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Isolation and Characterization of Lactic Acid Bacteria Involved In the Fermentation of Millet and Sorgum Sold In Nkwo-Achara Market, Abia State

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Abstract: *The isolation and characterization of lactic acid bacteria responsible for the fermentation of kunu-zaki and burukutu were studied in order to access their use as starter culture. Representatives of the lactic acid bacteria were isolated at 12hours interval during the course of fermentation characterized and identified using morphological, physiological and biochemical examination. Changes in pH, titratable acidity, LAB counts were also investigated during the fermentation of the two cereals at different fermentation time (12hrs and 24hrs) . The isolates obtained were accessed to have some level of starter culture. Based on the comparative performance of single isolates to a substrate and pH reduction. Isolates identified are lactobacillus plantarum, lactobacillus cellobiosus, lactobacillus pentosus, leuconostoc mesenteriods, and pediococcus pentosaceus. Results indicates a predominance of lactobacilli among isolates. Lactic acid bacterial counts in the kunu-zaki and burukutu were isolated at levels of 10^6, 10^7, cfu/ml. A very high reduction in pH was associated with an increase in titratable acidity. Analysis of variance show significance difference between pH of all the isolates in the two substrates used at $P < 0.05\%$ of freedom.*

I. Introduction

Microorganisms have been used to produce food for thousands of years. Fermented foods are not only perceived as pleasant-tasting but the acids produced as a by-product of microbial metabolism inhibit the growth of many spoilage organisms as well as food- borne pathogen (Nester et al., 2004). Thus, fermentation historically has been and continue to be today, an important method of food preservation, particularly when modern convenience such as refrigeration are lacking. High level fermentation are difficult to achieve and are not predictable in terms of length of fermentation and quality of products. Unwanted products could be achieved at the end of the fermentation (Nout, 1992). To achieve a good fermentation products, the most predominant microorganisms found in an acceptable product are isolated and purified according to Marshall, (1993). The medium used for such process will then be pasteurized to exclude most unwanted microorganisms and the purified microorganism is introduced to initiate the fermentation process. This procedure could be manipulated in such a way to be able to predict the amount and quality of products formed and the length of fermentation period (Chamunorwa et al., 2002). Millets are group of highly variable small seeded grasses, widely grown around the world as cereal crops or grains to both human food and fodder (MacDonough et al., 2000). Millets are important crops in the semi-arid tropics of Asia and Africa, with 97% of millet production in developing countries. The most widely grown millet is pearl millet, which is more pronounced in India and parts of Africa. Finger millet, proso millet and fox tail millet are also important crop species (Lawler, 2009).

Sorghum is a genus of grasses with about 30 species, one of which is raised for grain and many of which are used as fodder plants, either cultivated or as part of pasture. The plants are cultivated in warm climates worldwide. They are native to the tropic and subtropics of the old world and one species is endemic to Mexico, a number have been introduced into other parts of the world (Wikipedia, http//www.efloras.org/floratoxin.aspx).

Some species of sorghum can contain level of hydrogen cyanide, hordenin and nitrates lethal to grazing animals in the early stages of the plant growth. When stressed by draught or heat, plants can also contain toxic levels of cyanide and or nitrates at later stages in growth (Holzapfel, 1997).

Kunuzaki is a millet based non-alcoholic fermented beverage widely consumed in the northern parts of Nigeria. This beverage is however becoming more widely consumed in the southern Nigeria, because of the refreshing taste (FAO, 1996).

Burukutu is one of the indigenous alcoholic beverages and is produced mainly from the grains of guinea corn (Sorghum vulgare and sorghum bicolor). Sorghum grains are steeped in water overnight, washed and excess water drained (Kolawole et al., 2007). Microorganisms are needed during the fermentation process but a few usually determines quality of the end products. If favourable environmental condition is allowed, a particular community of microbes can determine the quality of a specific food (Kolawole, 2007). Isolation and characterization of lactic acid bacteria with a selection of starter cultures that are adapted to cereal based

production would be important. This can help to develop a starter culture and devise a means of affordable technology. Therefore, the objective of this work is to isolate lactic acid bacteria during the cause of traditional fermentation of millet and sorghum and to characterize them using morphological and physiological technique.

II. Materials And Methods

Source Of Cereal Grains

Cereal grains millet (Elausine coraccina), and sorghum (Sorghum vulgare) were purchased from Nkwo Achara main market, Abia State, Nigeria.

