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Lactococci dominate the bacterial communities of fermented maize, sorghum and millet slurries in Zimbabwe

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ABSTRACT

Maize, sorghum and millet fermented porridges are important as complementary foods for young children in Africa. The objective of the present study was to gain some insights into the bacterial communities of fermented slurries prepared from maize, sorghum and millets originating from different locations in Zimbabwe, and prepared either at household or laboratory level. A deep sequencing approach targeting the hypervariable V4 region of the 16S rRNA gene was used and yielded about 100,000 sequences per sample. *Lactococcus* dominated all the fermented slurries, flanked by other lactic acid bacteria such as *Weissella*, *Leuconostoc* and *Enterococcus*. *Enterobacteriaceae* detected in the water samples persisted throughout all the fermented cereals. Other subdominant bacteria identified in the fermented slurries included *Aeromonas*, *Pseudomonas* and *Acinetobacter*. In addition, some Proteobacteria, Actinobacteria and Bacteroidetes associated with the raw materials and environment were also detected. Fermented slurries could not be differentiated based on their origin nor on the type of fermentation, but clear differences were observed between red sorghum fermented slurries and fermented slurries prepared from other cereal flours. A thorough understanding of the functional capacities of the microbiota in African fermented slurries is highly needed in order to steer the fermentation for the production of standard, safe and nutritious fermented products.

1. Introduction

Fermented cereal products constitute a vital part of the African diet providing a low cost, energy efficient method of food processing and preservation. A wide range of fermented cereal products are consumed in Africa and include both thin and thick porridges, alcoholic and non-alcoholic beverages, and bread like products (Blandino et al., 2003; Gabaza et al., 2017). These products are important as dietary staples, complementary foods for young children, refreshments, and condiments, and are also essential for cultural ceremonies. The production of fermented foods is typically done at household level and in small production units under rudimentary conditions, which pose hygienic, toxicological risk and product inconsistency (Achi and Ukwuru, 2015; Holzapfel, 2002). Therefore, the quality of the products is uncontrollable and unpredictable such that the final quality and safety is highly variable.

Some of the African fermented cereal products, particularly the porridges and gruels used as complementary foods for young children, need improvements in terms of their safety and nutritional content. The use of functional starter cultures offers a great potential and has been successful in the production of high energy pearl millet gruel after using a starter culture with high amylolytic activity (Songré-Ouattara et al., 2009). Starter cultures with the ability to degrade mineral binders for improved mineral bioavailability among others are also urgently needed (Gabaza et al., 2017). A prerequisite to the production of starter cultures is a thorough characterization of the microbial diversity of the fermented foods concerned. The functional capacity of different microbial consortia found in each product (Oguntoyinbo et al., 2011) and the impact of process conditions and cereal substrates on the microbial diversity needs to be carefully studied.

The emergence of next generation sequencing methods brought new opportunities to the study of food fermentations as they are fast, cost effective and give a deeper understanding of the microbial ecology of fermented foods (Bokulich and Mills, 2012; Van Hijum et al., 2013).

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While the bacterial communities of African fermented foods has mainly been studied using traditional molecular techniques (Assohoun-Djeni et al., 2016; Madoroba et al., 2011; Mukisa et al., 2012; Oguntoyinbo et al., 2011), to date, only the microbial community of African pearl millet slurries has been described comprehensively through the application of modern day sequencing techniques (Humblot and Guyot, 2009). Sour porridge is an important type of fermented porridge in Zimbabwe that is mainly consumed by children as a complementary food, and is made from maize, sorghum and millets. The bacterial diversity of sour porridge is not known. We therefore applied 16S rRNA amplicon sequencing to decipher the bacterial communities of different types of fermented cereals typically used in the preparation of porridge. The objective of the present study was to describe the bacterial communities of fermented slurries from maize, sorghum and millets and to carry out a comprehensive comparative analysis of the bacterial diversity based on type of fermentation, type of cereal substrate and origin of fermented cereal.

2. Materials and methods

2.1. Materials

The finger millet grains used in the preparation of household fermented slurries were provided by farmers from Ushe communal area, in Hwedza, Zimbabwe, and were harvested during the 2013/2014 season. Cereals (maize, sorghum, pearl millet and finger millet) were provided by farmers from five locations in Zimbabwe and were harvested during the period 2015/2016. Power Microbiome™ Isolation kits and MagAttract suspension G was procured from Qiagen, Belgium. The Qubit™ dsDNA assay kit was obtained from Life Technologies.

2.2. Methods

2.2.1. Preparation of household fermented finger millet slurries

Four groups of women from the Hwedza communal area, Zimbabwe, prepared finger millet fermented slurries according to their traditions between July 1 and 12, 2014. The products were prepared from four different varieties of finger millet. Below, each group of women is referred to according to the variety used i.e. red variety 1 (RV1),[1] red variety 2 (RV2), white variety 1 (WV1) and white variety 2 (WV2). The grains were milled at the local mill to have a particle size of 1–2 mm as normally practiced and each group prepared twice, both a spontaneously fermented slurry (SFS) as well as a backslopped fermented slurry (BFS). Briefly, fermented slurries were prepared by adding water to an aliquot of flour and this mixture was left to ferment for 24–36 h in plastic or metal containers at ambient temperature in the women's households (Gabaza et al., 2016). Frothing at the surface of the fermenting slurry identified a successful fermentation after which the fermented slurry was cooked to make porridge. At the end of the fermentation, the pH of the household fermented slurries ranged between 3.89 and 4.62. A detailed flow chart showing the production process and estimates of ingredients used by each group during the preparation of the fermented slurries is shown in Fig. 1.

2.2.2. Sample collection of household fermented slurries

Samples of fermented slurries were collected at the end of fermentation; all fermentations were performed in the period July 1 to July 12, 2014. Samples (50 mL) were collected in sterile falcon tubes and transported under cooled conditions to the laboratory. Samples for DNA extraction were stored at −80 °C and transported to Belgium under dry ice for 16S rRNA amplicon sequencing.

2.2.3. Collection of raw materials for the preparation of laboratory fermented slurries

Five locations were chosen for the collection of raw materials based on availability of all or most of the cereal grains in those locations. From each location, cereal grain samples (maize, sorghum, finger millet and pearl millet) were collected from 5 to 7 households. One water sample was collected from the most used water source in each area. Raw material and water samples were collected between July 10 and August 12, 2016. Samples were transported to the laboratory at the University of Zimbabwe under cooled transportation where the water was immediately stored in the cold room. Table 1 shows the type of cereal grains collected from each location and also the water source from each location.

