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Full Length Research Paper

Lactic acid bacteria in fermentation of cereals for the production of indigenous Nigerian foods

E. Nwachukwu*, O. K. Achi, I. O. Ijeoma

Department of Microbiology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Nigeria

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The lactic acid bacteria (LAB) involved during the spontaneous fermentation of cereals (maize,millet, sorghum) for the production of indigenous foods ('akamu', 'kunu-zaki') were quantified and identified. The lactic acid bacteria counts varied during the fermentation of the cereals while the titratable acidity (TTA) increased with reduction in pH. Identification of isolates using API 50CHL system was *Lactobacillus plantarum*, *L. pentosus*, *L. cellbiosus*, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*. The potential of the isolates as starter cultures was determined by inoculating pure culture of lactic acid bacteria on sterilized cereals. The pH of the inoculated sterilized cereals dropped steadily while the total viable counts of the lactic acid bacteria especially *L. plantarum* increased. Analysis of variance (ANOVA, p=0.05) indicated the significant difference between total viable counts of different isolates during fermentation. This study showed potentials of *L. plantarum* as a starter culture for industrial fermentation of maize for 'akamu' production.

Key words: Cereals, Fermentation, Lactic acid bacteria, Akamu, Kunun-zaki

INTRODUCTION

Sorghum, maize and millet beverages in Africa possess similar features in which the lactic acid bacteria fermentation plays a key role in safety and acceptability of these products in tropical climate (Haggblade and Holzapfel, 1989). Cereal beverages are popular in Africa because of the social, religious and therapeutic values associated with them. Many African foods that are prepared by the action of diverse species of mould, bacteria and yeast such as "merissa" in Sudan, "busaa" in Uganda, "kaffir" in South Africa "bouza" in Egypt (Efiuvwevwere and Akoma, 1998) are little known outside their native countries.

Akamu is a locally prepared food from fermented maize, sorghum or millet in Nigeria. It serves as a weaning food for infants. The preparation of akamu involves soaking of corn in water for 1 to 3 days followed by wet milling and sieving (Odunfa, 1985). The filtrate is fermented for 2-3 days to produce white starchy sediment. Akamu is often marketed as a wet cake wrapped in leaves or transparent polythene bags. It can be boiled into a pap or cooked into a semi solid form prior to consumption (FAO, 1996). Kunun-zaki is millet based non-alcoholic fermented beverage widely consumed in the northern parts of Nigeria. The traditional processing of kunun-zaki involves the steeping of millet grains, wet milling with spices (ginger, clovers and pepper), wet sieving and partial gelatinization of the slurry, followed by addition of sugar and bottling (Adeyemi and Umar, 1994).

In general, a wide spectrum of micro organism is involved during fermentation process. But a few types usually determine the quality of end products (Kleerebezem and Hugenholtz, 2003; Abegaz, 2007). Therefore, isolation, characterization and identification of the microorganisms involved in fermentation of cereal with a prospective selection of starter cultures would be important to support the technical process and to obtain a predictable end-product with a desired quality. This may help in development of starter culture and devising appropriate and affordable technology that could modernize cereal based product preparation. The objective of the present study was to isolate lactic acid bacteria during fermentation of cereals, to characterize them using

*Corresponding Author, Email: drejik@yahoo.com

morphological, physiological and biochemical techniques with the aim of starter culture development.

MATERIALS AND METHODS

Cereal grains such as maize (Zea *mays*) and millet (*Eleusine coracana*) were purchased from Vom in Plateau state of Nigeria. The experiment was carried out at Federal College of Veterinary and Medical Laboratory Technology of the National Veterinary Research Institute Vom, Plateau state.

Fermentation of cereal based products

The cereal based products ('akamu' and 'kunun-zaki') were prepared using different cereals by modified methods (Odunfa, 1985; Adeyemi and Umar, 1994; Omemu *et al*., 2007).

Steeping of maize grains (2-3 days)
↓
Wet milling
↓
Sieving to remove bran, hulls and germs
↓
Souring of filtrate (2-3 days)
↓
Akamu (Odunfa, 1985; Omemu *et al*., 2007).

