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Microbes associated with fermentation of home-made cereal based beverages in Tanzania

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Abstract

Spontaneous fermentation has been used in Tanzania and other African countries for preservation and processing of food for many centuries. Most of the fermented cereal-based beverages in Tanzania lack information on quality aspects and functionality when ingested in the human gut. In addition, and specifically, studies on microflora taking part in the fermentation of locally available cereal beverages in Tanzania are scarce and microbes isolated and identified from such beverages are also limited. An experiment involving culturing, isolation and, morphological, physiological and biochemical characterisation of fermenting microbes from locally made fermented samples of beverages namely Kindi, Kimpumu, Togwa and Mbege originating from Mbeya, Kilimanjaro and Morogoro regions was conducted from January to August 2019. The results revealed that fermentation of Togwa involves activities by *L. pentosus, L. brevis, L. plantarum, L. lactis ssp. lactis* and *C. tropicalis* whereas fermentation of Kindi involves activities of *P. pentosaceus, Cryptococcus gattii, C. tropicalis* and *R. minuta*. Microbial species taking part in Kimpumu fermentation are *L. plantarum, C. zeylanoides, C. albicans*, and *C. tropicalis* and those in Mbege are *L. plantarum, C. ciferri,* and *C. dubliniensis*. The presence of probiotic Lactic Acid Bacteria (LAB) in the four local beverages indicate the potentiality of such beverages as fermented probiotic cereal-based beverages in Tanzania.

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Introduction

Beneficial microbes associated with fermentation of cereal-based beverages are normally of diverse nature and originate from different genera of bacteria and fungi (Oyedeji et al., 2013; Enujiugha and Badejo, 2017). Lactic acid bacteria (LAB) has been reported to comprise a major genus of bacteria which is involved in cereal fermentation (Achi and Asamudo, 2019; Aka *et al*., 2014; Blandino *et al*., 2003; Mokoena *et al*., 2016; Mukisa *et al*., 2017) whereas the most predominant group of fungi involved in such fermentation is yeast (Mukisa *et al*., 2017; Ogunremi *et al*.,2015; Todorov and Holzapfel, 2014). For many decades milk and milk products have been almost exclusively used as vehicles for beneficial microbes (probiotics) delivery to human being (Bansal *et al*., 2015; Dey, 2018; Nyanzi and Jooste, 2012; Salmerón, 2017). However, the growing concern in the world over increased lactose intolerance, cholesterol content of milk, milk allergy, vegetarianism, veganism, cultural food taboos, and religious belief has led to more research on other food matrices in order to overcome such limitations (Angelov *et al*., 2006; Bansal *et al*. ,2015; Gupta & Bajaj, 2017). Cereal grains are among the food matrices being investigated as possible delivery media for probiotics (Angelov *et al*., 2006; Rivera-Espinoza and Gallardo-Navarro, 2010; Gupta and Kumar Bajaj, 2016; Panghal *et al*., 2018). Cereals are important to human being as they contribute 60% of the world food production and contribute significantly in the supply of important nutrients to human being such as carbohydrates, protein, fiber, minerals and vitamins (Cheryl *et al*., 2018; Shori, 2016; Todorov and Holzapfel, 2014). Cereals occupy 73% of the world's total cultivated area (Todorov and Holzapfel, 2014).

As early as 1800s scientists began noticing the beneficial effects of consuming fermented milk though no scientific explanation was put forward at that time (McFarland, 2015).

In the same period, a renowned scientist Louis Pasteur discovered bacteria and yeasts responsible for milk fermentation but failed to explain the health effects such microbes impart to human being (McFarland, 2015). In mid 1800s a Russian scientist Elie Metchnikoff showed the link between the *Lactobacilli* present in the human gut and the longevity of life for people who consumed fermented milk (McFarland, 2015).

In a decade 1922-1932 human studies were carried out on the use of *Lactobacillus acidophilus* and positive health effects were observed in people with chronic diarrhea, constipation and mental disease. In the following years the study of beneficial microbes fermenting food slowed down significantly and resumed again in the years 1950s – 1980s focusing on screening potential beneficial microbes or probiotics isolated from nature and human being (McFarland, 2015).

