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Evaluation of *OGI* (CORN CARAMEL) from Maize and Sorghum for Isolation and Characterisation of Lactic Acid Bacteria (LAB)

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Abstract: Isolation and characterisation of Lactic Acid Bacteria (LAB) associated with the fermentation of maize and sorghum for the production of *ogi* were evaluated for the development of starter cultures. Changes in pH and LAB counts were investigated during the cereal based-product fermentation. A decrease in pH was observed during the natural fermentation from 0hr to 48hrs. The LAB were isolated, characterised and identified using cultural, morphological and physiological methods. The isolates were: *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus acidophilus, Lactobacillus cellobiosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus casei.* The isolates from maize–produced *ogi* were capable of growing on sterilised maize grains, with *Lactobacillus plantarum* having the highest potential as a starter culture for *ogi* production from maize. Also, the isolates from sorghum-produced *ogi* were capable of growing on sterilised sorghum grains, with *Lactobacillus delbrueckii* subsp. *bulgaricus* having the higher potential as starter culture for *ogi* production from sorghum. Sterilised maize grains inoculated with pure culture of *Lactobacillus plantarum* showed the highest counts of 2.1×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacteria. In the same vein, sterilised sorghum grains inoculated with pure culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* showed the higher counts of 1.12×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacterium. There was no significant difference ($p \leq 0.05$) in the mean of the pH values obtained during the spontaneous fermentation of maize and sorghum. The results obtained indicated that all the six LAB isolates could be used singly as starter cultures to produce *ogi* within a shorter fermentation period.

Keywords: *Ogi*, Maize, Sorghum, Fermentation, Microbial Evaluation, Lactic Acid Bacteria, Starter Culture

1. Introduction

Microorganisms have been used to produce food for thousands of years. Lactic acid bacteria constitute an important group of these organisms and have been associated with production of fermented foods and feeds for many centuries [1]. Some of the reasons for their widespread use are the ability to retard spoilage, preserve food as well as improve flavour and texture of foods. They also play fundamental role in microbial ecology of foods by synthesizing a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins [2-3]. They are increasingly being recognised for their health and nutritional benefits, hence some strains are used as probiotics [4-7].

One of the popular indigenous fermented foods in Nigeria is *ogi* (Corn caramel), which is a fermented cereal porridge made from maize (*Zea mays*), sorghum (*Sorghum vulgare*) or millet (*Pennisetum typoideum*). The *ogi* porridge is very smooth in texture, and has a sour taste reminiscent of that of yoghurt [8]. Traditional fermentation processes of *ogi* production are usually spontaneous and uncontrolled [9]. *Ogi* is popularly produced by grinding cereals such as maize, sorghum or millet, sieving it wet and then allowing the starch to sediment and ferment in a liquid menstruum [10]. In

Nigeria, *ogi* is either prepared into a smooth porridge called *pap* or a solid gel known as *eko* or *agidi*. The consistency of the *pap* varies from thick to watery according to choice. The *pap* can be sweetened with sugar and milk; it is then eaten with bean cake. The *pap* is used as the first native food for weaning babies [11-12]. It also serves as breakfast meal for pre-school, school children and adults [13]. In a more concentrated form, it is boiled into a thick gel and then allowed to set stiff in leaf moulds as *eko* or *agidi*. In either form, it is usually preferred to many other indigenous foods by the aged and the convalescence.

The traditional method of *ogi* production is labour intensive, time consuming and has low productivities, with success depending upon observation of good manufacturing practice. Several traditional fermentations from Asia have been upgraded to high technology production system because of the strong research tradition in fermented food technology. Their experience can be used to upgrade some African's indigenous fermented foods [14]. *Ogi*, being important cereal porridge in the West African sub-region, has for some time, been a subject of scientific evaluations. Many workers have reported on different aspects of *ogi* production. Particular attention has been given to the various aspects such as process variations and mechanization, and nutritional improvement [11, 15]. The microbiology of *ogi* and related products during the processing stages up to the finished products have equally been studied [15-16]. New attention is presently on the use of starter cultures, which is solving numerous problems associated with the product. There are many studies about different fermentation techniques during *ogi* production such as accelerated batch fermentation, starter culture fermentation, and dry milling before fermentation with the aim of replacing the traditional (spontaneous) fermentation technique. Hence, this research was carried out to isolate and characterize Lactic Acid Bacteria (LAB) associated with the fermentation of maize and sorghum for the production of *ogi* for the development of starter cultures.