Steeping of millet grains
↓
Wet milling with spice
↓
Sieving of the slurry and partial gelatinization
↓
Addition of flavor and bottling
↓
Kunun-zaki (Adeyemi and Umar, 1994)

Enumeration of lactic acid bacteria

Samples were collected at 12 h intervals during fermentation of selected cereals till the end of fermentation. The cereal gruel was agitated for 2 minutes before sampling to ensure uniform mixing. Peptone water was used for serial dilutions of 10 ml samples followed by overnight incubation at 35ºC. Serial dilutions of peptone water solutions containing bacterial suspension from fermenting cereals were plated out on De Man, Rogosa and Sharpe (MRS) agar by using 1ml portions of appropriate dilutions. The inoculated plates were incubated anaerobically for 48 h at 37ºC and 42ºC respectively for the enumeration of thermophilic and mesophilic Lactic acid bacteria (LAB). Total viable counts of the LAB isolates were recorded as colony forming unit per ml (cfu/ml) after 24 to 48 h of incubation (Abegaz, 2007).

Identification of lactic acid bacteria

For identification of LAB, all isolates from the plates having discrete colonies were isolated and subcultured repeatedly on MRS agar till pure colonies were obtained. The isolates were examined by colony and cell morphology, Gram reaction, catalase test, nitrate reduction test, oxidase test and urease production. Growth at 10ºC, 15ºC and 45ºC in MRS broth for 24 to 48 hours were determined. Growth in the presence of 6%, 8% and 10% NaCl in MRS broth incubated at 37ºC for 24 to 48 hours were determined. Identification by API 50 CHL (Bio merieux SA, Marcy-l' Etoile, France) was carried out according to the instruction of the manufacturer. The suspensions of test organism were made using sterilized peptone water until the result of turbidity standard. Exactly 1 ml proportion of the suspension of test organism was inoculated into vials containing the sugar powders of the various carbohydrates of the API 50 CHL kit. Sterile normal saline was sprinkled on the tray on which the vials were placed to create a humid environment. Sterile liquid paraffin was used to seal the top of the vials to create an anaerobic environment and incubated at 37ºC for 24 to 48 h. A total of 50 representative gram positive, catalase negative, oxidase negative, urease positive, non-sporing, non-motile rods, coccobacillus, cocci and tetrad were formed into clusters based on similarity in morphology, physiology and biochemical reaction.

Chemical analysis

The changes in pH (pH meter - Surgifield medical England Sm - 6021A) and titratable acidity (TTA) (method of Amoa-Awua et al., 1996) of fermenting samples were monitored for every 12 h till the end of fermentation.

Fermentation of sterile cereals

The cereal grains were packed in air tight bottles and sealed into autoclave and sterilized at 121ºC for 15 minutes. The sample was allowed to cool to room temperature before inoculation. Pure cultures of each of the lactic acid bacteria isolates were plated on MRS agar at 30º C for 24 h. The cultures were washed off by pouring 10 ml of sterile peptone water onto the agar plates and the cell suspensions withdrawn with sterile syringes. The cell suspensions were used to prepare a10 fold serial dilution such that 1ml of inoculums produced a concentration of approximately10^6-10^7cfu/ml^{-1}using a prefixed absorbance read at 650nm against sterile peptone water. Thereafter 1 ml portion of the respective suspensions was used as inoculum for the sterilized grains steeped in sterile distilled water. Ten (10) grams of the respective inoculated grains was homogenized with 90 ml of sterile 0.1% peptone water in a stomacher. The homogenate was serially diluted and plated aseptically using spread plate method. Viable counts were made on MRS agar incubated anaerobically for 24-72 h (Omemu, *et al*., 2007). The pH changes during the controlled fermentation were also monitored.

Statistical analysis

The data obtained were analyzed using Analysis of variance (ANOVA) at p≤ 0.05 and correlation using Vassar Stats (Lowry, 1999-2007).