2.2.4. Preparation of laboratory fermented slurries

For each location and cereal grain type, a pooled sample was prepared by mixing equal aliquots of the cereal grain from each household in order to produce a pooled sample representative of each location. For example, in Chiweshe, maize grains were collected from five households; a pooled maize grain sample representative of Chiweshe was made by mixing equal aliquots of the maize grain from each household. The grain samples were further dried in an oven and then milled using a laboratory mill equipped with sieve of size 0.5 mm. A total of 20 such 'composite' samples were made from the five locations (Table 1). Fermented slurries were prepared using the composite cereal flours and the water specific for each location following the method as illustrated in Fig. 1. Based on the preparation of household fermented slurries, fermented slurries were made by mixing an aliquot of cereal flour and water (1:3) in autoclaved glass jars. The water was used without further treatment as normally practiced in the preparation of the porridges (Gabaza et al., 2016). The mixture was left to ferment at 25 °C in a temperature-controlled room for 26 h. The pH of the fermented slurries was monitored until the end of the fermentation and ranged between 4.12 and 5.75. All fermentations were done in triplicate. Fermented slurries were stored at −80 °C and transported under dry ice to Belgium on September 14–19, 2016.

2.2.5. Extraction of DNA

DNA was extracted from fermented cereals and water. An aliquot of thawed fermented slurry (50 mL) was first centrifuged at 1000 $\times g$ for 10 min. The supernatant was collected and subjected to another centrifugation at 1000 $\times g$ for 10 min to remove the starchy pellet and then at 10000 $\times g$ for 10 min to pellet the bacterial cells. This process was repeated four times in order to collect as many bacterial cells as possible. Water samples were centrifuged at 10000 $\times g$ for 20 min to pellet bacterial cells. DNA was extracted using the Power Microbiome™ Isolation kit according to the manufacturers protocol with an additional lysis step at 90 °C for 10 min after the fourth step. Purification of the DNA was done by adding 5 μL sodium acetate and 125 μL absolute ethanol to 50 μL of DNA extract and precipitating the DNA overnight at −20 °C. Centrifugation was performed at 13000 $\times g$ for 30 min after which the DNA was washed twice with 500 mL of ice-cold ethanol (75%). The DNA was air dried and suspended in DNase free water. To ensure the DNA conformed to the required specification for sequencing, the quality of the DNA was assessed with Nanodrop (NanoDrop ND-1000, Thermo Fischer) (OD260/280–1.8–2.0), agarose gel electrophoresis (no DNA degradation and RNA contamination) and the concentration was measured using a Qubit 3.0 Fluorimeter (Life Technologies, Carlsbad, CA, USA) ($>$ 5 ng/μL and amounting to $>$ 100 ng). Out of 81 DNA samples, 15 samples did not meet the quality criteria and were further purified by suspending the DNA in 100 μL high salt TE buffer and incubating for 30 min at 60 °C, followed by adding 5 μL of MagAttract suspension G and 120 μL of absolute ethanol. This

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[1] RV1: red variety 1, RV2: red variety 2, WV1: white variety 1, WV2: white variety 2, SFS: spontaneously fermented slurry, BFS: backslopped fermented slurry, f/millet: finger millet, p/millet: pearl millet, LAB: lactic acid bacteria, DGGE: denatured gradient gel electrophoresis, TTGE: temporal temperature gel electrophoresis

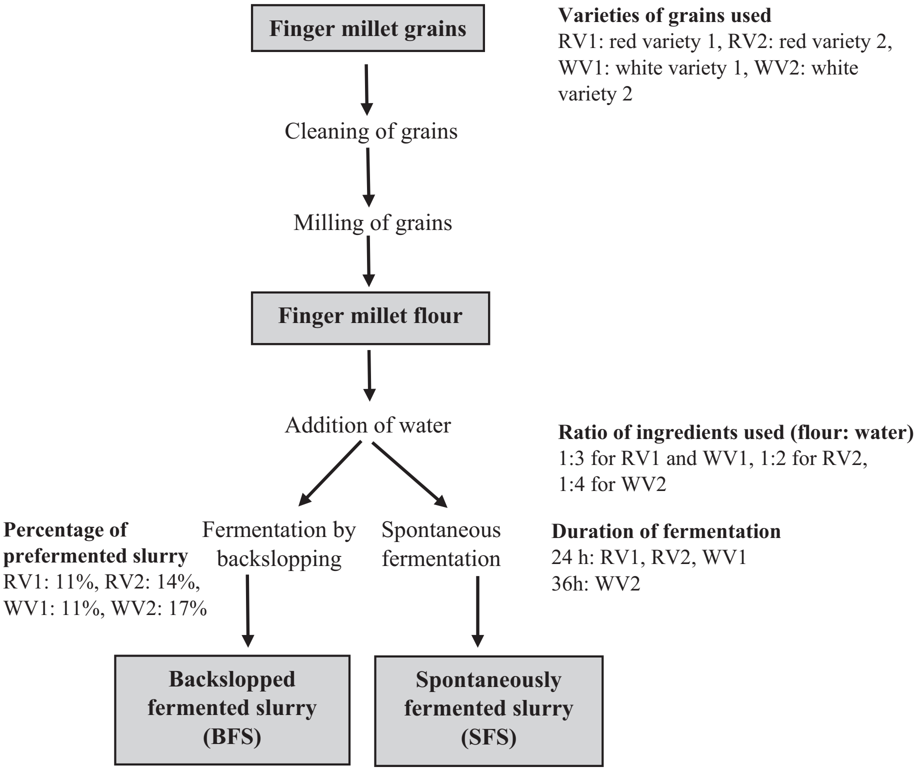

Fig. 1. Schematic showing the flow chart for the preparation of fermented slurries.

Table 1
Sampling locations and type of cereal grains collected.