Probiotics are "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002; Gil-Rodríguez *et al*., 2015; Marsh *et al*., 2014; Murevanhema and Jideani, 2013; WHO, 2001). More research on probiotic strains shed more knowledge on the complex interactions of colon flora and their bactericidal actions against colonization by pathogenic bacteria (McFarland, 2015).

The study and effective research on probiotics gained momentum in the decade 1980-1989 through clinical trials and from that period to the year 2014, more than a thousand articles have been published on probiotics (McFarland, 2015).

In Tanzania and many other African countries, fermentation has been used as a food processing and preservation method from time immemorial (Nyanzi and Jooste, 2012; Franz *et al*., 2014). However, this has been done without the knowledge of what causes and the type of microorganisms involved in food fermentations (Olasupo *et al*., 2010). Most of the fermented foods particularly cereal-based beverages have been produced through spontaneous fermentation which make them of unpredictable quality and functionality when ingested in the human

gut (Aka *et al.*, 2014). Such beverages have been produced without knowing the mechanism involved and the byproducts of the process as it was just an art handed down from one generation to another (Anukam and Reid, 2009; Olasupo *et al.*, 2010).

There is a wide variety of fermented local alcoholic and non-alcoholic cereal beverages in Tanzania. These include Kimpumu (made from finger millet), Togwa (maize, finger millet), Kindi (maize, finger millet), Mbege (finger millet and ripe bananas), Mapuya (maize, millet or sorghum), Komoni (Maize) and others just to mention a few. These beverages are prepared at household level particularly by women.

Most of the cereal beverages in Tanzania are produced through the saccharification of starch from cereals and germinated cereals which provide cereal malt (Kitabatake *et al.*, 2003). Of the many types of local fermented beverages, those made from cereal malt; especially millet malt is the commonest and considered original among others. These drinks have long been in existence and are popular in the communities. Regardless of their important role they play in the society; alcoholic and non-alcoholic beverages in Tanzania are still much turbid than modern drinks because of the crude filtration done after fermentation (Kitabatake *et al.*, 2003). The beverages are produced in various parts of Tanzania and may differ slightly in the way they are prepared particularly on the use of additives, ingredient varieties and mixing ratios as well as fermentation times.

In Tanzania studies on microflora taking part in the fermentation of locally available cereal beverages are scarce and microbes isolated and identified from such beverages are also limited. Attempts to use beneficial microbes (probiotics) in Tanzania to impart health benefits to people was only done in 2004 by the West Heads East (WHE) project in Mwanza at Mabatini area to improve the health condition of people diagnosed with HIV/AIDS (Reid, 2010). However, the bacteria used namely *Lactobacillus rhamnosus GR-1* was not isolated from indigenous sources. As such this study focused on isolation and identification of microflora taking part in the fermentation of four cereal-based beverages namely Kindi, Kimpumu, Togwa and Mbege with the objective of knowing the dominant genera of bacteria and yeast involved in the cereal fermentation of the beverages and their diverse species and phenotypic characteristics in order to determine the probiotic potential of the beverages in Tanzania.

Materials and methods

Survey and sampling procedure

Collection of samples of each cereal-based beverage (Kimpumu, Kindi, Togwa, and Mbege) was done from local producers in Mbeya, Morogoro and Kilimanjaro regions. One district per region was purposively selected from which one village was identified based on availability of the local beverage. In each of the village, four samples (each 100ml) from different producers were aseptically collected using a sterile syringe for each type of beverage. Glass bottles with the samples were labelled and kept in a cool box for transportation to the microbiology laboratory at the Sokoine University of Agriculture Morogoro, Tanzania. In the lab, pH of each sample was measured and the average recorded. Samples were then stored at +4^{o}C for further analysis.

Enrichment, Growth and Isolation of Microbes

For each of the collected beverage samples, four enrichment broths (each 20ml) were prepared by using Nutrient Broth (Oxoid, UK) following the manufacturer's protocol. Then 16 bottles containing the broth were sterilized and cooled in water bath at 45^{o}C for 10 to 15 minutes.

The broths were inoculated with 1ml of the stock sample such that each beverage sample had four enrichment solutions which were then incubated at 37^{o}C for 24 hours. Thereafter, 0.5µl of each of the enrichment culture was inoculated on plates with solid Nutrient agar; NA (Oxoid, UK) and incubated at 37^{o}C. Each sample being studied had four replications and observations of the growing microbes on agar were done after 24hrs, 48hrs, 72hrs and 96hrs.