Traditional Fermentation Of The Sample

The traditional fermentation were achieved using a modified methods of Kolawole et al., (2007) . 250g was soaked for each of the millet and sorghum in one litre of tap water. Ten milliliters of the cereal gruel was collected at 12hrs intervals from the start of the traditional fermentation to the termination for the determination of the fermentation parameters.

This includes the isolation of lactic acid bacteria, determination of pH and titratable acidity. Production of kunu-zaki starts from steeping the millet grains followed by wet milling with spices, sieving of the slurry and partial gelatinization. flavour will thereafter be added and bottled (Adeyemi and Umar, 1994). The production of sorghum starts from the steeping of the grain overnight followed by malting of the grains for 5 days. The malted grains are sundried, grind and mixed with water and boiled for hours, further fermentation for up to 45hrs will follow and the last product is burukutu (Kolawole et al., 2007).

III. Isolation And Enumeration Of Lactic Acid Bacteria (Lab)

The cereal gruel was agitated for 2mins before sampling to ensure mixing. Surface plating on MRS agar was used anaerobically using the method of Okereke and Okereke, (2013). Identification of discrete colonies and gram reaction was by the modified method of Cheezbrough, (2005).

Chemical Analysis

The changes in pH of fermenting samples were monitored every 12 hours for each of the grains using a pH meter (Surgified Medical England SM – 6021A).

The titratable acidity was determined by titrating 20ml of liquid samples using the method described by Amoa-Awua et al., (1996).

Samples of the cereal grains were sterilized using 1 litre Erlenmeyer flask and aluminum cotton wool described by Oluwafemi and Ibeh, (2011). Controlled fermentation was conducted and total viable count obtained using the procedure of Amoa-Awua et al., (2007).

Statistical Analysis

Analysis of various and mean separation was determined by the method of Duncan's multiple range or the least square difference (LSD) test at $P< 0.05$.

IV. Results and discussion

Fig 1.0 Changes In Lactic Acid Bacteria (Lab) (Cfu/Ml^{-1}), pH And Titratable Acidity During Fermentation Of Millet

Time (hrs)	LAB	pH	TTA (%)
0 hours	$11.5 \times 10^7 \pm 5.00^a$	6.4 ± 0.01^a	0.03 ± 0.01^c
12 hours	$6.45 \pm 10^7 \pm 15.50^b$	5.2 ± 0.01^a	0.51 ± 0.01^a
24 hours	$6.55 \pm 10^7 \pm 15.50^b$	6.0 ± 0.21^a	0.47 ± 0.01^b
36 hours	$6.10 \times 10^7 \pm 10.00^b$	3.75 ± 0.04^b	0.53 ± 0.03^a
48 hours	$5.75 \times 10^7 \pm 6.50^b$	3.75 ± 0.13^b	0.59 ± 0.01^a

Means in the same column with different superscript are significantly different ($P < 0.05$).

Changes in the lactic acid counts during the fermentation of kunu-zaki showed an initial high microbial count of 11.5×10^7 cfu/ml at 0hour but decreased during the fermentation as shown in figure 1.0. A variation in the pH and titratable acidity was observed during the fermentation. Analysis of variance show significant difference between the pH, lactic acid bacteria counts and TTA during the fermentation at $P < 0.05$.

The decrease in lactic acid bacteria could be as a result of the processing steps such as water replacement and wet milling (Omemu et al., 2007).

Fig 2.0: Changes In Lactic Acid Bacteria (Lab) (Cfu/Ml^{-1}), pH And Titratable Acidity (Tta) During The Fermentation Of Sorghum.

Time (hrs)	LAB	pH	TTA (%)
0	$3.50 \times 10^6 \pm 25.00^d$	5.85 ± 0.02^b	0.15 ± 0.01^b
12	$3.65 \times 10^6 \pm 6.50^d$	5.45 ± 0.01^b	0.04 ± 0.01^c
24	$3.60 \times 10^6 \pm 16.00^d$	5.85 ± 0.01^b	0.04 ± 0.01^c
36	$3.15 \times 10^6 \pm 0.50^d$	5.45 ± 0.01^b	0.21 ± 0.02^{ab}
48hrs	$3.50 \times 10^6 \pm 5.00^d$	5.50 ± 0.01^b	0.03 ± 0.01^c

Means in the same column with different superscript are significantly different (P<0.05)

Fig 2 show the results of lactic acid bacteria (LAB), pH and titratable acidity during the fermentation of sorghum. There was a gradual increase in counts of lactic acid bacteria from 3.50×10^6 cfu/ml during the 0 hour to 3.60×10^6cfu/ml during the 24 hours. A reduction of 3.15×10^6 cfu/ml was observed at 36 hours and a sharp increase again during the 48 hours (3.50×10^6cfu/ml).

