South Africa. J. Food Prot. 57(4):305-310.
Wutor VC, Togo CA, Pletschke BI (2009). Suitability of total coliform β-D-galactosidase activity and CFU counts in monitoring faecal contamination of environmental water samples. Water South Africa 35(1):85-88.
Zhang C, Brandt MJ, Schwab C, Gänzle MG (2010). Propionic acid production by co-fermentation of *Lactobacillus buchneri* and *Lactobacillus diolivorans* in sourdough. Food Microbiol. 27:390-395.