Growth on NA was intended to be a yardstick to gauge the presence/absence of microbes in the four beverages. Simultaneously, slants were prepared in universal bottles using Nutrient Agar and individual isolates of the selected colonies were slanted and preserved at + 4°C for further analysis.

Preliminary Identification of Microbes

Macro-observations by sub culturing of microbes: Agar plates containing Nutrient Agar, Sabouraud Dextrose Agar (Oxoid, UK), De Man Rogosa and Sharpe Agar (Biomérieux SA, France) and MacConkey Agar (Himedia Laboratories, India) were prepared according to the manufacturers' protocols as well as routine lab techniques and inoculated with isolates from the slants. The plates were labelled and incubated at 37°C for 24-48 hours. The colonies were then observed (macro-morphology) using naked eyes for appearance or colour, edge and degree of growth. Information recorded included appearance (whitish or creamy), edge (rough, smooth, round).

Micro observations (Micro-morphology): Smears of the isolates were prepared on microscope slides and stained following routine laboratory Gram Stain procedure. The slides were finally observed under a light microscope (Model CX21FS1, Olympus Corp. Tokyo, Japan) by using 100x objective lens. Micro-observations provided information on microbial shape (rod, ovoid, coccus), Gram Stain reaction (purple or red), microbial arrangement (single, pair, triple, chains) and facilitated selection of working strains through repeated streaking of a single colony on selective media to obtain pure isolates. Purification of isolates from a mixed culture paved the way for actual observation of isolates morphological characteristics such as shapes, arrangements, Gram Stain status, and some other phenomena like similarities and differences among them. These characteristics were recorded and used as criteria to make initial distinction among the isolates (purple colour- Gram positive microbes, red colour – Gram negative microbes, rod-shaped – likely lactobacilli and spherical shaped – likely yeasts) pending further tests to presume and affirm identity of isolates. Similarity of microbes led to random selection of representative isolates for further analysis. The results were recorded in tabular form.

Presumptive identification of isolates to genus level

Probable identification of microbial isolates to genus level was done based on key morphological and physiological characteristics such as appearance, shape, size and growth as described by Desiye and Abegaz (2013). Isolates were tentatively identified as Lactobacilli based on methods adopted by other researchers (Desiye and Abegaz, 2013; Kavitha *et al.*, 2016; Deshpande *et al.*, 2017) and use of Bergey's Manual of Systematic Bacteriology (Hammes and Hertel, 2009). Isolates were tentatively identified as yeasts based on morphological characterisation by Kurtzman *et al.* (2011). Briefly, yeast colonies were mucoid, glittering, creamy colonies with entire edge. Presumptive yeasts also grew on Sabourad Dextrose Agar (SDA) as dome-like colonies, were Gram positive, spherical or ovoid in shape with a fermenting malt smell. Tentative identification to genus level facilitated the grouping of the isolates into two groups; lactobacilli and yeasts for appropriate identification using the API® systems.

Identification of isolates to genus and species level using Analytical Profile Index (API® systems)

Gram positive rods presumed to be Lactobacillus spp. were identified using the API® 50 CHL medium and the API® 50 CH strips following the procedure described by the manufacturer; Biomérieux SA, Marcy - l'Etoile, France. Likewise, Gram positive, ovoid or spherical cells with the likelihood of being yeasts were identified with the aid of API® 20 C AUX which comprises the API® 20 C AUX strips and the API® C medium and following the manufacturer's procedure. The identification results were then tabulated.

Results

Acidity or pH measurement

The pH of the cereal-based fermented beverages; Kindi, Kimpumu, Togwa and Mbege were 3.6, 4.0, 3.17 and 4.0, respectively.

Table 1. Morphological, Physiological and Biochemical characterisation of LAB isolates.

