# Starter Selection from Fermented Cereals for the Production of Weaning Blend

**Adeyemo, S. M. and Abimbola, O. G.**
Food and Industrial Microbiology Unit, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria
Corresponding author: adeyemostella@gmail.com

**Abstract**: This study focused on the isolation and identification of lactic acid bacteria (LAB) from fermented cereals and selection of the same with best technological properties as starter in the production of weaning blend. Yellow maize and sorghum were purchased at Atakunmosa market, Ilesa, Nigeria. The cereals were steeped in water for five days and were processed into *Ogi* . Lactic acid bacteria were isolated from the *Ogi* using de Mann Rogosa and Sharpe by pour plate techniques. The isolates were screened for desirable technological properties such as exopolysaccharide production, antagonistic properties against pathogenic organisms, inability to produce biogenic amines, production of ammonia from arginine, growth at different pH and production of antimicrobials and amylase enzyme using standard procedures. The results showed that a total of 10 lactic acid bacteria were isolated and were identified as *Lactobacillus plantarum* 4(40%), *L. composti* 2(20%), *L. fermentum* 2(20%), *L. casei* 1(10%) and *L. delbrueckii* 1(10%). *L. plantarum* and *L. delbrueckii* have the best technological properties with high exopolysaccharide production (3+), diacetyl, lactic acid and hydrogen peroxide production (0.2100, 0.3718 and 0.0008 g/mL) and (0.200, 0.3692 and 0.0007 g/mL) respectively. The two LAB also showed highest degree of antagonistic activities against pathogenic organisms (*Staphylococcus aureus* ATTC 43300 and *Escherichia coli* NCIB 86). The study evaluated the techniques that can be used to select starter culture for the production of weaning blends for infant. *L. plantarum* and *L. delbrueckii* showed the best technological properties with high antimicrobial and exopolysaccharide production.
**Keywords**: Technological properties, Cereals, Lactic acid Bacteria (LAB), Starter culture

## INTRODUCTION

The traditional cereal-based foods that are consumed in West Africa are processed by the natural fermentation of maize, sorghum and/or millet and are particularly important as weaning foods for infants and as dietary staples for adults (Mbata *et al.,* 2009). Lactic acid fermentation at the household level is a natural process brought about by lactic acid bacteria present in the raw food, or those derived from a starter culture. The fermentation process is often carried out on small or household scales and are characterized by the use of simple, non-sterile equipment, random or natural inoculums, unregulated conditions, sensory fluctuations, poor durability and unattractive packaging of the processed products (Olanrewaju *et al*. 2009).

Many studies have focused on the characterisation of the microorganisms that are commonly used in the processing of these products. Such research has demonstrated that fermentation was natural and involved mixed cultures of lactic acid bacteria (LAB), yeasts and fungi. The lactic acid bacteria species identified included *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, *Lactobacillus delbrueckii*, *Lactobacillus amylolyticus*, *Lactobacillus reuteri*, *Lactobacillus paraplantarum*, *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Pediococcus acidilactici*, *Pediococcus pentosaceus*, *Streptococcus gallolyticus* and *Weissella confusa*. (Nwachukwu *et al.* (2010); Sawadogo-Lingani *et al*. (2010); Oguntoyinbo *et al*. (2011); Turpin *et al.* (2011); Adimpong *et al*. (2012); Oguntoyinbo and Narbad, (2012).

The modern large-scale production of fermented cereal-based foods is almost entirely dependent on the use of defined strains of microorganisms, which could replace the undefined strain mixtures traditionally used for the manufacture of these products (Oguntoyinbo *et al*. (2011); Turpin *et al*. (2011). The development and improvement of inoculants containing high concentrations of live microorganisms, referred to as starter cultures, is a subject of increasing interest in efforts to standardize the fermentation step.

