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# Identification of bacteria associated with pap (Ogi/Akamu) stored at room and refrigerated temperatures

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## Abstract

Stored pap (ogi/akamu) has been reported to harbor several microorganisms including bacteria. The study was focused on identification of bacteria associated with pap (ogi/akamu) stored at room and refrigerated temperatures. The assayed pap was produced by soaking yellow maize (500 g) in sterile water and allowed to ferment for 72h and then grinded using home blender, sieved with a muslin cloth to obtain the pap. The pap was divided into two equal parts. One part was stored at room temperature and the other stored in a refrigerator for 9days respectively. At every 24h, each of the samples was taken to the laboratory for examination. The serially diluted pap samples were inoculated into De man rogosa and sharpe agar, nutrient agar, mannitol salt agar, salmonella shigella agar and MacConkey agar respectively and were incubated at 37 °C for 24h. The numbers of microbial colonies on the nutrient agar plates were counted using a colony counter. The phenotypic characterizations were carried out on the isolates and molecular identification done on the predominant lactic acid bacteria. The phenotypic characterization revealed the isolated bacteria as *Lactobacillus* sp.*, Escherichia coli, Salmonella* sp. and *Staphylococcus aureus.* The molecular characterization confirmed the predominant bacteria as *Lactobacillus plantarum* FPS. The total bacterial count was more at room temperature samples (±3.78CFU/ml) than refrigerated temperature (±0.41CFU/ml). There was significant difference between the recovered bacteria at room and refrigerated temperature ($p>0.05$). The obtained result confirmed room temperature unsafe for storing pap and refrigerator temperature better option for storing them.

**Keywords**: Isolation; Pap; Characterization; Temperature

## 1. Introduction

Pap(ogiakamu) is a gel-like traditional fermented starchy food which can be produced from maize, millet or sorghum and popularly consumed in Nigeria[1] .This food had undergone a desirable change due to the action of the invading microorganisms or their metabolic products [2]. Traditionally, pap is made by soaking any of these grains in water for 72h, then wet-milling and sieved to remove husks. The fermentation process of pap is achieved by several lactic acid bacteria including *Lactobacillus* spp. and various yeasts including *Saccharomyces* and *Candida* spp. [3]

Pap may be mixed with milk or grinded crayfish and is used as a weaning food for babies. It can also be consumed by adult with bean cake, fried ripe plantain, or mixed with little sugar and milk [4]. Pap is very nutritious as it contains many essential nutrients. It was reported that pap significantly contained the high protein content, potassium (14.50–19.10 mg/100 g) [5]. The phytochemical composition (mg/g) of the experimental pap samples was at the range of 0.34–1.62, 53.82–177.09, 0.00–0.10, 3.71–69.22 and 2.08–6.08 mg/g in oxalate, saponin, flavonoid, phytate and tannin, respectively [5]. The pap samples, especially the one made with yellow maize was high in calcium, Ca/P ratio, iron, zinc but low in phytate and tannin content. Pap is very rich in dietary fiber and carbohydrates. It is 100% natural as it has

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no additives or any artificial content. It contains high moisture and helps to supply the daily requirement of water.As a result, it is used by nursing mothers to help the easy flow of breast milk (lactation) after delivery [6].

*Lactobacillus*, *Bacillus*, *Corynebacterium*, *Streptococcus* and *Clostridium* spp. were isolated bacteria from stored pap [7]. The isolated fungi genera include; *Aspergillus species, Fusarium, Pencillium, Saccharomyces*and *Candida* spp. Several pathogens including *leuconostoc* spp, *Escherichia* spp., *Staphylococcus aureus,* Klebsiella sp*., Enterococcus faecalis*, *Pseudomonas* and *Proteus* spp. were also isolated by [8,9].

Ogi can be contaminated during its production. The contamination of ogi by pathogens could be through the water used for soaking, during grinding and through the muslin clothes used for sieving. To avoid these contaminants in order to increase the shelf life of ogi, good and personal hygienic practices should be maintained at every stages of production. Also, appropriate training about the safety standards and good manufacturing practices during production should be adopted to ensure good health of the consumers.