	LAB Isolates and results										
Characteristics	T1a (3)	T2b	T3d	KM2a (3)	KM4b (1)	KD1a (2)	KD2b	KD4c (2)	MB1a (2)	MB2b	MB4d (2)
Morphological											
Colour/appearance	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish	Whitish
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	+	+	+	+	+	+	+	+	+	+	+
Physiological											
Growth at different temperatures											
15°C	+	+	+	+	+	+	+	+	+	+	+
45°C	-	-	-	-	-	+	+	+	-	-	-
Growth in NaCl											
4%	-	-	-	-	-	+	+	+	-	-	-
6.5%	-	-	-	-	-	+	+	+	-	-	-
Catalase test	-	-	-	-	-	-	-	-	-	-	-
Biochemical (49 sugars involved only important shown)											
N-AcetylGlucosamine	+	+	+	+	+	+	+	+	+	+	+
Amygdalin	+	+	+	+	+	+	+	+	+	+	+
Arbutin	+	+	+	+	+	+	+	+	+	+	+
Esculine ferric citrate	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	+	+	+	+	+	+	+	+	+
D-Cellobiose	+	+	+	+	+	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+	+	+	+	+
D-Melibiose	+	+	+	+	+	+	+	+	+	+	+
D-Saccharose	+	+	+	+	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+

Note: + = positive, - = negative. T1a, T2b, ...MB2b, MB4d = codes for *Lactobacillus* isolates.
T1a (3) = *L. pentosus*, T2b = *L. plantarum*, T3d = *L. brevis*, KM2a (3) = *L. plantarum*, KM4b (1) = *L. plantarum*, KD1a (2) = *P. pentosaceus*, KD2b = *P. pentosaceus*, KD4c (2) = *P. pentosaceus*, MB1a (2) = *L. plantarum*, MB2b = *L. plantarum*, MB4d (2) = *L. plantarum*. T3c = *Lactococcus lactis ssp. lactis* not in the table. Bracketed number like (3) shows number of other isolates with same identification.

Isolation

Using NA, a total of 64 bacterial isolates were obtained from cereal-based fermented beverages collected from different locations in Tanzania.

Macro observation on various agar media

The plates after 24-48 hours of incubation at 37°C were macro-morphologically observed. It appeared that all colonies growing on Nutrient Agar (NA), De Man Rogosa and Sharpe Agar (MRS) and Sabourad Dextrose Agar (SDA) were smooth round colonies with whitish or creamy colour but colonies growing on MacConkey agar appeared smooth and colourless or transparent. The degree of growth of microbes on all media was higher. Appearances of colonies are given in Table 1 and 2.

Micro-observations

The Gram Stain test results indicate that Togwa and Mbege isolates contain both Gram-positive and Gram-negative microbes. However, Gram negative microbes are not found in Kindi and Kimpumu isolates.

Gram positive rods (singles, pairs, triples) grew predominantly in NA and MRS for all sample isolates whereas Gram positive, ovoid cells (singles, pairs, triples, clusters) grew mostly in SDA for all isolates. Gram negative coccoid rods which are colorless and transparent grew predominantly in MacConkey agar for Togwa and Mbege isolates only. Of the 64 isolates microscopically observed; 12 were Gram negative coccoids and 52 were Gram positive rods and ovoid

cells. Table 1 summarizes characteristics of 27 LAB isolates and Table 2 summarizes characteristics of 25 yeast isolates.

Microbial Isolates Identification to Genus and Species Level

Identification of Lactobacillus species

After obtaining the biochemical profiles of the isolates and identifying the strains; the results indicate that Togwa isolates contain *Lactobacillus* spp. namely *Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus plantarum,* and *Lactococcus lactis ssp. lactis*. In the case of Kindi isolates; these mostly contain a LAB called *Pediococcus pentosaceus*. The species identified in Kimpumu isolates is *L. plantarum* while in Mbege isolates the microbe identified is *L. plantarum* as well. Table3 summarizes the results narrated.

Table 2. Morphological and Biochemical characterisation of yeast isolates.