It is added to a raw material to produce a fermented food by accelerating and steering its fermentation process. Lactic acid bacteria occupy a central role in fermentation processes, and have a long and safe history of application and consumption in the production of fermented foods and beverages (Caplice and Fitzgerald, 2009). They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. Also, their production of acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharide, and several enzymes is of importance. They are reported to enhance shelf life and microbial safety, improve texture, and contribute to the pleasant sensory profile of the end product (De Vuyst *et al.,* 2004). The production of fermented foods and beverages through spontaneous fermentation and back-slopping represents a cheap and reliable preservation method in less developed countries. The direct addition of selected starter cultures to raw materials has been a breakthrough in the processing of fermented foods, resulting in a high degree of control over the fermentation process and standardisation of the end product (De Vuyst, *et al.,* 2004).

The use of defined strains or starter culture during cereal dough fermentation was reported to minimize dry matter loss, enhance control over the fermentation step, enhance acid production or reduction in pH levels, contribute to aroma and taste formation, as well as to increase the overall acceptability of the product and enhance the nutritional quality of the product through the formation of preservative compounds or a reduction in mycotoxins, such as aflatoxins and fumonisins (Enwa *et al.* 2011). The industrial use of LAB starter cultures in the food industry depends on the concentration and preservation technologies employed, which are required to permit long-term delivery of stable cultures in term of viability and functional activity (Carvalho *et al*. 2003). The use of LAB starter cultures by small-scale processing units or small-scale industrial agro-food enterprises continues to be limited. The study aimed at evaluating the procedures and techniques in selecting starter culture form *Ogi* using desirable technological properties for the production of traditional weaning blends for infants.

## MATERIALS AND METHODS

### Collection of samples

Yellow maize (*Zea mays L.)* and sorghum (*Sorghum bicolor* L.), were purchased from Atakunmosa market, Ilesha, Osun state, Nigeria. They were collected in clean polyethene bags and were transported to the laboratory for processing into *Ogi* slurry and used for the isolation of lactic acid bacteria.

### Isolation of Lactic Acid Bacteria

One gramme (1g) of well-macerated yellow maize and sorghum *Ogi* samples were homogenized with 9 mL of sterile maximum recovery diluent (MRD, Oxoid) in test-tube and serially diluted in the same diluent. Exactly 0.1 mL of appropriately diluted samples were pour – plated on MRS agar (de Man, Rogosa, and Sharpe agar), Plates were then incubated under anaerobic conditions at 30°C for 48 h, colonies obtained were purified by successive subculturing on MRS agar and then subjected to Gram staining and catalase test. Gram positive and catalase negative isolates were confirmed as LAB and stored on MRSA slant in the refrigerator (Adebayo-Tayo and Onilude, 2008).

### Physiological and Biochemical Characterization of Lactic Acid Bacteria

The isolates were examined for their physiological and biochemical characteristics such as growth at different pH, growth at varying temperature, growth at 4% NaCl, production of ammonia from arginine, nitrate reduction, production of hydrogen sulphide, production of gas, starch hydrolysis test, casein hydrolysis, gelatin hydrolysis, citrate, indole, oxidase, methyl red test and Voges-Proskauer (MRVP), and sugar fermentation tests by using standard procedure. (Harrigan and McCance, (1978); Olutiola *et al*. (1991); Harrigan, (1998); Klein *et al*.( 2003); Axelsson *et al.*(2004).

**Determination of Technological Properties of Lactic Acid Bacteria**

**Preparation of cell suspension of Lactic Acid Bacteria**

The LAB isolates were grown on MRSA slant for 24 h at 30°C. The growth on the slant was washed with 10 mL of sterile normal saline (0.85% w/v). The washed cell suspension was diluted further in sterile normal saline to give an absorbance of 0.08g/cm$^3$ in a spectrophotometer set at 540 nm wavelengths (Ojokoh, 2009).