Location	Cereal grains collected	Water source
Chiweshe	Maize Red finger millet Red sorghum	Borehole
Chiredzi	Maize Red finger millet White sorghum Pearl millet	Open well
Mutoko	Maize Red finger millet White sorghum Pearl millet	Borehole
Hwedza	Maize Red finger millet White finger millet Red sorghum White sorghum Pearl millet	Borehole
Victoria Falls	Red finger millet White sorghum Pearl millet	Borehole

Only 3 types of cereal grains could be collected from Chiweshe and Victoria Falls as pearl millet and maize, respectively, were not cultivated at those locations.

mixture was vortexed gently and was incubated for 5 min at room temperature. The tubes were placed on a magnetic rack in order to separate the beads binding the DNA from the suspension. The beads were washed three times with wash buffer (30% TE and 70% ethanol) and air dried at room temperature for 10 min. DNase free water was then added to the beads to suspend the DNA and this was incubated for 5 min at 60 °C followed by separation of the beads from the DNA on the magnetic rack.

2.2.6. 16S rRNA amplicon library preparation and amplicon sequencing

DNA samples were shipped to Novogene Bioinformatics Technology Co., Ltd., Hong Kong (https://en.novogene.com/next-generation-sequencing-services/microbial-genome/amplicon-sequencing/) where library preparation and amplicon sequencing was performed. Amplification of the 16S rRNA gene was performed by targeting the hypervariable V4 region using the primers 515F and 806R tailed with Illumina adapters with indexing barcodes, to generate fragment sizes of approximately 292 bp. Paired-end sequencing was carried out on HiSeq PE250 to generate sequences of high quality ($Q30 > 80\%$) and a sequencing depth of at least 30,000 raw tags per sample.

2.2.7. Sequence analysis

Demultiplexing, denoising and removal of chimeric sequences was performed by means of the LotuS pipeline (Hildebrand et al., 2014) after which the reads were binned into operational taxonomic units (OTUs) based on a 97% OTU similarity cutoff. The taxonomic origin of each OTU was determined by blasting the sequences against the Ribosomal Database Project database. Alpha diversity (Observed, Chao1, Shannon and Simpson diversity) indices were calculated to estimate the bacterial biodiversity in each sample. The percentage of bacterial OTUs in each sample were calculated as a proxy to their relative abundances. Based on the relative abundances, bacterial genera were classified into three population groups where abundances of < 1% were considered as rare genera, 1 to < 10% as subdominant genera, and finally the genera with relative abundances of > 10% as dominant (Nam et al., 2012).

2.2.8. Statistical data analysis

The relative abundances of taxa present in at least 25% of the samples and the diversity measures were compared using the Kruskal Wallis test and q-values < 0.05 after Bonferroni correction for multiple testing were considered significant. In household fermented slurries, bacterial populations in spontaneous and backslopped fermentations of each finger millet variety were compared. In laboratory fermented slurries, comparisons of bacterial populations were made based either on cereal type or location. Also, bacterial populations of spontaneously fermented finger millet household slurries (white varieties) and laboratory fermented finger millet slurries from Hwedza were compared. Principal coordinate analysis (PCoA) using weighted Unifrac distance matrix was carried out to visualize similarities and dissimilarities among all the samples sequenced i.e. water, household and laboratory fermented slurries. All statistical analysis were carried out using R version 3.3.2 using R packages ggplot2 (Wickham, 2009) and phyloseq (McMurdie and Holmes, 2013).

3. Results

3.1. Diversity estimates of bacterial communities

After the quality control process which included the removal of sequences that could not be attributed to phyla within the domains of bacteria and archaea, and of sequences attributed to chloroplasts, a total of 6,861,665 high quality sequences were retained for the laboratory fermented slurries (an average of 114,361), 1,852,973 sequences for household fermented slurries (an average of 115,810) and 386,281 sequences for the water samples (an average of 96,570). Data of water samples from only 4 locations was available as the water sample from Chiweshe did not yield enough DNA material to meet sequencing requirements. Richness estimator (observed and Chao1) and diversity indices (Shannon index and Simpson index) are presented in Fig. 2. The observed number of OTUs ranged between 86 and 594 for the household fermented slurries, 57 and 610 for the laboratory fermented slurries and 464 and 1010 for the water samples. There were significant differences among the observed number of OTUs ($q < 0.05$) with major differences between water samples and fermented cereals, and between red sorghum slurries and other types of fermented cereal slurries. The same trend was also observed when using the richness estimator Chao1, and in all samples the total number of OTUs estimated by Chao1 was slightly higher than the observed number of OTUs which covered an average of 70 to 80% of the estimated richness suggesting that there could be a few unseen OTUs. The highest diversity calculated using the Shannon index ($q < 0.05$) was found in the water samples, followed by the household fermented slurries and lastly the laboratory fermented slurries. In contrast, the Simpson-index ($q < 0.05$), which is a measure of both diversity and evenness, was highest in the water samples and household fermented slurries and lowest in the laboratory fermented slurries.

3.2. Bacterial communities of household fermented slurries

In general, the most abundant bacterial phylum in household fermented slurries was the Firmicutes (72%), followed by Proteobacteria (27%). Other phyla represented very minor overall fractions. Of these, Actinobacteria had the highest abundance (0.7%). The relative abundance at the phylum and genus levels for the different finger millet household fermented slurries is shown in Fig. 3. Finger millet household fermented slurries were dominated by unclassified *Enterobacteriaceae* (14%) and the genera *Lactococcus* (39%), *Weissella* (14%) and *Leuconostoc* (14%), while the subdominant genera included *Aeromonas* (6%), *Enterococcus* (3%), *Pseudomonas* (3%), *Lactobacillus* (2%) and *Acinetobacter* (1%). An average of 5% of the total abundance was attributed to rare genera some of which were present in the water and belonged to Proteobacteria such as *Sphingomonas*,