There are many ways ogi can be stored. It can be stored at room temperature and can be soaked in water but may not be a good option as it can last for a couple of days . It can also be stored in a refrigerator at temperature ranging between 37 °F (3 °C) and 40 °F (5 °C) and can last longer unless there was failure in power supply. Pap can be stored in a freezer(temperature at 0 °F or -18 °C) and one of the best ways to preserve pap. One of the oldest methods of storing pap is drying method. This method can be used to store pap as long as the pap is free of moisture [10].

Therefore, it is necessary to assess this stored pap for predominant bacteria to ensure consumers safety.

## 2. Materials and methods

### 2.1. Source of Sample

The assayed yellow maize grains were purchased from Garikki Market Enugu State, Nigeria.

### 2.2. Preparation of Samples

The yellow maize grains were sorted to remove stones and spoilt grains. The maize (500 g) was weighed, washed using sterile water and then steeped in water for 72h. Thereafter, it was grinded using home blender (corona), sieved with a muslin cloth and was allowed to sediment to decant the water to obtain the pap. The pap was divided into two equal parts. One was stored at room temperature and the other part was kept in refrigerator. All the samples were left for 9days. At every 24h, each of the samples was taken to the laboratory for examination.

### 2.3. Bacterial Isolation

A loop full of each of the pap samples were serially diluted using sterile water. Thereafter, they were inoculated into De man rogosa and sharpe agar, nutrient agar, mannitol salt agar, salmonella shigella agar and MacConkey agar respectively and were incubated at 37 ℃ for 24h. The numbers of microbial colonies on the nutrient agar plates were counted using a colony counter.

### 2.4. Characterization of the Isolates

#### *2.4.1. Phenotypic Characterization*

The colonial appearances on each medium were observed and were subjected to other identification tests including Gram staining and biochemical tests.

### 2.5. Molecular Identification

The molecular characterization on predominant lactic acid bacteria was carried out as below;

#### *2.5.1. DNA Extraction*

The DNA extraction, Electrophoresis for DNA and PCR, 16SrRNA gene amplification and sequencing of the predominant *Lactobacillus* sp. was done as described by [11].

The organism (2mL) was added to a ZR BashingTMLysis Tube and 750ul lysis solution was added to the tube. It was secured in a bead fitted with 2 ml tube holder assembly and processed at maximum speed for > 5 minutes. The ZR

Bashing BeadTMLysis Tube was centrifuged in a microcentirifuge at > 10,000 x g for 1 min. Then 400 ul supernatant was transferred to a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuged at 7,000 x g for 1 minute.

Bacterial DNA Binding Buffer (1,200 ul) was added to the filterate in the Collection Tube. The 800 ul of the mixture was transferred to a Zymo-SpinTM IIC Column in a Collection Tube and was centrifuged at 10,000 x g for 1 min.Then the flow through was discarded from the Collection Tube. The 800 ul of the mixture was transferred to a Zymo-SpinTM IIC Column in a Collection Tube and was centrifuged at 10,000 x g for 1 minute..

The DNA Pre-Wash Buffer (200ul) was added to the Zymo-Spin TM IIC Column in new Collection Tube and was centrifuged at 10,000 x g for 1 min.Bacterial DNA Wash Buffer (500 ul) was added to the Zymo-SpinTM IIC Column and centrifuged at 10,000 x g for 1 min. The Zymo-SpinTM IIC Column was transferred to a clean 1.5 ml microcentrifuge tube and 100ul (35 ul minimum) DNA Elution Buffer was added directly to the column matrix. It was centrifuged at 10,000 x g for 30 seconds to elute the DNA.

*2.5.2. Electrophoresis for DNA and PCR*

The agarose powder (for DNA 1g) and (for PCR 2g) was dissolved in 100 mL 1xTAE in a microwavable flask and was microwave for 3 min, then allowed to cool down to 50 °C. Then 10µL EZ vision DNA stain was added and the agarose was poured into a gel tray with the well comb in place and was left at 4 °C for 15 min to solidify.

Loading samples and running an agarose gel were then carried out for80-150 V for1h, then the gel was carefully removefrom the gel box. Then DNA fragments or PCR product was visualizeunder UV transilluminator.

*2.5.3. 16SrRNA gene amplification of the bacterial isolate*

The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM forward (27F: AGAGTTTGATCMTGGCTCAG)and reverse (1525R: AAGGAGGTGWTCCARCCGCA)primer; 2µL of DNA template and then made up with 8.5µL Nuclease free water.