**Exopolysaccharides (EPSs) Production by Lactic Acid Bacteria Isolates**

EPSs productions by the isolates were carried out following the method described by Giraud (1998). The LAB strains were precultivated on MRS agar and streaked onto LTV agar (0.5% (w/v) tryptone, 1% (w/v) meat extract, 0.65% (w/v) NaCl, 0.8% (w/v) $KNO_3$, 0.8% (w/v) sucrose, 0.1% (v/v) Tween 80 (Merck), 1.7% (w/v) agar, pH 7.1 ± 0.2 (Sawadogo- Lingani *et al.,* 2007)) and incubated at 30°C for 48 h. The sticky aspects of the colonies were determined by testing them for slime formation using the inoculation loop method. The isolates were considered positively slime producer if the length of the slime was above 1.5mm (Knoshaug *et al.,* 2000). Positive results were confirmed using MRS-sucrose broth without glucose and peptone. The isolates were cultured in MRS-sucrose broth and incubated at 30°C for 24 h. Exactly 1.5 mL of 24 h old culture of the test organism was centrifuged at 5000g for 10 minutes (4°C) and 1mL of the supernatant was put in a glass tube and an equal volume of ethanol was added. An opaque link is formed at the interface of the cell supernatant and ethanol in the presence of EPS. The positive isolates were described according to the intensity of the opaque link.

**Production of Biogenic Amines by the Lactic Acid Bacteria Isolates**

The ability of the lactic acid bacteria isolates to produce biogenic amine were determined qualitatively on an improved screening medium (decarboxylase medium) as described by Joosten and Northolt (1989) using a cocktail of four precursor of amino acids (histidine, lysine, tryptophan and tyrosine). The concentration of each amino acid was 1% and bromocresol purple (0.006%) was used as pH indicator. The pH of the medium was adjusted to 5.3 and then sterilized by autoclaving for 10 min at 121°C to avoid excessive hydrolysis of the agar at low pH. The sterile agar was cooled to 45°C, poured in sterile petri dishes, allowed to set and incubated overnight at 30$^o$C. Twenty-four hours old culture of the test organisms were streaked in duplicate on the decarboxylase medium plates with and without amino acids (as control) and were incubated for 4 days at 37°C under aerobic and anaerobic conditions in parallel. A change in bromocresol indicator to purple following the growth of the test organism was considered as index of significant amino acid decarboxylase activity, corresponding to > 350 mg of a particular amino acid L$^{-1}$ (Olasupo *et al.,* 2001).

**Antagonistic Activity of the Lactic Acid Bacteria Isolates against *Escherichia coli* and *Staphylococcus aureus***

The lactic acid bacteria isolates were screened for antagonistic activity against *Escherichia coli* and *Staphylococcus aureus* using the agar well diffusion assay technique (Schlinger and Lucke, 1989). The LAB isolates were grown in MRS broth for 24 h at 30°C. The cell free supernatants of the broth culture were obtained (by centrifugation at 15,000 rpm for 15 min). The supernatant were divided in to two portions, one of the portion was adjusted to pH 6.2 using 2.5 N NaOH to rule out inhibition due to pH reduction caused by organic acids while the other portion was used unadjusted. The pH adjusted supernatants were filtered through a syringe filter with a pore size of 0.22 µm (Satorius Millipore, UK). Antagonistic activities of both pH adjusted and unadjusted cell free supernatant of LAB were tested. Two milliliter (10$^6$cfu/mL) of the indicator organisms (*Staphylococcus aureus* and *Escherichia coli*) cultured in nutrient broth for 24 h at 37°C was inoculated (seeded) in to 18 mL of sterile molten Mueller Hinton

agar maintained at 45°C and the resulting mixture was poured in to Petri-dish. After solidification of the agar, wells of 4 mm in diameter were cut into it using sterile cork borer. Fifty microlitres (50µL) of the cell free supernatant (adjusted and unadjusted) of the test organism (LAB) were added to each well and incubated at 35°C for 48 h. The diameter of the zone of inhibition was measured and recorded. Inhibition occurring with the use of the pH adjusted supernatant was assumed to the presence of inhibitory substance other than pH.