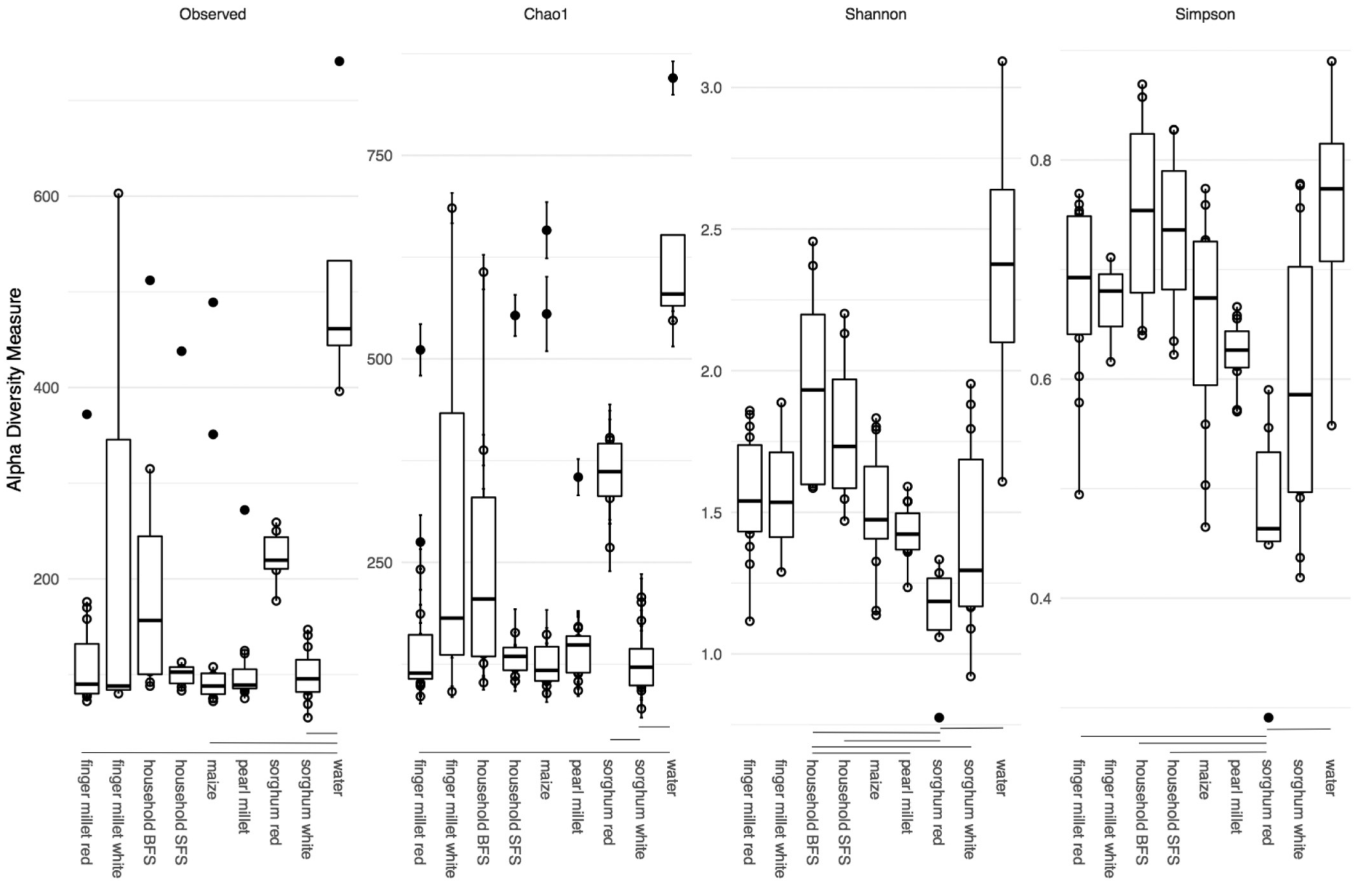

Fig. 2. Diversity measures of bacterial 16S rRNA sequences in water, household fermented slurries (spontaneous and backslopped) and laboratory fermented slurries originating from five locations in Zimbabwe

SFS: spontaneously fermented slurries, BFS: backslopped fermented slurries. All household fermented slurries were based on finger millet (both red and white varieties). Horizontal lines just above the legend indicate pairwise significant statistical differences (2-sided and Bonferroni corrected $q < 0.05$).