	Yeast Isolates and results											
Characteristics	YIA (2)	YIB (2)	YIC	YID	YIE (1)	YIF	YIG	YIH (2)	YII (1)	YIJ (2)	YIK (1)	YIL (2)
Morphological												
Colour/appearance	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy	Creamy
Shape	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+
Biochemical (19 sugars involved only important shown)												
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	+	+	+	-	+	+	+	+	+
D-Adonitol	+	+	+	-	-	-	-	+	-	+	-	+
Xylitol	-	-	-	-	-	-	-	+	-	+	-	+
D-Galactose	+	+	+	+	-	+	+	+	-	+	+	+
D-Maltose	+	+	+	+	-	+	+	+	-	+	+	+
D-Saccharose	-	-	-	+	-	+	+	-	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+	-	+
D-Melezitose	+	+	+	+	-	-	-	-	-	-	-	-
D-Raffinose	-	-	-	+	-	-	-	-	-	-	-	-

Note: + = positive, - = negative. YIA, YIB, ...YIK, YIL = codes for yeast isolates.

YIA (2) = *C. tropicalis*, YIB (2) = *C. tropicalis*, YIC = *C. tropicalis*, YID = *C. tropicalis*, YIE (1) = *Candida zeylanoides*, YIF = *Candida albicans*, YIG = *Cryptococcus gattii*, YIH (2) = *C. tropicalis*, YII (1) = *Rhodotorula minuta*, YIJ (2) = *Candida ciferrii,* YIK (1) = *Candida dubliniensis,* YIL (2) = *C. ciferrii.* Numbers in brackets like (2) shows number of other isolates with same identification.

Identification of yeasts/fungi

The seven-digit numerical profiles of yeasts were key in identification of yeast species or strains.

The results indicate that Togwa isolates were proliferated by *Candida tropicalis*; Kindi isolates showed growth of *Cryptococcus gattii*, *Candida tropicalis* and *Rhodotorula minuta*. On the other hand, *Candida zeylanoides*, *Candida albicans*, and *Candida tropicalis* were identified from Kimpumu isolates while *Candida ciferrii* and *Candida dubliniensis* were identified from Mbege isolates. These results are depicted in Table 4.

Discussion

The low acidity or pH of all the fermented cereal beverages sampled implies that the production of such beverages results to products and by products with low pH values. Most microbes grow well at optimal pH values or neutral pH values: 6.5-7.0 (James *et al.*, 2005) than those observed in the sampled beverages. It has been empirically shown that some microbes such as LAB can grow and survive at pH values ranging from 5 and lower (Waters *et al.*, 2015). LAB activities during the fermentation of carbohydrates also produces lactic acid as the main end product thus decreasing the pH of the resulting

product (Achi and Asamudo, 2019; Kohajdová and Karovičová, 2007) .On the other hand, literature indicates that yeasts grow well in a wide range of acidic pH (Mukisa *et al.*, 2017; Todorov and Holzapfel, 2014; Kurtzman *et al.*, 2011). LAB and yeasts have also been observed to be the major groups of microorganism which participate in the fermentation of most of the cereal based foods and beverages (Aka *et al.*, 2014; Achi and Asamudo, 2019).

Table 3. *Lactobacillus* species in beverages.

Beverage type	Fermenting species
Togwa	*Lactobacillus pentosus*
	Lactobacillus brevis
	Lactobacillus plantarum
	Lactococcus lactis ssp. lactis
Kindi	*Pediococcus pentosaceus*
Mbege	*Lactobacillus plantarum*
Kimpumu	*Lactobacillus plantarum*

The presence of low pH in the end products due to production of lactic acid and other organic acids is ideal for their growth and exponential increase. Consequently, the observation of low pH values in the four sampled local cereal-based beverages indicate the possibility of the predominant growth and proliferation of acidophilic microbes such as LAB and yeasts during the spontaneous fermentations.

Following enrichment of beverage samples; more diverse microbial growth on plates of Nutrient agar was expected with prolonged incubation time; however, this never happened as the degree of growth of the same type of colonies persisted. The existence of similar colonies for all beverage samples on NA showed the possibility of having the same types of microflora participating in the fermentation of the respective beverages. Macro observation showed absence of colonies with red or pinkish colour (characteristic of lactose fermenters) on MacConkey agar implying that lactose fermenters particularly Enterobacteriaceae such as Escherichia coli, Klebsiella or Enterobacter spp. were absent in the test samples. There was possibility of growth of Salmonella spp. and Shigella spp. which are non-lactose fermenters in Togwa and Mbege due to the growth of colourless and transparent colonies. On the other hand, colonies growing on NA, SDA and MRS were smooth round colonies with whitish/creamy colours. As such, it was possible to distinguish the colonies based on appearance/colour and shape/edge. The appearance of many colonies on the four-agar media indicated that they had the necessary nutrient requirements for their growth and can be used to culture such microbes.