**Determination of Lactic Acid Produced by Lactic Acid Bacteria Isolates**

The method of AOAC (2000) was used in the determination of lactic acid produced by the lactic acid bacteria isolates. This was determined by titrating 25 mL of broth cultures of the test organism (24 h old) with 0.1N NaOH until a pink colour appeared with three drops of phenolphthalein (1 % w/v) as indicator. Each ml of 0.1N NaOH is equivalent to 90.08mg of lactic acid (A.O.A.C, 2000).

$$\text{Titratable Acidity} = \frac{\text{Volume mL of NaOH} \times \text{Normality of NaOH} \times \text{Lactic acid equivalent}}{\text{Volume of the sample used}} \ldots\ldots \text{Eqn. 2}$$

**Determination of Diacetyl Produced by the Lactic Acid Bacteria Isolates**

The amount of diacetyl produced by the lactic acid bacteria isolates was estimated following the method of Sanni *et al.*, (1995), 25 mL of MRS broth culture of the test isolates (24 h) poured in conical flasks and 7.5ml hydroxyl amine solution which served as substrate for residual titration. The content of the flasks were then titrated with 0.1N HCl to a green-yellow end-point using bromophenol blue as indicator. The equivalent factor of HCl to diacetyl is 21.52mg (Sanni *et al*., 1995).

$$\text{AK} = \frac{(\text{B} - \text{S})(100 - \text{e})}{\text{W}} \ldots\ldots \text{Eqn. 3}$$

AK=Percentage of diacetyl B = mL of 0.1N HCl consumed in titration of the sample E=Equivalent factor of HCl to diacetyl=21.52mg W=Volume of sample

S=No of ml 0.1N HCl consumed in titration of 7.5mL Hydroxyl amine.

**Determination of Hydrogen Peroxide Production by Lactic Acid Bacteria Isolates.**

Twenty millilitres of 0.1M $H_2SO_4$ was added to 25mL of the MRS broth cultures of the test isolates (24h).Titration was carried out with 0.1N potassium permanganate. Each mL of 0.1M $H_2SO_4$ is equivalent to 1.70 mg of Hydrogen peroxide and decolorization of the sample was regarded as end point (A.O.A.C. 1990).

Hydrogen Peroxide Concentration = $\frac{mL\ KMnO4 \times NKMnO4 \times M.E}{mL\ H2SO4} \times 100 \times Volume\ of\ sample\ used \ldots\ldots Eqn. 4$

mL $KMnO_4$=Volume of $KMNO_4$ used $NKMNO_4$=Normality of $KMNO_4$,

mL $H_2SO_4$=Volume of $H_2SO_4$ added M.E=Equivalence factor of $H_2O_2$=1.701mg

**Assay for Enzymes**

**(i) Determination of inoculum size**

The lactic acid bacteria isolates were standardized according to 0.500 MacFarland standard using $BaCl_2$ and HCl at the right proportion. The culture supernatant was also standardised to 0.500 optical densities using sterile MRS broth (Olutiola *et al.,* 1991).

**(ii) Medium preparation, inoculation and incubation**

MRS-Starch broth in which the glucose had been substituted with equivalent amount (w/v) of soluble starch was used for inoculation. The medium was dissolved and homogenized in a water bath (Uniscope 801A Model, England) after which it was dispensed into Erlenmeyer flasks in aliquots of 250 mL, plugged with non-absorbent cotton-wool and aluminium foil.

It was sterilized using membrane filteration. With a sterile pipette, 10 mL of the standardized isolates were inoculated into the medium and incubated at 30$^{o}$C on a shaker for 48 h.The culture–broth was centrifuged at 10,000 rpm for 15mins using refrigerated centrifuge. The cell-free culture supernatant was labelled as the crude enzyme while the sediment (microbial cells) was stored in the refrigerator for further use. This was then assayed for amylase production (Lealem and Gashe, 1994).