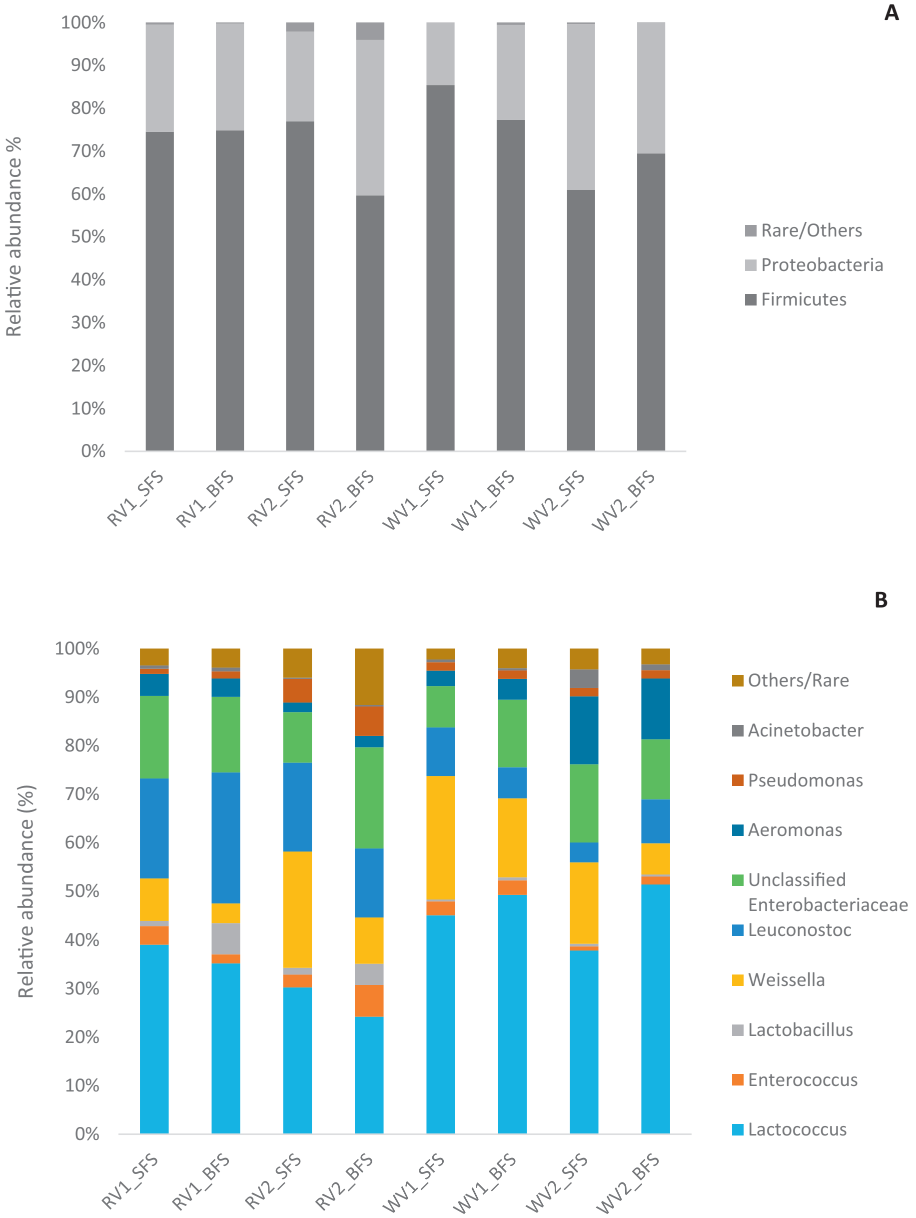

Fig. 3. Relative abundance of bacterial communities from household fermented slurries at phylum (A) and genus level (B)
RV1: red variety 1, RV2: red variety 2, WV1: white variety 1, WV2: white variety 2, SFS: spontaneously fermented slurry, BFS: backslopped fermented slurry. Others/rare represent all phyla or genera with relative abundance of < 1%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Stenotrophomonas, unclassified *Comamonadaceae*, *Acetobacter* and *Tolumonas*. Some Firmicutes commonly found in fermented cereals were also found among the rare genera and included *Pediococcus* and *Streptococcus*. Among > 50 rare genera, *Curtobacterium* (Actinobacteria phylum) and *Sphingobacterium* (Bacteroidetes phylum) were the most dominantly detected.

3.3. Bacterial communities of water and laboratory fermented slurries

The laboratory fermented slurries were prepared using the flour and water from the different locations as these were expected to harbor the inocula for the fermentation and to be the main drivers of the fermentation. The relative abundance of the bacterial communities of the water and of the laboratory fermented slurries at the phylum and genus level is shown in Figs. 4 and 5. Unlike in the fermented slurries, Proteobacteria constituted the major phyla in water samples, accounting for approximately 70% of the phyla, followed by about 23% Firmicutes, 4% Bacteroidetes and about 2% Actinobacteria, while about 1% of the phyla were considered as rare. Water from Mutoko had the highest abundance of Proteobacteria and the lowest abundance of rare phyla, while water from Victoria Falls had almost equal abundances of Firmicutes and Proteobacteria. Hwedza water contained the highest level of Actinobacteria. Four taxa were classified as dominant and included unclassified *Comamonadaceae* (19%), *Acinetobacter* (18%), *Lactococcus* (15%) and unclassified *Enterobacteriaceae* (10%), while 9 taxa were classified as subdominant i.e. *Aeromonas* (5%), *Flavobacterium* (3%), *Aquabacterium* (2%), *Enterococcus* (2%), *Leuconostoc* (2%), *Pseudomonas* (2%), an unclassified clade of Actinobacteria referred to as the Hgcl clade (Glöckner et al., 2000) (1%), *Hydrogenophaga* (1%) and *Dechloromonas* (1%). Most of the Firmicutes identified in water samples belonged to the order Lactobacillales and also included *Weissella*, *Lactobacillus* and *Streptococcus* among the rare genera. Water from different

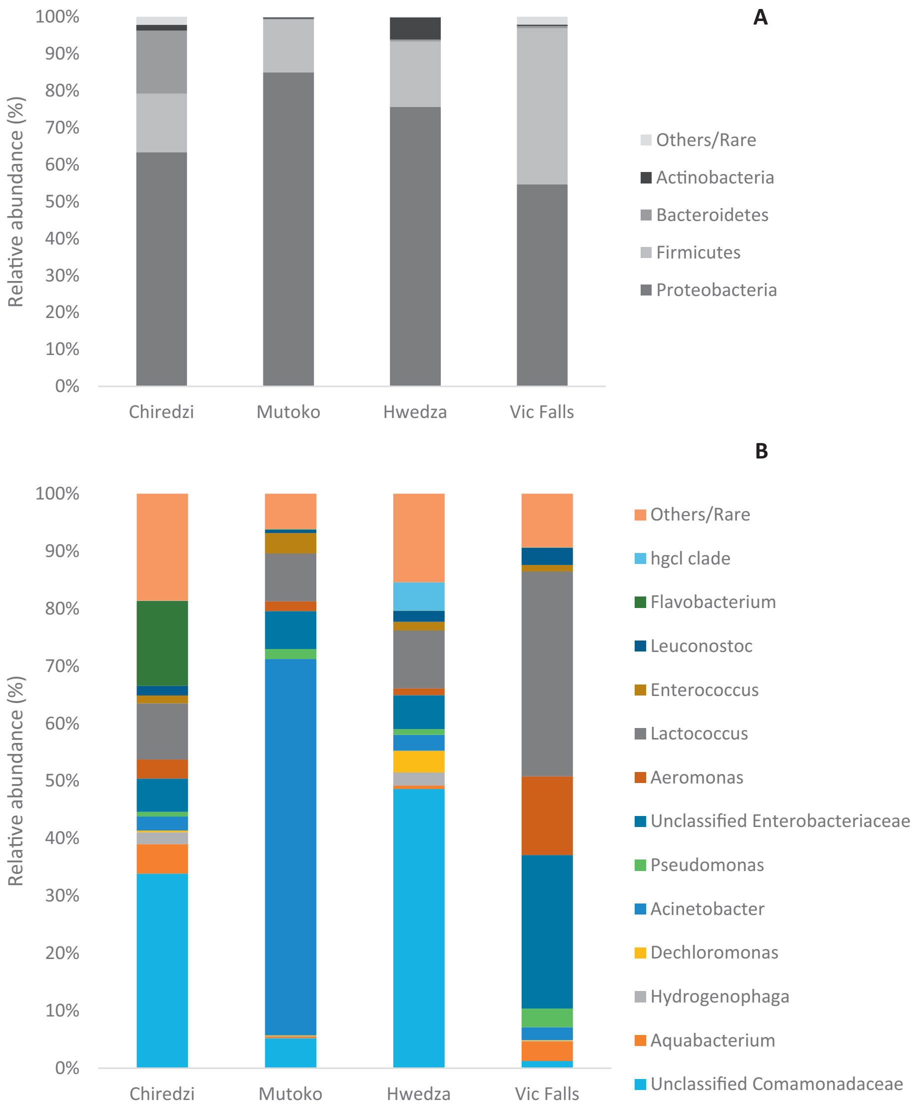

Fig. 4. Relative abundance of bacterial communities from water samples at phylum (A) and genus level (B) Others/rare represent all phyla or genera with relative abundance of < 1%.

locations showed markedly different profiles in their bacterial communities at the genus level (Fig. 4). Water from Chiredzi and Hwedza had more unclassified *Comamonadaceae* than the other two locations while water from Mutoko had more *Acinetobacter,* and water from Victoria Falls had more *Lactococcus* and unclassified *Enterobacteriaceae*. *Flavobacterium* was particularly abundant in Chiredzi water.

