

## Identification of Bacteria Isolated from Traditionally Fermented, Pearl Millet (*Pennisetum glaucum*) Remnant in Namibia

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Fermented pearl millet (*Pennisetum glaucum*) known as *mahangu* in Namibia, is traditionally produced grain in four northern regions of Namibia; *Omusati*, *Oshana*, *Ohangwena* and *Oshikoto* where it is used for thick porridge or fermented nonalcoholic brew preparation known as *oshikundu*. *Eendjeke* is a collection of shredded pearl millet grains obtained as remnants when *mahangu* grains are traditionally processed into flour. *Eendjeke* is added to decorticated pearl millet before moistening, incubation and processing into flour. Addition of *eendjeke* to decorticated *mahangu* grains speeds up the fermentation process.

Traditionally pearl millet remnants (*eendjeke*) are prepared by cleaning the grains of solid particles and dirt. The grains are decorticated by adding minimal water and pounding with a mortar and pestle. The cortex particles are sieved off and the cleaned grains mixed with *eendjeke* are moistened. The mixture is covered and allowed to stand at room temperature for between six (6) and eight (8) hours. The grains are successively pounded with intermitted steps of sieving to separate flour from grain particles. The process is stopped when the producer assumes that the amount of grains remaining do not yield a significant amount of flour. The grain remnants are sun dried and stored in the shade till used for the next back slopping process.

In general fermentation is known to impart improved health and sensory qualities on food. It improves digestibility and detoxifies anti-nutrients (phytates and polyphenols) [1]. It improves the availability of minerals [2], enhances flavour by generating molecules with desirable aroma [3]. It improves palatability and extends shelf life of processed food. In addition fermenting microorganisms lower the pH of the food hence limiting the growth of pathogenic microorganisms. However in Namibia the predominant microorganisms responsible involved in traditional pearl millet fermentation have not been identified.

Fifty three (53) Lactic acid bacteria (LAB) were isolated from *eendjeke* using de Man, Rogosa and Sharpe (MRS) medium (Oxoid) and M17 (Sigma-Aldrich) agars [4]. All cultures were preserved in MRS broth (Oxoid) supplemented with 20% glycerol and stored at -20°C until identified. Bacteria were retrieved from Nutrient broth by streaking on same solid media. After two successive steps of streaking to ensure their purity isolates were identified using Gram stain differentiation with aqueous 10% KOH solution, Catalase test and API 50 CH identification kits [1]. The API LAB PLUS database (Bio Merieux,) was used to determine the identity of the isolates to species level.

Good identification (up to 99.9%) was obtained with APIwebtm assigning the isolates to four genera of Lactic acid bacteria. *Lactobacillus plantarum* (32%) was the most predominant species isolated followed by *Lactococcus Lactis* (24.5). *Weisella confusa* (22.6%) was the third highest isolate. Other LAB isolated were *Lactobacillus pentocaseus* (13%), and *Lactobacillus pentosus* (7.5%).

Figure

**Figure 1:** Steps used in the generation of eendjeke. (a)= Whole pearl millet grains; (b) = Pearl millet flour obtained from successive rounds of pounding; (c) = shredded pearl millet grains remnants

However the high frequency of isolates in the fermentation starter material cannot be directly interpreted as the predominant fermenting organisms during the decorticated pearl millet flour fermentation. The material (*eendjeke*) was subjected to factors that might have altered the microbial composition during storage. Since after flour preparation, *eendjeke* is preserved by drying, the proportion of surviving organisms also depends on their differential ability to withstand low water activity. Also the specific fermentation capacities of the identified cultures have not been determined.

Secondly, a study done in *oshikundu* [5] (a gruel prepared using pearl millet flour) isolated *L. fermentum*, *L. acidifarinae*, *L. spicheri*, *L. namurensis*, *L. zymae*, *L. brevis*, *Leuconostoc qurlium*, *Pediococcus acidilactic*, and Yeasts, *Saccharomyces* spp., and *Pichia kudriavzevii* in addition to the species isolated by this study. In addition studies carried out in other parts of Africa that determined the identity of microorganisms involved in the fermentation of a number of food commodities processed from sorghum and pearl millet showed a much more diverse LAB species repertoire (*L. fermentum*, *Lactobacillus salivarius*, *L. delbrueckii*, *L. amylolyticus*, *L. reuteri*, *Leuconostoc*, *Pediococcus* and *Streptococcus* species as well as yeasts and moulds) [6] that form part of the fermenting population.

*Weissella confusa* a Gram-positive spherical or rod, within the family Leuconostocaceae, can cause bacteremia, endocarditis, and abscess in immunocompromised patients however in some cases it has as a probiotic effect [7-9]. Despite the benefits of fermentation and the apparent preference for fermented pearl millet flour by consumers, the microbial repertoire responsible for pearl millet fermentation in Namibia has not been fully elucidated.

It therefore becomes important to analyse for the presence of fungi (yeasts and moulds) in the same materials (*eendjeke*) and to examine traditionally fermented and processed, freshly prepared pearl millet flour for same. The relative fermentation capacities of bacterial and fungal isolates, the effect of fermentation on the proximate nutritional content and the role of presumptive pathogenic probiotic isolates such as *Weissella confusa* in fermented pearl millet need to be clarified.

### Conflict of Interest

The author needs funding to carry out extensive microbiological study and identify bacterial and fungal isolates from the fresh flour, and on the pearl millet grains remnants traditionally used in the back sloping process.

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