RESULT

During fermentation of maize for akamu (Figure 1) the lactic acid bacteria count at 0 h was 7.05×10^6cfu/ml which decreased with a sharp rise at 108 h (9.65×10^6 cfu/ml). There was an increase in TTA with reduction in

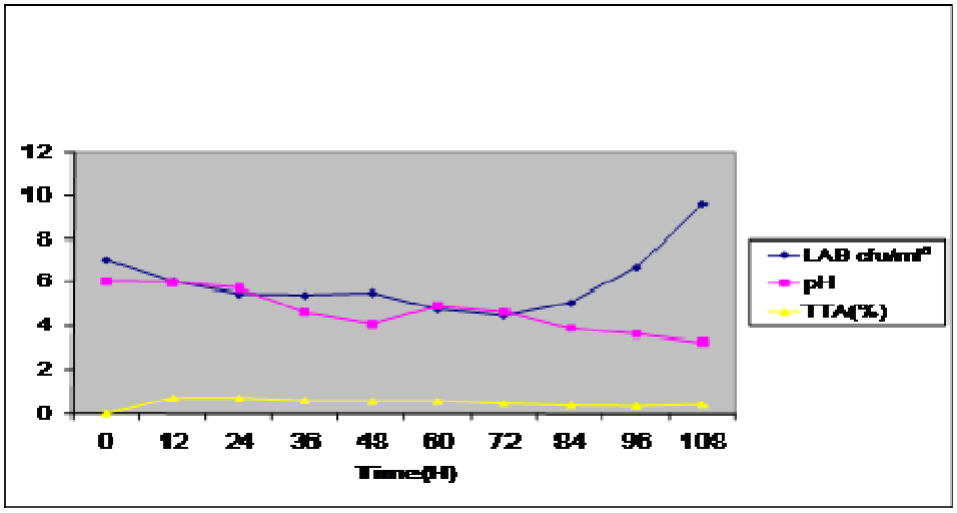

Figure. 1 Changes in lactic acid bacteria (LAB) (cfu/ml), pH and titratable acidity (TTA) during fermentation of maize

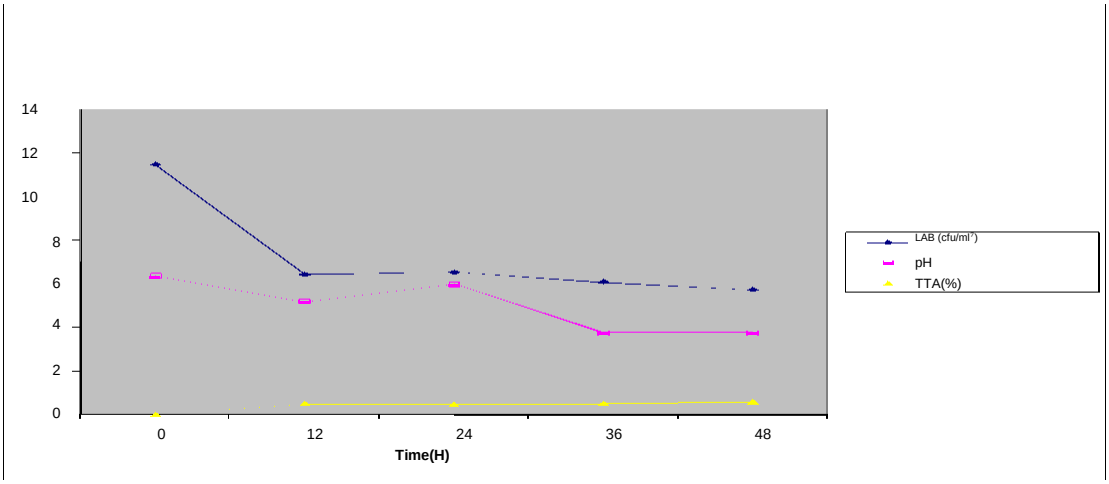

Figure. 2 Changes in lactic acid bacteria (LAB) (cfu/ml), pH and titratable acidity (TTA) during fermentation of millet

pH. There was no significant correlation between lactic acid bacteria count, pH and titrable acidity on fermentation.

The changes in lactic acid bacteria counts during the fermentation of millet for kunun-zaki showed a high microbial count at 0 h but decreased during the fermentation (Figure. 2). The pH and TTA levels were fluctuated during the fermentation. There was a significant positive correlation between TTA and LAB count at $p \leq 0.05$.