**(iv) Amylase assay**

Amylase activities of the organisms were determined using 3, 5-dinitrosalicylic acid (DNSA) reagent method as modified by Giraud *et al.* (1991). One millilitre of culture supernatant was added to 1mL of the substrate containing 1.2% (w/v) soluble starch in 0.1M phosphate buffer, pH 6.0. The enzyme-substrate mixture was incubated at 30 $^{o}$C for 10 minutes. The reaction was stopped by the addition of 5M NaOH. One millilitre of the DNSA reagent was added to the filtrate-substrate-reaction mixture which helps to raise the pH of the solution to an approximately 12.3- 12.4 and produce ANS which absorb the light at 540nm, it was then heated in boiling water at 100$^{0}$C for 10mins to inactivate the enzymes and cooled with distilled water. The absorbance was measured at 540nm using Cecil 2031 spectrophotometer, England. One millilitre of uninoculated blank similarly treated was used to set the spectrophotometer at zero (Giraud *et al.,* 1991).

**Production of Ammonia from Arginine**

A modified MRS broth containing 3% arginine (MRS-arginine) as described by Harrigan and McCance (1978) and Harrigan, (1998), was prepared and 5 mL was dispensed into test tubes and sterilized by autoclaving at 121° C for 15 min. The MRS-arginine broth was inoculated with 0.1 mL of 24 h old MRS broth culture of the test isolate and incubated at 30°C for 5 days anaerobically. At the end of the incubation period, 1.0 mL of Nessler's reagent was added to 1.0 mL of MRS-arginine broth culture in a clean test tube. Production of orange to brown colouration indicated the presence of ammonia from arginine which signified positive reaction to the test while negative result was indicated by no alteration in colour.

**Statistical Analysis**

Data obtained in replicates of two or three and were analyzed by using ANOVA, Mean, and Standard deviation. Significant differences between Means were determined at 95% confident limit ($p< 0.05$) and were compared using Duncan Multiple Range Test with the aid of SAS program (Snedecore *et al.,* 2015).

## RESULTS

**Identification of Lactic Acid Bacteria Isolates from the Fermented *Ogi* Samples**

The result of the biochemical identification and percentage occurrence of the isolates is shown in Table 1 and Figure 1. A total number of ten (10) lactic acid bacteria were isolated from the yellow maize and sorghum *ogi*. They were all gram-positive rods, negative to catalase test, gelatin hydrolysis, citrate utilization, methyl red test and Voges-Proskauer test and they also showed varied reaction to other biochemical tests. Based on probable identity of the LAB isolate, 4 (40%) of the isolates were identified as *Lactobacillus plantarum*, 2 (20%) of the isolates were identified as *Lactobacillus fermentum*, 2 (20%) of the isolates was identified as *Lactobacillus composti*, 1 (10%) (Fig. 1) of the isolate was identified as *Lactobacillus delbrueckii* and 1 (10%) of the isolate was identified as *Lactobacillus casei.*

**Exopolysaccharide production by lactic acid bacteria isolates**

The results of production of exopolysaccharide by the *L. plantarum* (A8) and *L. delbrueckii* (B1) produced a very good level of EPS LAB isolates is shown in Table 2. It was observed that 20.0 %, (2 of 10) produced a very good level of EPSs, 20.0% (2 of 10) produced moderately, and 60.0 % (6 of 10) showed slight production of EPSs.

**Production of biogenic amine by the lactic acid bacteria isolates**

The result of biogenic amine of the lactic acid bacteria isolates is shown in Table 3. Out of the lactic acid bacteria isolates, *Lactobacillus composti* (A1 and B3) exhibited decarboxylase activity.

**Antagonistic activity of the lactic acid bacteria isolates against *Staphylococcus aureus* NCIB 8588 and *Escherichia coli* NCIB 86**

The result of the antagonistic properties of the lactic acid bacteria isolates is shown in Table 4. The LAB isolates were tested against *Staphylococcus aureus* NCIB 8588 and *Escherichia coli* NCIB 86. Inhibitory activities were shown by all the lactic acid bacteria in varying concentrations.