2. Methods

2.1. Sample Collection

Maize and sorghum grains that were used were purchased from Gwagwalada Market, FCT, Nigeria.

2.2. Laboratory Preparation of Ogi

White variety of maize grain and red variety of Sorghum used for the study were sorted to remove dirts and spoilt ones from healthy ones. The sorted ones were soaked in water for 2 days, followed by wet-milling, and sieving to remove bran, hulls and germ. The pomace was then retained on the sieve, and later discarded. The filtrate was then fermented for 2 days to yield *ogi,* which is starchy sediment, by leaving the filtrate in a covered container.

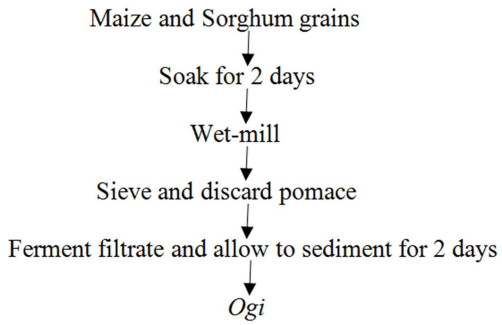

Figure 1. *Flow diagram for the preparation of ogi from maize and sorghum.*

2.3. Physicochemical Analysis

The hydrogen ion concentration (pH) changes of the fermenting maize and sorghum grains were measured at 24h intervals for 48 h using a pH meter.

2.4. Isolation of Microorganisms

Stock culture was prepared from each slurry by aseptically adding 5ml of the *ogi* into sterile 250ml conical flask containing 45ml of sterile distilled water. The mixture was then shaken to form a suspension. A serial dilution of eachslurry was carried out using the Ten-fold dilution method, and pour plate method using deManRogosa Sharpe (MRS) agar. The MRS agar plates were then incubated anaerobically at 30°C for 48h.

2.5. Growth Evaluation

The numbers of microbial colonies were counted. The total viable count was then determined using the expression:

$$\frac{\text{Number of microbial colonies} \times \text{Volume inoculated}}{\text{Dilution factor}} \quad (1)$$

Colonies with distinct cultural characteristics such as colour, size and shape were randomly picked from the MRS agar plates as presumptive lactic acid bacteria isolates and streaked on fresh MRS agar plates to purify the isolates. They were then maintained on appropriate slants at 4°C for further analyses.

2.6. Characterisation and Identification

Each of the lactic acid bacteria isolate was initially examined for colonial and cell morphology, cell arrangement, spore formation and motility. Only the gram positive, catalase negative and non-spore forming isolates were characterised by phenotypic and biochemical tests. The lactic acid bacteria isolates were tested for fermentation of the following carbohydrates: D-glucose, lactose, sucrose, galactose, maltose, mannose and fructose. Bromocresol purple broth base was used as basal medium. One percent filter-sterilised sugar solution using 0.2µm Millipore filter (corning) was added aseptically into sterilized bromocresol purple broth base before inoculation with culture of each lactic acid bacteria strain. The results were assessed with reference to an uninoculated control after anaerobic incubation at 30°C for 5 days. Test tubes in which

bromocresol purple colour changed yellow indicated utilization of sugar or acid fermentation. The various lactic acid bacteria strains were then identified by reference to the Bergey's Manual of Systemic Bacteriology [17] and the Genera of Lactic Acid Bacteria based on the results of the various tests.

2.6.1. Gram Staining of the Isolates

The Gram staining was conducted as follows: Smear of culture was prepared on clean slide by emulsifying a little quantity of the growth on a drop of normal saline. The smear was then allowed to air-dry. After which, heat-fixing was done. Crystal violet was then added as a primary stain for 30s and then drained off with distilled water. Lugol's iodine was then added and allowed to react for 30s. After which, it was washed off with distilled water. Acetone alcohol was added, and immediately, it was washed off with distilled water. After which, the smear was counter-stained with safranin for 1 minute, and washed with distilled water. The smear was then allowed to dry. A drop of oil immersion was placed on the stained smear and viewed with a high objective lens of microscope.