The appearance of Gram-positive rod-shaped bacteria in pairs, triples or singly in all beverage samples provides the clue of the existence of various species of lactobacilli which are important in carrying out the acidic fermentations of the beverages(Abegaz, 2007; Oyedeji *et al.*, 2013). Ovoid or spherical gram-positive cells in clusters, pairs or singly with a fermenting malt smell were also observed in all beverage samples showing the possibility of the participation of fungi/yeasts in the fermentations of these beverages(Mugula, Nnko *et al.*, 2003; Mukisa *et al.*, 2017). The presence of gram-negative coccoid rods in togwa and mbege isolates show the possible existence of pathogenic or spoilage microbes in the beverages (Mugula *et al.*, 2003; Abegaz, 2007). However, these are normally eliminated by the decreased pH of the substrate due to production of lactic acid and yield of other antimicrobial metabolites as well as the by-products of the fermentation process.

The major implication of the identified lactobacillus species from the collected samples of local cereal beverages; Kindi, Kimpumu, Togwa and Mbege, is that spontaneous fermentation in such beverages is carried out by species of the three genera of lactic acid bacteria (LAB) namely *Lactobacillus, Lactococcus* and *Pediococcus*. However, the LAB most dominant in togwa are *L. pentosus, L. brevis,* and *L. plantarum*. In the case of Kindi, Kimpumu and Mbege, the leading LAB participating in the fermentations of the beverages are *P. pentosaceus* and *L. plantarum* respectively. Mukisa *et al.* (2017) isolated some LAB namely *L. plantarum, Weissella confusa, Lactococcus*

lactis and Lactobacillus fermentum from Obushera; a traditional sorghum malt fermented beverage in Uganda. Other studies on fermented cereal beverages like that of Ogi from fermented maize (*Zea mays*) has shown the presence of *L. plantarum* and *L. lactis* among others in the end product (Oyedeji *et al*., 2013). Abegaz (2007) was able to isolate LAB of the genera *Lactobacillus*, *Pediococcus* and *Weissella* as dominant LAB from the fermentation of Borde; an Ethiopian spontaneously fermented low or non-alcoholic cereal beverage.

Table 4. Yeast species in beverages.

Beverage type	Fermenting species
Togwa	*Candida tropicalis*
Kindi	*Cryptococcus gattii*
	Candida tropicalis
	Rhodotorula minuta
Mbege	*Candida ciferrii*
	Candida dubliniensis
Kimpumu	*Candida zeylanoides*
	Candida tropicalis
	Candida albicans