The identification of isolates using API 50CHL system was *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus cellobiosus*, *Pediococcus pentosaceus* and *Leuconostoc mesenteroides*.

The changes in pH during fermentation of sterilized cereals (maize or millet) using pure cultures of lactic acid bacteria are shown in Figure 3 and 4. The pH of the two cereals (maize and millet) inoculated differently with pure cultures of each of *Pediococcus pentosaceus, Lactobacillus plantarum* and *Lactobacillus cellobiosus* dropped from initial pH value after 24 hrs indicating fermentation of the cereals. A significant difference in pH was observed during the fermentation of maize with different pure cultures. This was not significant for millet

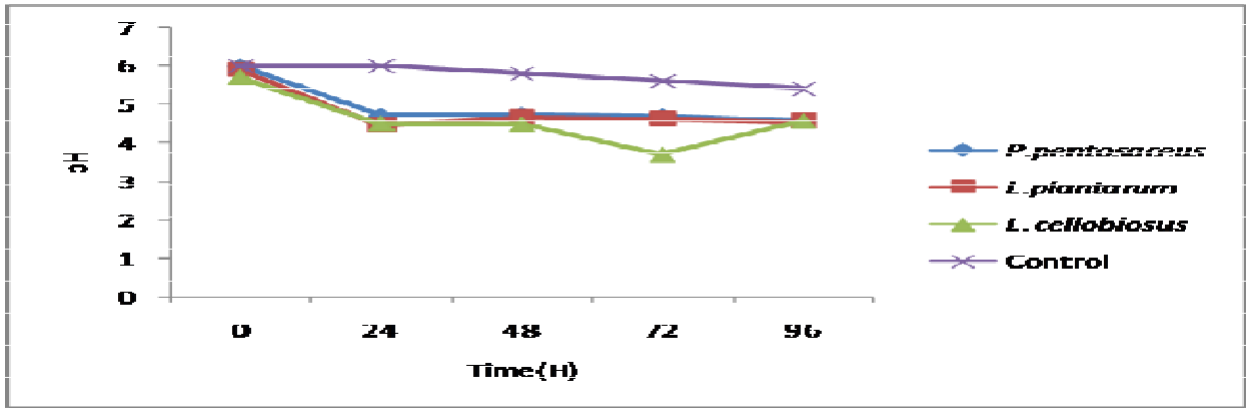

Figure. 3 pH value changes of millet inoculated with different isolates

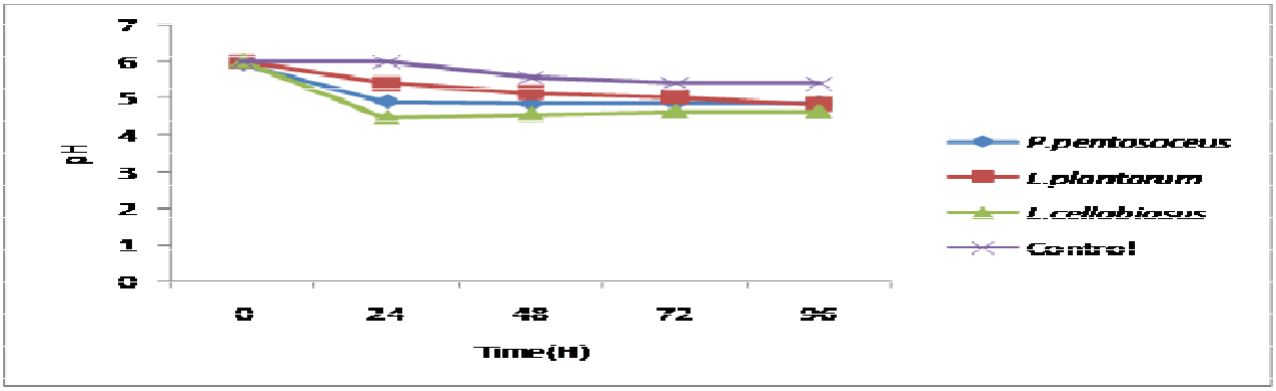

Figure. 4 pH value changes of maize inoculated with different isolates

and sorghum.