In general, laboratory fermented slurries, like the household fermented slurries showed a dominance of Firmicutes (72%) and Proteobacteria (27%) (Fig. 5). At the genus level, only *Lactococcus* (44%) and unclassified *Enterobacteriaceae* (29%) were considered dominant while the subdominant groups comprised *Leuconostoc* (8%), *Aeromonas* (7%), *Weissella* (3%), *Enterococcus* (3%) and *Pseudomonas* (2%). Among > 50 rare genera, the most dominantly detected included *Lactobacillus* (Firmicutes phylum), *Acinetobacter* (Proteobacteria phylum), *Tolumonas* (Proteobacteria phylum) and *Paenibacillus* (Firmicutes phylum). In the laboratory fermented slurries, the *Leuconostocaceae* family comprised of 73% *Leuconostoc* and 27% *Weissella* in contrast to 50% *Leuconostoc* and 50% *Weissella* in the laboratory fermented slurries.

3.4. Comparison of bacterial communities of fermented slurries

Among household finger millet fermented slurries, there was no significant difference between spontaneously fermented slurries and backslopped fermented slurries of the same variety. The bacterial communities of household fermented finger millet slurries comprised generally the same bacterial consortia, which slightly differed in their relative abundances. The spontaneously fermented white finger millet from the household fermentation were compared with the laboratory fermented white finger millet slurries. Differences were observed in the relative abundances of unclassified *Enterobacteriaceae* and *Pseudomonas* which were both higher in the laboratory fermented slurries compared to the household fermented slurries ($q < 0.05$).

Pertaining to the laboratory fermented slurries originating from 5 different locations, there were significant differences among the genera *Tolumonas*, *Plesiomonas* and unclassified *Aeromonadaceae* ($q < 0.05$). These potentially pathogenic bacteria were highest in the fermented slurries from Chiredzi compared to other locations. Indeed, these genera were also more abundant in the water from Chiredzi than from other locations. However, there were no differences in dominant and subdominant genera based on location. Based on the type of cereal, significant differences were observed in the relative abundances of

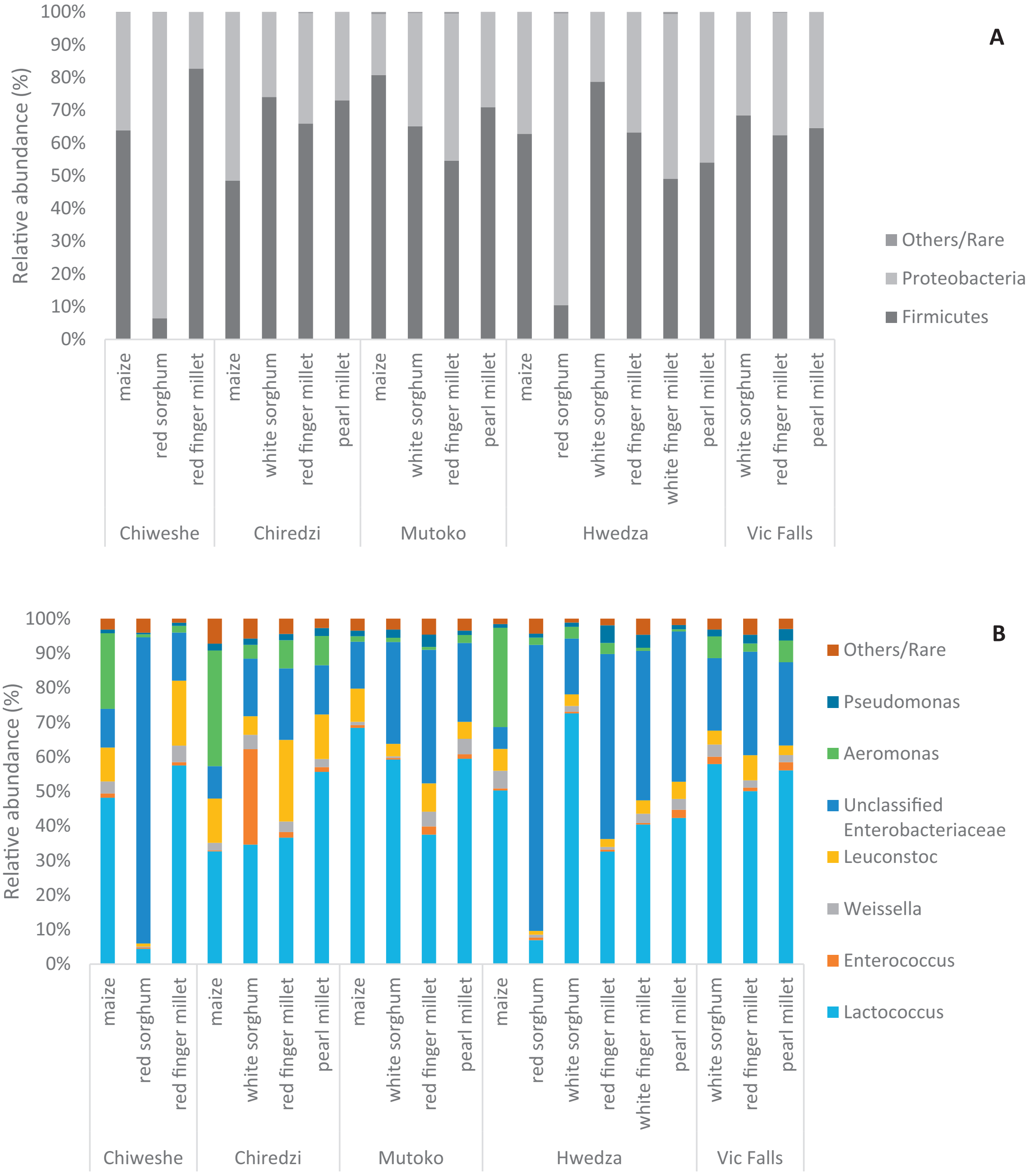

Fig. 5. Relative abundance of bacterial communities from laboratory fermented slurries at phylum (A) and genus level (B) f/millet: finger millet, p/millet: pearl millet, Others/rare represent all phyla or genera with relative abundance of < 1%.

unclassified *Enterobacteriaceae*, *Lactococcus*, *Leuconostoc* and *Pantoea* ($q < 0.05$). In all these cases, a distinct difference was observed between red sorghum and maize whereby red sorghum had the highest abundance of unclassified *Enterobacteriaceae* and the lowest abundance of *Lactococcus,* whereas maize had the lowest abundance of unclassified *Enterobacteriaceae* and the highest abundance of *Lactococcus*. *Leuconostoc* abundance was also lowest in red sorghum and highest in maize and red finger millet.