2.6.2. Test for Spore Formation

Smear of the organism was made on clean microscope slide. After which, the slide was air-dried and the smear was heat-fixed. Small piece of blotting paper (absorbent paper) was placed on the slide (smear side up), and then placed on a wire gauze on a ring stand. The smear was flooded and kept saturated with malachite green. The slide was heated gently till it started to evaporate via Bunsen burner and the heat was removed. After which, the slide was reheated to heat needed to keep the slide steaming for about 3-5 minutes. As the paper began to dry, two drops of malachite green was added to keep it moist. After 5 minutes, the slide was carefully removed from the rack. The blotting paper was removed and the slide was allowed to cool to room temperature for 2 minutes. The slide was thoroughly rinsed with tap water (to wash the malachite green from both sides of the microscope slide). The smear was stained with safranin for 2 minutes. Both sides of the slide were then rinsed to remove the secondary stain. After which, the slide was blotted and air-dried. The bacterium was observed under 100× (oil immersion) total magnification.

2.6.3. Catalase Test

A colony of culture was picked using a sterile wire loop and then emulsified in a few drop of hydrogen peroxide on a clean microscope slide. Absence of effervescence indicated catalase-negative.

2.6.4. Motility Test

Discrete colony of overnight culture was placed on microscope slide containing a drop of peptone water, and covered with a cover slip after a minute. Then, viewing microscopically with 40× objective was done.

2.6.5. Sugar Fermentation

Exactly 10ml of peptone water was introduced into different sterile test tubes. One gram of respective carbohydrate, i.e., D-glucose, lactose, sucrose, galactose, mannose, fructose and maltose was added into each of the test tubes that contained the peptone water and labelled accordingly. They were stirred to dissolve completely by warming over a Bunsen burner.

Bromocresol purple broth base was used as basal medium. One percent filter-sterilised sugar solution using 0.2µm Millipore filter (corning) was added aseptically into sterilised bromocresol purple broth base. The tubes were plugged with cotton wool and sealed with foil before sterilisation in autoclave at 115°C for 15 minutes. After the sterilisation of the medium, the cultural organisms were inoculated into each of the tubes respectively and Durham's tubes were inserted in inverted positions into each of the tubes. The results were assessed with reference to an uninoculated control after anaerobic incubation at 30°C for 5 days. Tubes in which bromocresol purple colour changed to yellow indicated utilization of sugar or acid production. Gas production was shown by the presence of bubbles on the surface of the medium and on upward movement of the inverted Durham's tubes.

2.7. Fermentation of Sterile Cereals

The cereal grains were packed in air-tight bottles and sealed into autoclave and sterilised at 121°C 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 30°C for 48h. The cultures were washed off by pouring 10ml of sterile peptone water onto the agar plates and the cell suspensions withdrawn with sterile syringes. The cell suspensions were used to prepare a 10-fold serial dilution. Thereafter, 1ml portion of the respective suspensions was used as inoculum for the sterilised grains steeped in sterile distilled water. Ten grams of the respective inoculated grains were homogenated, serially diluted, and plated aseptically using pour plate method. Viable counts were made on MRS agar incubated anaerobically for 48h.

2.8. Statistical Analysis

pH values obtained from the spontaneous fermentation of maize and sorghum were analysed by one-way analysis of variance (ANOVA) at P = 0.05 and P = 0.01.

3. Results

Table 1. *Changes in pH during the Natural Fermentation of Maize.*

Time (hrs)	pH
0	6.6
24	4.9
48	3.4

Table 2. *Changes in pH during the Natural Fermentation of Sorghum.*

Time (hrs)	pH
0	6.8
24	5.7
48	4.2

Table 3. *Cultural Characteristics of the Lactic Acid Bacteria from ogi Prepared from Maize.*

Code	Colony character				
	Colony Shape	**Edge**	**Elevation**	**Surface**	**Pigmentation**
MF	Filamentous	Undulate	Umbonate	Smooth	Creamy
MC	Circular	Entire	Convex	Smooth	Creamy
MP	Punctiform	Entire	Convex	Smooth	White
MR	Rhizoid	Lobate	Raised	Smooth	Yellow

Key: MF = Filamentous microorganism isolated from *ogi* prepared from maize; MC = Circular microorganism isolated from *ogi*prepared from maize; MP = Punctiform microorganism isolated from *ogi* prepared from maize; MR = Raised microorganism isolated from *ogi* prepared from maize.