The total viable counts of the different isolates during fermentation of sterilized millet and maize respectively are shown in Figure 5 and 6. Maize and millet inoculated

DISCUSSIONS

Counts of lactic acid bacteria were high at the onset of the fermentation of maize for akamu with a decrease in counts as the fermentation progressed (Figure. 1). The decrease in lactic acid bacteria counts could be attributed to the processing steps such as the replacement of the steeped liquor prior to wet milling, removal of chaff and sieving. This agreed with a study by Omemu *et al* (2007) who noted similar reduction in yeast population in the spontaneous fermentation of akamu. The sharp increase in counts (9.65×10^{6}cfu/ml^{-1}) noted after 48 h may be with *Lactobacillus plantarum* showed the highest microbial counts at 96 h of fermentation. The changes in counts of *Pedicoccus pentosaceus* during fermentation of maize and millet was less than other two isolates. The microbial population of the different isolates in the fermentation of maize was significant at p≤ 0.05. attributed to the acidification of the fermentation medium (Abegaz, 2007).

A decrease in pH as the fermentation progressed (5.75 – 3.26) was possibly because of the accelerated growth rate of lactic acid bacteria (Inyang and Idoko, 2006). The decrease in pH and increase in LAB followed the same trend as reported for other natural fermented foods (Sulma *et al*., 1991; Choi *et al*., 1994). However a slight decrease in lactic acid concentration was observed in the later stages of fermentation. The decrease could be attributed to the utilization of lactic acid by yeast (Muyanja *et al*., 2003).

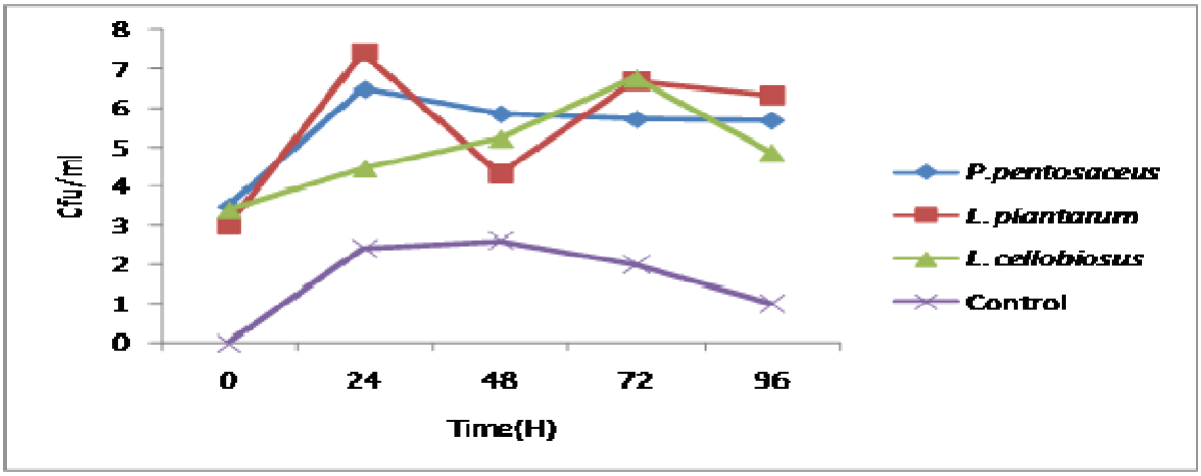

Figure. 5 Total viable counts (cfu/ml) of the different isolates during fermentation of sterilized millet