In fact, red sorghum had the highest abundance of Proteobacteria and the lowest abundance of Firmicutes, which is reflected in the PCoA plot (Fig. 6A) where the red sorghum is distinguished from other fermented cereals. The PCoA plot shows three distinct groups. Group 1 shows the water samples from three locations except the water from Victoria falls. The latter grouped with the fermented slurries as it had almost 50% Proteobacteria and 50% Firmicutes. Group 2 is the red sorghum from Hwedza and Chiweshe and group 3 is that of the fermented slurries, from both household and laboratory fermentation. Fermented slurries which are more different from each other are those of maize and red sorghum which are positioned on the extremes of axis 1.

A separate PCoA analysis of the group 3 samples revealed a clustering of the maize fermented slurries along axis 1 and that of the sorghum white and pearl millet fermented slurries along axis 2 (Fig. 6B). A clear separation of the finger millet fermented slurries and that of the household finger millet fermented slurries could not be observed.

4. Discussion

African cereal fermented slurries are important in the diet of many

(caption on next page)

Fig. 6. A: Principal Coordinate Analysis (PCoA) based on weighted UniFrac analysis of 16S rRNA gene sequences from household finger millet fermented slurries, laboratory fermented slurries originating from different locations and water

B: Principal Coordinate Analysis (PCoA) based on weighted UniFrac analysis of 16S rRNA gene sequences from all fermented slurries excluding red sorghum fermented slurries

Samples shown are from the Group 3 samples shown in Fig. 6A and include household finger millet fermented slurries and laboratory fermented slurries originating from different locations excluding red sorghum fermented slurries (group 2, Fig. 6A) and water samples (group 1, Fig. 6A). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

population groups such that an understanding of their microbial ecosystem is crucial to their improvement. Two studies were carried out; one involving household fermented finger millet slurries and another involving laboratory fermented slurries using different types of cereals typically used in Zimbabwe for the production of porridges. In addition, the preparation of the laboratory fermented slurries was conducted in a way that mimicked the production of fermented slurries from different locations by using the raw materials from the respective locations but controlling the fermentation conditions.

There was no statistical difference between microbiota of spontaneously fermented slurries and backslopped slurries in the households examined. Although a higher level of contaminating or undesirable microbiota in the household fermented slurries compared to laboratory fermented slurries could be expected (Minervini et al., 2015), their microbiota compositions were not significantly different either. The minor differences observed may have been caused by minor differences in fermentation environment and slight variations in the fermentation conditions. Moreover, the sample size may have been too small to detect significant microbial differences. The differences in microbiota composition of fermented slurries from different locations were attributed to rare genera only, which is consistent with studies that failed to show a region specificity in the microbiota composition of sourdoughs (Gobbetti et al., 2016; Van Kerrebroeck et al., 2017). The absence of specific location microbiota suggests that other parameters in particular the type of raw materials used were the most important drivers of the composition of the cereal fermentation.

In both household and laboratory fermented slurries, the dominant and subdominant microbiota generally consisted of *Lactococcus*, unclassified *Enterobacteriaceae*, *Weissella*, *Leuconostoc*, *Aeromonas*, *Enterococcus* and *Pseudomonas* (Figs. 3 and 5). Although relative abundances of these genera varied among the different cereal flour fermentations, the bacterial community at the end of these fermentations was fairly simple. Out of 13 dominant and subdominant genera detected in water samples, only seven were detected in the fermented slurries, and were mostly Firmicutes which, compared to the water samples, increased in abundance and Proteobacteria which generally decreased in abundance. Of the latter, only the unclassified *Enterobacteriaceae* increased in abundance in the fermented slurries. Bacteria associated with cereal flours such as *Acinetobacter*, *Pantoea*, *Comamonas*, *Enterobacter*, *Erwinia* and *Sphingomonas* (Ercolini et al., 2013) were all detected but constituted minor fractions. Although the raw materials used in cereal fermentations may harbor a rich diversity of microbiota, cereal fermentations are often characterized by a succession of dominant and subdominant lactic acid bacteria (LAB) (Gobbetti et al., 2016). This LAB succession involves the suppression of gram-negative bacteria and the eventual dominance of lactobacilli. In addition, such microbial ecosystems become less complex due to the adaptation of a limited number of species to the microbial ecosystem (Minervini et al., 2015; Van Kerrebroeck et al., 2017).

The presence of *Lactococcus, Enterococcus* and *Leuconostoc* in the present study is in tandem with the early stages of sourdough fermentation (Figs. 3 and 5). These LAB are cereal endophytes associated with the outer layers of the cereal kernel, and do not tolerate long term acidification (Corsetti et al., 2007; Gobbetti et al., 2016; Minervini et al., 2015; Weckx et al., 2010a). In addition, these organisms were also present in the water implying that the water used from the five locations studied was likely a rich inoculum of these LAB. The genera *Weissella* and *Leuconostoc* were ubiquitous in both household and laboratory fermented slurries. They are indigenous to plant material, widely associated with liquid sourdoughs, and prevail in sourdoughs with a pH above 4 and fermented at low temperatures of < 30 °C, which is characteristic of the fermentation process in the present study (Ampe et al., 1999; Van Kerrebroeck et al., 2017). However, the strong dominance of *Lactococcus* in the present study was unexpected. This organism comprises only a minor part of the dominant LAB in sourdough fermentation (De Vuyst et al., 2014; Nout and Rombouts, 1992) and among African cereal fermentations, only one study indicated the prevalence of *Lactococcus lactis* throughout a 54 h fermentation of *ting* where it was considered well adapted to the sorghum environment (Madoroba et al., 2011). *Lactococcus* has been infrequently observed during the fermentation of *obushera,* sorghum/millet based gruels (Mukisa et al., 2012; Muyanja et al., 2003), *ben-saalga,* pearl millet slurries (Humblot and Guyot, 2009), maize and sorghum *ogi* and *kunu zaki* (Oguntoyinbo et al., 2011*)* and in some rye, wheat and spelt sourdoughs (Ercolini et al., 2013; Weckx et al., 2010a; Weckx et al., 2010b). The presence of *Lactococcus* as one of the dominant genera in the water samples may also have contributed to its competitive advantage over other LAB (Fig. 4).