Table 4. *Cultural Characteristics of the Lactic Acid Bacteria from OgiPrepared from Sorghum.*

Code	Colony character				
	Colony Shape	**Edge**	**Elevation**	**Surface**	**Pigmentation**
SP	Punctiform	Entire	Convex	Smooth	White
SC	Circular	Entire	Convex	Smooth	Creamy

Key: SP = Punctiform microorganism isolated from *ogi* prepared from sorghum; SC = Circular microorganism isolated from *ogi* prepared from sorghum.

Table 5. *Changes in Lactic Acid Bacteria (LAB) (cfu/ml) for 48 hours of Fermentation of Maize.*

Code	Time (hrs)	TVC (cfu/ml)
MF	48	1.0×10^7
MC	48	3.0×10^6
MP	48	7.0×10^6
MR	48	6.4×10^6

Key: TVC = Total Viable Count.

Table 6. *Changes in Lactic Acid Bacteria (LAB) (cfu/ml) for 48 hours of Fermentation of Sorghum.*

Code	Time (hrs)	TVC (cfu/ml)
SP	48	4.8×10^6
SC	48	2.1×10^6

Table 7. *Morphological Characteristics of Lactic Acid Bacteria (LAB) Isolated from Ogi Prepared from Maize.*

Code	Morphological Characteristics	
	Cell form	**Cellular arrangement**
MF	Short rods	Chains
MC	Cocci	Chains
MP	Short rods	Clusters
MR	Short rods	Clusters

Table 8. *Morphological Characteristics of Lactic Acid Bacteria (LAB) Isolated from Ogi Prepared from Sorghum.*

Code	Morphological Characteristics	
	Cell form	**Cellular arrangement**
SP	Short rods	Single
SC	Short rods	Chains

Table 9. *Physiological Properties of Lactic Acid Bacteria (LAB) Isolated from Ogi Prepared from Maize.*

Code	Gram Reaction	Spore Formation	Catalase Test	Motility Test	Sugar Fermentation						
					D-g	**L**	**S**	**Ga**	**Man**	**F**	**Mal**
MF	+	-	-	-	A	A	A	A	A	A	AG
MC	+	-	-	-	A	A	A	A	A	A	A
MP	+	-	-	-	AG	A	A	AG	A	AG	A
MR	+	-	-	-	A	AG	A	A	A	A	A

Key: += Positive; -= Negative; A = Acid; AG = Acid and Gas; D-g = D-glucose; L = Lactose; S = Sucrose; Ga = Galactose; Man = Mannose; F = Fructose; Mal = Maltose.

Table 10. *Physiological Properties of Lactic Acid Bacteria (LAB) Isolated from Ogi Prepared from Sorghum.*

Code	Gram Reaction	Spore Formation	Catalase Test	Motility Test	Sugar Fermentation						
					D-g	L	S	Ga	Man	F	Mal
SP	+	-	-	-	A	AG	A	A	A	A	A
SC	+	-	-	-	A	A	A	A	A	A	A

Table 11. *Identified Lactic Acid Bacteria (LAB) Isolated from Ogi prepared from Maize.*

Code	Identified LAB
MF	*Lactobacillus acidophilus*
MC	*Lactococcus lactis*
MP	*Lactobacillus cellobiosus*
MR	*Lactobacillus plantarum*

Table 12. *Identified Lactic Acid Bacteria (LAB) Isolated from Ogi prepared from Sorghum.*

Code	Identified LAB
SP	*Lactobacillus delbrueckii* subsp. *bulgaricus*
SC	*Lactobacillus casei*

Table 13. *Bacterial Counts of the Different Lactic Acid Bacteria (LAB) in Sterilised Maize during Fermentation.*

Inoculum	Time (hrs)	TVC (cfu/ml)
Lactobacillus acidophilus	24	1.5×10^7
Lactococcus lactis	24	7.7×10^7
Lactobacillus cellobiosus	24	1.8×10^8
Lactobacillus plantarum	24	2.1×10^8