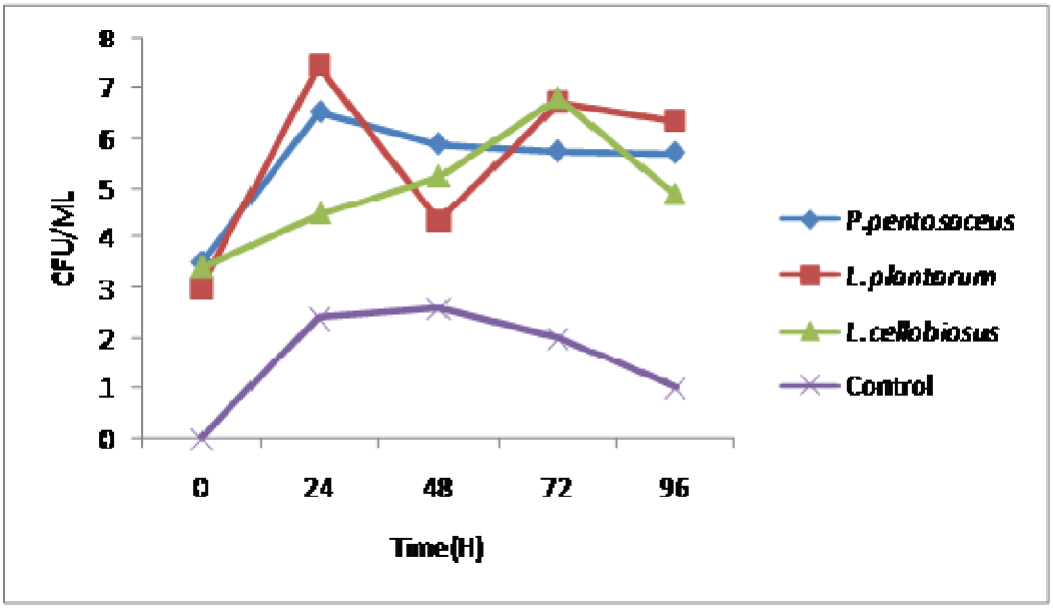

Figure. 6 Total viable counts (cfu/ml) of the different isolates during fermentation of sterilized maize

The decrease in lactic acid bacteria count in spontaneously fermented millet for kunun-zaki could be probably due to depletion in nutrient available in the cereal slurry. During the onset of fermentation, pH value was close to neutrality but in the course of the fermentation a drop in pH value was noted. This drop in pH agreed with the previous studies by Lei and Jakobsen (2004). The low pH value of 3.75 noted at the end of the fermentation was agreed with result noted by Efiuvwevwere and Akoma (1998). Generally acidity

increased as fermentation advanced (Akpinar-Bayizit *et al*., 2007).

The lactic acid bacteria isolated were *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus cellobiosus*, *Leuconostoc mesenteroides* and *Pediococcus pentosaceus*. Hounhouigan *et al*. (1993) isolated *L*. *brevis*, *L*. *curvatus*, *P*. *pentosaceus* and *Pediococcus acidilactici* during spontaneous fermentation of mawe from maize. Uchimura *et al*., (1991) reported *P. pentosaceus* in fermentation of Indonesian ragi.

The isolates were accessed for technological potentials in terms of substrate utilization and pH reduction. Using pH as an index of fermentation, it was noted that all the isolates of lactic acid bacteria used as starter cultures for fermentation of maize and millet. A decreased acidification was noted during the fermentation. The pH values recorded for the different starters during fermentation of cereals was within 4.80 - 4.50 though the spontaneous fermentation had pH value of 3.20. The lower pH noted for the spontaneous fermentation could be attributed to the combined action of yeast and lactic acid bacteria which brought about a more significant decrease in pH and a simultaneous increase in acidity in fermented cereal than use of single cultures (Khetarpual and Chaunan,1990).

L. *plantarum* used as pure cultures was noted to have the highest counts after 96 h of fermentation and this agreed with a similar study by Omemu *et al* (2007). Possible reason for the high counts of *L*. *plantarum* could be temperature of 30^{o}C of incubation which favors growth of the organism. The reduction noted in microbial counts of *P*. *pentosaceus* during fermentation of the two cereals agreed with findings of Mugula *et al*. (2002) in a similar study. Generally the reduction noted in microbial counts of the pure cultures after 48 h and 72 h of fermentation of millet and maize could be attributed to dehydration which possibly adversely affected the biota of the cereals (Amoa-Awua *et al*., 2005; Amoa-Awua *et al*. 2007).

This study reviewed the presence of different lactic acid bacteria in the fermentation of cereals. The fermentation for production of akamu showed *L*. *plantarum* as the best starter because of increase in its counts as a single culture. This study showed potentials of *L*. *plantarum* as a starter culture for industrial fermentation of akamu.

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