The water samples also harbored a high abundance of unclassified *Enterobacteriaceae* which persisted at the end of the fermentation. Other Proteobacteria which were abundant in water such as unclassified *Comamonadaceae* and *Acinetobacter* were detected in the fermented slurries but in minor fractions, suggesting a potential competitive advantage of *Enterobacteriaceae* over other Proteobacteria. *Enterobacteriaceae* have been found to persist in sourdoughs because of their ability to metabolize acids and tolerate acidic stress (Ercolini et al., 2013; Gobbetti et al., 2016). In wheat sourdough, *Enterobacteriaceae* only started disappearing after 5 days of propagation coinciding with the formation of the mature sourdough (Ercolini et al., 2013). *Enterobacteriaceae* have also been observed in pearl millet slurries sampled from traditional production units of Burkina Faso (Humblot and Guyot, 2009), Mexican maize *pozol* (Ampe et al., 1999), *obushera* (Mukisa et al., 2012), *doklu* (Assohoun-Djeni et al., 2016), *poto poto* (Abriouel et al., 2006) and in Portuguese *broa* made from maize and rye flour (Rocha and Malcata, 2012).

Pertaining to cereal flour type, a major difference was observed between fermented slurries prepared using red sorghum flour and those prepared using the other cereal flours i.e. maize, white and red finger millet, pearl millet and white sorghum (Fig. 6A). Fig. 6B further shows a clear clustering of maize, white sorghum and pearl millet fermented slurries, demonstrating that the type of cereal flour may be a major determinant of bacterial communities. Unclassified *Enterobacteriaceae* constituted 83–89% of the total microbiota of the red sorghum slurries in contrast to 6–54% in other cereal fermented slurries (Fig. 5). Fermentation of flours from whole cereal grains may result in differences in microbial communities given the presence of dietary fiber and bioactive compounds in the bran fraction (Katina et al., 2012; Katina et al., 2007). More specifically, certain types of phenolic compounds have antibacterial properties that may select for certain bacteria. Maize, sorghum and millets all contain different levels and types of phenolic compounds (Dykes and Rooney, 2006; Gabaza et al., 2018). Among the vast array of phenolic compounds contained in these cereals, red sorghum additionally contains condensed tannins which are not present in the other cereals examined in the present study (Gabaza et al., 2018).

Condensed tannins can suppress growth of other bacteria purportedly through their ability to complex with polymers. Low concentrations of 0.2–2% can have a significant effect on the diversity of bacterial populations of an ecosystem (Smith et al., 2005). The fecal microbiota of rats shifted from the predominant bacterial species towards species of *Enterobacteriaceae* and Bacteroidetes after rats were fed a high condensed tannin diet and the prevailing species were considered to be "tannin resistant" (Smith and Mackie, 2004).

In addition, the antimicrobial activity of red sorghum whole grains was 100–200 times higher than that of the white sorghum and prevented the growth of *Lactobacillus sanfranciscensis* isolated from wheat sourdough (Sekwati-Monang et al., 2012). Only strains of *Lactobacillus casei* and *Lactobacillus parabuchneri* isolated from the sorghum sourdough could grow on the phenolic extracts because of their ability to metabolize phenolic compounds (Sekwati-Monang et al., 2012; Svensson et al., 2010). *L. buchneri* and *L. casei* were isolated from red sorghum that had been fermented for 2–3 days (Sekwati-Monang and Gänzle, 2011) and since the laboratory fermentation in the present study was done for 26 h, lactobacilli with the ability to metabolize phenolic compounds may not had started to proliferate. The use of autochthonous starter cultures with fast acidification properties may thus be important in the case of red sorghum or a longer fermentation may be required to allow the growth of lactobacilli which can metabolize red sorghum phenolic compounds. Pertaining to other cereals i.e. maize, finger millet and pearl millet, perhaps clear differences in their microbial diversity and the influence of flour type in these cereals will be more apparent at the species level.

Although *Enterobacteriaceae* may in fact have a positive functional role in the fermentation, their dominance is a cause of concern as opportunistic pathogenic genera such as *Shigella*, *Salmonella* and *Escherichia* belong to this family. *Aeromonas* and *Pseudomonas* still persisted at end of the fermentation indicating another potential hazard associated with consumption of these products. The fermented cereal slurries will undergo a final cooking process to prepare the porridge and this will eliminate most pathogens but some *Aeromonas* and *Pseudomonas* species can produce toxins that can still render the porridge unsafe. Other cereal fermentations, particularly the non-alcoholic beverages such as *mahewu* from Southern Africa and *obushera* from Uganda, harbor the same potentially pathogenic microbial communities but are consumed without a further cooking step making them high risk products. These products are also used as complementary foods for young children and some of the detected microorganisms could cause diarrhea in infants (Mukisa et al., 2012).

5. Conclusion

Amplicon sequencing of the V4 region of 16S rRNA gene showed the dominance of *Lactococcus* in Zimbabwean maize, sorghum and millet fermented slurries after a fermentation that lasted for 24–36 h. Other LAB commonly associated with these cereal fermentations included *Weissella*, *Leuconostoc* and *Enterococcus* along with some Proteobacteria, Bacteroidetes and Actinobacteria that are typically associated with the raw materials and environment. Large differences in the bacterial communities of red sorghum compared with other cereal fermented slurries were observed. Fermented slurries could not be differentiated based on origin, nor on whether slurries were fermented at laboratory or household level. Although the sequencing in the present study did not allow species-specific identification of the bacterial communities, it led to a deeper understanding of the bacterial composition underlying Zimbabwean maize, sorghum and millet fermented slurries. Yeasts communities have also been observed in African cereal fermented foods so future studies should also consider the use of modern day sequencing techniques to describe the yeasts microbial consortia in order to have a complete view of the microbial ecosystem of African cereal fermented foods. The method used in this study could not differentiate between metabolically active and non-metabolically active microorganisms as such future studies could focus on this aspect in order to build upon the findings from this study.

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Declaration of conflict of interest

None.

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