Table 14. *Bacterial Counts of the Different Lactic Acid Bacteria (LAB) in Sterilised Sorghum during Fermentation.*

Inoculum	Time (hrs)	TVC (cfu/ml)
Lactobacillus delbrueckii subsp. *bulgaricus*	24	1.12×10^8
Lactobacillus casei	24	1.06×10^8

4. Discussion

The results obtained in this research showed that during the spontaneous fermentation of maize, the pH of fermented maize decreased from 6.6 to 3.4 within 48hrs (Table 1). The pH of fermented sorghum decreased from 6.8 to 4.2 within 48 hrs (Table 2). These results are in agreement with the results of Hamad *et al*.; Asmahan and Muna; Ojokoh; and Olaoluwa *et al.* [18-21]. The fermenting organisms do contribute to acidity attributable to the production of lactic acid and acetic acid during the processes which exert a depressive effect on the pH of the fermenting materials [22-23]. The decrease in pH followed the same trend as reported for other natural fermented foods [24]. There was no significant difference in the mean of the pH during the natural fermentation of maize and sorghum. Different cultural characteristics were observed for the different isolates (Table 3 & 4).

The cell studies revealed short-rods LAB, as well as a coccus-shaped isolate (Table 7 & 8). The isolates were gram-positive, non-sporing, non-motile and catalase-negative. Fermentation tests revealed the isolates possessing the ability to ferment sugars used, with some producing gas, while others did not possess such ability (Table 9 &10). All the bacteria isolated from the fermented foods fit the classification of LAB as Gram- positive, catalase-negative, non-sporing, and non-motile.

Six Lactic Acid Bacteria (LAB) were obtained from *ogi* prepared from maize and sorghum. The isolates were initially differentiated on the basis of their cultural and morphological studies, after which, they were subjected to various physiological tests. The LAB isolates were: *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus acidophilus, Lactobacillus cellobiosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus casei* (Table 11 and Table 12).

Sterilised maize grains inoculated with pure culture of *Lactobacillus plantarum* showed the highest counts of 2.1×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacteria. In the same vein, sterilised sorghum grains inoculated with pure culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* showed the higher counts of 1.12×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacterium (Table 13 & 14). The results agree with a similar study by Omemu *et al.;* and Nwachukwu *et al.* [25-26]. Possible reason for the high counts of *Lactobacillus plantarum* could be temperature of 30°C of incubation which favours growth of the organism [26].

5. Conclusion

This study evaluated *ogi* prepared from maize and sorghum the presence of different Lactic Acid Bacteria.

Changes in pH and LAB counts were investigated during the cereal based-product fermentation. A decrease in pH was observed during the natural fermentation from 0hr to 48hrs. The isolates obtained were: *Lactobacillus plantarum*, *Lactococcus lactis*, *Lactobacillus acidophilus, Lactobacillus cellobiosus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus casei.* The isolates from maize–produced *ogi* wereobserved to be capable of growing on sterilised maize grains, with *Lactobacillus plantarum* having the highest potential as a starter culture for *ogi* production from maize. Also, the isolates from sorghum-produced *ogi* were capable of growing on sterilized sorghum grains, with *Lactobacillus delbrueckii* subsp. *bulgaricus* having the higher potential as starter culture for *ogi* production from sorghum. Sterilised maize grains inoculated with pure culture of *Lactobacillus plantarum* showed the highest counts of 2.1×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacteria. Meanwhile, sterilised sorghum grains inoculated with pure culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* showed the higher counts of 1.12×10^8 at 24 hrs of fermentation than the other Lactic Acid Bacterium. The fermentation for production of *ogi* from maize showed *Lactobacillus plantarum* as the best starter culture for fermentation because of increase in its counts as a single culture. Also, the fermentation for production of *ogi* from sorghum showed *Lactobacillus delbrueckii* subsp. *bulgaricus* as the better starter culture for fermentation. The results obtained indicated that, judging from the lowering of pH value and production of lactic acid; all the six LAB isolates could be used singly as starter cultures to produce *ogi* within a shorter fermentation period.

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