The increase at the initial time could be attributed to the utilization of nutrients in the medium (Choi et al., 1994). The decrease in initial high count of lactic acid in fig. 2.0 could be as a result of nutrient depletion in the slurry (Osuntogun and Aboaba, 2004).

Fig 3: pH Values Of Millet Inoculated With Different Isolates

Innoculum	0	24hrs	48hrs
Pediococcus Pentosaceus	6.00 ± 0.10^a	4.71 ± 0.01^b	4.70 ± 0.01^b
Lactobacillus Plantarum	5.90 ± 0.10^c	4.47 ± 0.01^d	4.65 ± 0.01^{bd}
Lactobacillus cellobiosus	5.70 ± 0.10^E	4.50 ± 0.01^h	3.69 ± 0.01^T
Control	6.00 ± 0.10^a	6.00 ± 0.10^a	5.80 ± 0.10^b

Means in the same row with different superscript are significantly different (P<0.05)

This research has confirmed the presence of lactic acid bacteria in the fermentation of cereal based products. The genera of lactic acid bacteria isolated included lactobacillus, leuconostoc and pediococcus. Most of the LAB isolated from the fermentation of these cereal products have also been isolated from other fermented foods (Abegaz, 2007).

The dominance of L. plantarum has been attributed to it's acid tolerance (Houhouigan et al., 1993) and has also been isolated from raw sorghum powder (Kunene et al., 2000) and also from vegetable fermentation (Oyewole and Odunfa, 1990). Julius et al., (2009) isolated L. plantarum from maasai and palm wine. Hounhuigan et al., (1993) isolated L. brevis, L. curvatus, P. pentosaceus and pediococcus acidilactici during spontaneous fermentation of mawe from maize.

Fig 4.O: Morphological And Physiological Properties Of Lactic Acid Bacteria (Lab) Isolated From The Cereal Based Foods

Cell form & arrangement	Gram reaction	MOT	CAT	OXI	URE	NIT	PROBABLE genus
Cell in clusters	+	-	-	-	+	-	Pediococcus
Short rod in tetrades or pairs	+	-	-	-	+	-	Lactobaciillus
Long rod in clusters	+	-	-	-	+	-	Lactobacillus
Short rod in pairs	+	-	-	-	+	-	Leuconostoc
Long/short rod in pairs or singles	+	-	-	-	+	-	Lactobacillus

Key:
MOT – Motility test
OXI – Oxidase test
NIT – Nitrate test
- Negative
+ Positive
URE – Urease test
CAT – Catalase test

From fig 4.0, it is clear that lactic acid bacteria generally grow and remain viable within a medium pH range of 4.5 to 7.0. MacDonald et al., (1990) reported similar results for cells of lactobacillus plantarum which grow until a final pH 4.6 to 4.8 was reached. Using pH as an index of fermentation, all the isolates of lactic acid bacteria used as starter cultures fermented millet and sorghum into it's end product. Lactic acid bacteria have been consistently demonstrated to be responsible for the traditional lactic acid fermentation of sorghum into beer (Steinkraus, 1996). This work has confirmed a role of lactic acid bacteria in burukutu fermentation as an important tool.

V. Conclusion And Recommendation

This work have shown the presence of a diverse species of lactic acid bacteria in the fermentation of millet and sorghum into kunun-zaki and burukutu.

According to Rolle and Satin, (2002), traditional small scale fermentation technologies offered considerable potential for stimulating development in the food industry of developing countries in light of their low cost, scalability, minimal energy etc. the production of traditional foods/drinks e.g kunun-zaki and Burukutu was processed in a clean, and conducive environment which avoided the inversion of extraneous microbes that could cause food poisoning as reported by Onyeagba and Isu, (2006).

Hence that achievement was recorded in this research, more efforts should be geared towards the use of molecular biology and biotechnology to confirm the identification of the dominant lactic acid bacteria in cereal based foods.

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