*2.5.4. Sequencing*

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA X were used for all genetic analysis.

### 2.6. Statistical Analysis

The data obtained were analyzed using IBM Statistical Product and Service Solutions (SPSS), version 18. One-way analysis of variance (ANOVA) with Duncan test for multiple comparism was used to compare means across the groups.

## 3. Results and discussion

Table 1 revealed the recovered isolates across the period of storage.The phenotypic identification including Gram reaction, biochemical tests and growth on their selective medium revealed four isolates in the stored pap. The isolated bacteria were *Lactobacillus* sp*., Escherichia coli, Salmonella* sp. and *Staphylococcus aureus.* The obtained result is similar with the report of [8, 9] that isolated *leuconostoc* specie, *Lacto bacillus* species , *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella sp., Enterococcus faecalis*, *Pseudomonas* species and *Proteus* species from assayed stored pap. The predominant isolate was *Lactobacillus* sp. The presence of these pathogens could be as a result of improper storage method, poor hygiene during production and use of contaminated muslin clothes used for sieving or contaminated water used during soaking, grinding and sieving of the fermented maize. The presence of these pathogens in the stored pap can cause health hazard when consumed as they may expose consumers to high risk of food borne infection.

**Table 1** Recovered isolates across the period of storage

| | Parameters | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Isolates** | **Gram reaction** | **Catalase** | **Oxidase** | **VogesProskauer** | **Urease** | **Indole** | **Methyl red** | **Citrate** | **Growth on MSA** | **Growth on SSA** | **Growth on MAC** | **Growth on MRS** | **Lactose** | **Sucrose** | **Maltose** | **Galactose** | **Fructose** | **Inference** |
| 1 | +rod shape | - | - | - | - | - | - | - | NA | NA | NA | + | + | + | + | + | + | *Lactobacillus* sp. |
| 2 | - Rod shape | + | - | - | - | + | + | - | NA | NA | + | NA | + | + | - | + | - | *Escherichia coli* |
| 3 | - rod shape | + | - | - | - | - | + | + | NA | + | NA | NA | - | - | + | + | - | *Salmonell*asp. |
| 4 | + spherical shape | + | - | + | + | - | + | + | + | NA | NA | NA | + | + | + | + | + | *Staphylococcus aureus* |

Legend: NA= Not Applicable, MRS= DemanRogosa Sharp, MSA= Mannitol Salt Agar, MAC= MacConkey Agar, SSA= Salmonella Shigella Agar

The total bacterial count from the stored pap at room and refrigerated temperatures (Figure1) revealed the number of bacteria across the period. The total bacterial count increased as the period of storage was increased in room temperature samples. There are significant difference between the recovered bacteria at room and refrigerated temperature ($p<0.05$). There was growth even at 0h (±0.1CFU/mL). This could be the presence of lactic acid bacteria or other pathogen. The highest bacteria recovered in room temperature could be as a result of improper storage method and the fact that foods spoil faster in room temperature compared to a refrigerator due to several reasons. At room temperature, bacteria, mold and yeast can multiply more quickly, leading to spoilage. In a refrigerator, the cold temperature slows down the growth of bacteria and the activity of enzymes and thus helps to preserve the food for a longer period. Furthermore, the controlled humidity in a refrigerator can also help to slow down the spoilage process of microorganisms.