LAB have also been found to dominate the fermentations of two Zambian beverages known as Chibwantu and Munkoyo which are made from maize and sometimes with addition of millet or sorghum. The LAB isolated from these beverages were from the genera *Lactobacillus, Lactococcus, Leuconostoc, Streptococcus* and *Weissella* (Schoustra *et al*., 2013). A study carried out by Mugula *et al.* (2003) on spontaneous fermentation of togwa showed the presence of *L. fermentum, L. plantarum, L. brevis, Lactobacillus cellobiosus, P. pentosaceus and Weissella confusa* as the fermenting LAB in togwa. The growth of LAB in the samples of Kindi, Kimpumu, Togwa and Mbege is encouraged or favoured by the low acid condition or pH in the four beverages. The average pH of the samples at the time of collection were kindi (pH 3.6), kimpumu (pH 4.0), togwa (pH 3.17) and mbege (pH 4.0). The genera of LAB taking part in the fermentations of the four-local cereal-based beverages; Kindi, Kimpumu, Togwa and Mbege are identical to those of the previous studies narrated above though there are some additional genera that participated in the fermentations of such products like *Weissella, Leuconostoc* and *Streptococcus*. The slight difference in the participating genera is attributed to substrate composition, microbial structure differences and environmental conditions in the sites of beverage preparation, methods of preparing the beverages, metabolites produced during fermentation, the pH of the end products and the initial microbial load in the substrates. It is also worth noting that among the LAB participating in the fermentations of the four local cereal beverages; *L. plantarum, L. brevis, L. pentosus,* and *P. pentosaceus* have been proven to be probiotics or of beneficial use to human being (Bourdichon *et al*., 2012).During the fermentation of the four cereal-based beverages mentioned above; the major genus of yeasts participating in such fermentations is empirically Candida. In Togwa the most predominant yeast is *C. tropicalis*, in Kindi the dominating ones are *R. minuta, Cryptococcus gattii*, and *C. tropicalis*. In Kimpumu the most proliferating yeasts are *C. zeylanoides, C. tropicalis* and *C. albicans* whereas in Mbege the predominant yeasts are *C. ciferrii* and *C. dubliniensis*. Although yeasts of the genus Candida have been associated with some diseases (fungal diseases) in human and animals; recent development in research for beneficial microbes have indicated that some candida species (family saccharomycetaceae) participate in various food fermentations and have been included in the microbial food culture list (updated in 2011) of species used for food fermentations (Bourdichon *et al*., 2012). Some studies have also shown that various yeast species (non-saccharomyces species) such as those of the genera Candida, Debaryomyces, Pichia and some others have probiotic potential because of their ability to withstand the harsh conditions and colonize the gastro-intestinal tract during assays of various mammalian cell models (Pedersen *et al*., 2012). Candida albicans is an opportunistic pathogen though a non-harmful member of the microflora in heathy individuals. Candida tropicalis has been isolated during spontaneous fermentation and in the end product of the West African fermented pearl

millet-based food called fura (Pedersen *et al.*, 2012). Dominance of the genera Candida has also been observed during isolation, characterization and identification of yeasts isolated from three Nigerian spontaneously fermented cereal beverages; Burukutu (fermented sorghum & cassava), Kunu-zaki (fermented maize & red sorghum) and Ogi (fermented maize & sorghum/millet) whereby almost 40% of the isolates were identified as Candida spp. (Ogunremi *et al.*, 2015). In previous studies involving non-culture dependent techniques; it has been shown that Candida spp. are among the predominant yeast species taking part in the fermentations of many traditional cereal based products (Mukisa *et al.*, 2017). Investigation done by Mugula *et al.* (2003) also shows similarity of results to this study as yeasts of the genus Candida were among those isolated from togwa; a maize and finger millet malt derived cereal beverage in Tanzania. The similarities of the results of this study with other previous studies is based on the empirical fact that fermentations of cereals involving LAB and yeasts results to decreased pH in the range 3.5-4.0 which favours the proliferation of such microbes while others including pathogenic ones are killed in such low acidity. The slight differences in some of the candida spp. observed in this study and the previous ones lies in the specificity of the microbial strains to the cereal substrates and other factors as elaborated in the case of lactobacillus species above. However, as only *Saccharomyces cerevisiae subsp. Boulardii* is the only known probiotic yeast to date (Ogunremi *et al.*, 2015; Pedersen *et al.*, 2012); there is need to do further research on isolation and characterization of yeast species from spontaneously fermented cereal beverages in order to come up with novel species that can be used safely in various fermented cereal-based formulations.

Conclusion

This study has indicated that the predominant genera of LAB participating in the fermentation of the four-cereal based beverages in Tanzania namely Kindi, Kimpumu, Togwa and Mbege are *Lactobacillus, Lactococcus* and *Pediococcus*. The major genus of yeasts involved in the fermentation of such beverages is *Candida*. The dominant LAB and yeast species in fermentation of Togwa are *L. pentosus, L. brevis, L. plantarum* and *C. tropicalis* while in the fermentation of Kindi the dominant species involved are *P. pentosaceus*, *Cryptococcus gattii*, *C. tropicalis* and *R. minuta*. Species of microbes mostly involved in the fermentation of Kimpumu are *L. plantarum*, *C. zeylanoides*, *C. albicans*, and *C. tropicalis*. Mbege fermentation is predominated by species of *L. plantarum*, *C. ciferrii,* and *C. dubliniensis*. Since probiotic bacteria participate in the fermentation of the four local cereal beverages; these are potential probiotic starter cultures for the beverages depending on where each was isolated during this study.

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Conflict of interest

The authors declare no conflict of interest.

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