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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 ×*g* for 10 min. The supernatant was collected and subjected to another centrifugation at 1000 ×*g* for 10 min to remove the starchy pellet and then at 10000 ×*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 ×*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 ×*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

[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