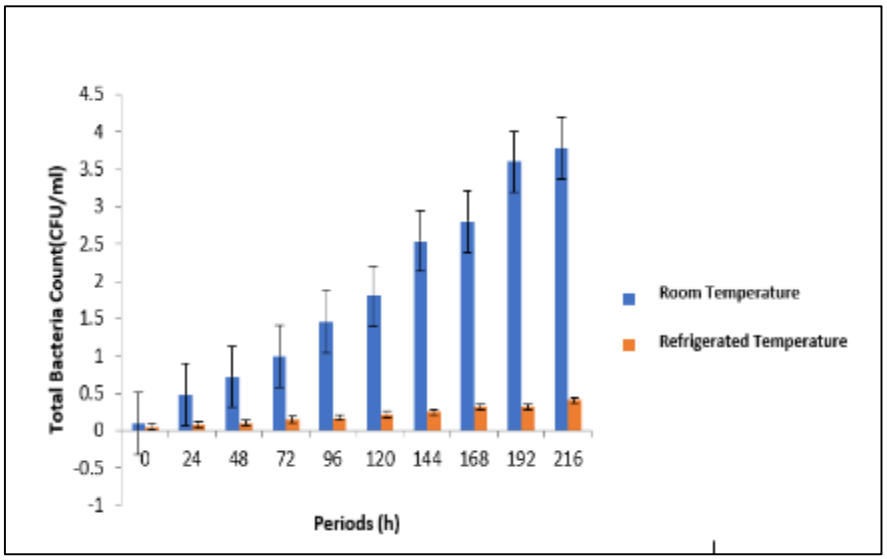
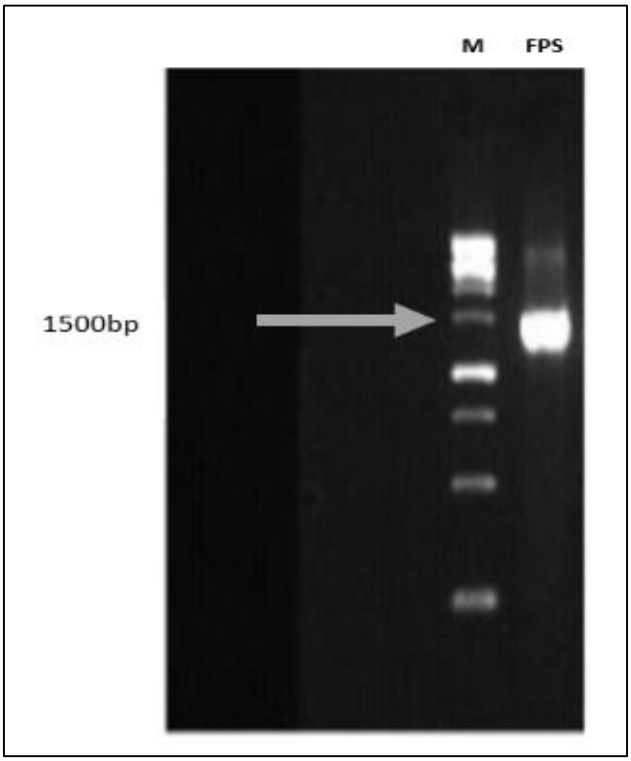



**Figure 1** Total Bacterial count on the Stored Pap at Room and Refrigerated Temperatures

This is in agreement with the reports of [12, 13] that reported that storing pap for longer period at room temperature have the tendency to experience a reduction in lactic acid below the minimum recommendation and growth of pathogens and this could not be safe for consumption due to increased sourness, changed aroma, consistency and colour.

### 3.1. Molecular Identification

The molecular identification of the predominant bacteria (*Lactobacillus* sp.) revealed the organism as *Lactobacillus plantarum* FPS. The isolated organism makes the processed pap a healthy food considering the benefits associated with this organism as [14] reported *Lactobacillus plantarum* CS with great probiotic potentials. The result is similar with the result of [15, 16] that isolated *Lactobacillus plantarum* from the assayed stored pap.




M = 1kbp DNA ladder

**Figure 2** Amplification of 16SrRNA Gene at 1500bp. by *Lactobacillus plantarum* FPS

GTCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGG
CTCGTAAAACTCTGTTGTTGTCTGATGGAGCAACGCCGCGTG
AGTGAAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGA
AGAACATATCTGAGAGTAACTGTTCAGGTATTGACGGTATTT
AACCAGAAAGCCAAAAGAAGAACATATCTGAGAGTAACTGTT
CAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTAC
GTGCCAGCAGCCGCGGTAATAACGGCTAACTACGTGCCAGCA
GCCGCGGTAATAA

**Figure 3** Partial Sequence of *Lactobacillus plantarum* strain FPS(16S ribosomal RNA gene)

## 4. Conclusion

The obtained results revealed room temperature as unsafe method for storing pap and refrigerator better method as it was revealed to increase the shelf life of the assayed stored pap.

## Compliance with ethical standards

*Disclosure of conflict of interest*

The authors have no conflict of interest to disclose.

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