**Production of lactic acid, diacetyl and hydrogen peroxide by lactic acid bacteria isolates**

The result of the lactic acid, diacetyl, and hydrogen peroxide produced by the LAB isolates is shown in Table 5. Lactic acid production ranged from 0.2725-0.3518 (g/mL) by *Lactobaccillus composti* (B3) and *Lactobacillus plantarum* (A8) respectively, the amount of diacetyl produced ranged from 0.1508-0.2100g/mL by *Lactobacillus composti* (A3) and *Lactobacillus plantarum* (A8) and hydrogen peroxide produced ranged from 0.0005-0.0007 g/mL by *Lactobacillus fermentum* (A4) and *Lactobacillus plantarum* (A8) respectively.

**Production of amylase enzyme by lactic acid bacteria isolates**

The result of the amylases production by the LAB isolates is shown in Table 6. The LAB isolates produced the enzymes in varying concentration. It ranged from 0.700-1.250 by *Lactobacillus composti* (A3) and *Lactobacillus plantarum* (A8) respectively.

**Growth of the Lactic Acid Bacteria Isolates at Different pH**

The result of the growth of the LAB isolates at different pH value is shown in Table 7. All the LAB isolates grew well at pH 5, 6 and 7. Few of the LAB isolates grew well at extreme acidic pH (pH 3) (*L. fermentum* A4), and extreme alkali pH (pH 9). (*L. fermentum* (A4), *L. composti* (B3), *L. plantarum* (B2) and *L. casei* (B6))

**Growth at Different Time Interval of the Selected Starter**

The result of the growth of the selected starter culture at different time interval between 0 h - 120 h. of inoculation at 540nm is shown in Table 8. The optical density of the selected starter (*Lactobacillus plantarum and Lactobacillus delbrueckii*) increases progressively from 0 h to 96 h and declined steadily at 120 h.

**Table 1: Biochemical and Physiol*ogi*cal Identification of the LAB Isolates**

| Isolates designation | Catalase | Gram reaction | Morphology | Starch hydrolysis | Gelatin hydrolysis | Casein hydrolysis | Citrate | Indole | Oxidase | MR | VP | $H_2S$ production | $C0_2$ production | Nitrate reduction | Prod. Of $NH_3$ from arginine | Growth at pH 3.5 | Growth at pH 9.5 | Growth at 20°C | Growth at 45°C | Growth at 4.% NaCl | Sugar fermentation test | | | | | | | | | | | | Probable identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | | Sorbitol | Lactose | Mannitol | Xylose | Dextrose | Sucrose | Fructose | Galactose | Glucose | Raffinose | Rhamnose | Trehalose | |
| A1 | - | + | SR | + | - | + | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | *L. plantarum* |
| A3 | - | + | SR | + | - | + | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | *L. composti* |
| A4 | - | + | SR | + | - | + | - | - | + | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | *L. fermentum* |
| A7 | - | + | LR | + | - | + | - | - | - | - | - | - | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | *L. fermentum* |
| A8 | - | + | SR | + | - | + | - | - | - | - | - | - | - | + | - | + | + | + | - | + | + | + | + | - | + | + | + | + | + | - | - | + | *L. plantarum* |
| B1 | - | + | SR | + | - | + | - | - | - | - | - | - | - | + | - | + | + | + | + | + | - | + | - | - | - | - | - | - | + | - | - | + | *L. delbrueckii* |
| B2 | - | + | SR | + | - | - | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + | + | *L. plantarum* |
| B3 | - | + | LR | + | - | + | - | - | - | - | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | *L. composti* |
| B5 | - | + | SR | - | - | + | - | - | - | - | - | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | *L. plantarum* |
| B6 | - | + | SR | + | - | + | - | - | - | - | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | + | *L. casei* |

**Key: + = Positive reaction, - = Negative reaction, SR= Short Rod, LR: Long Rod**

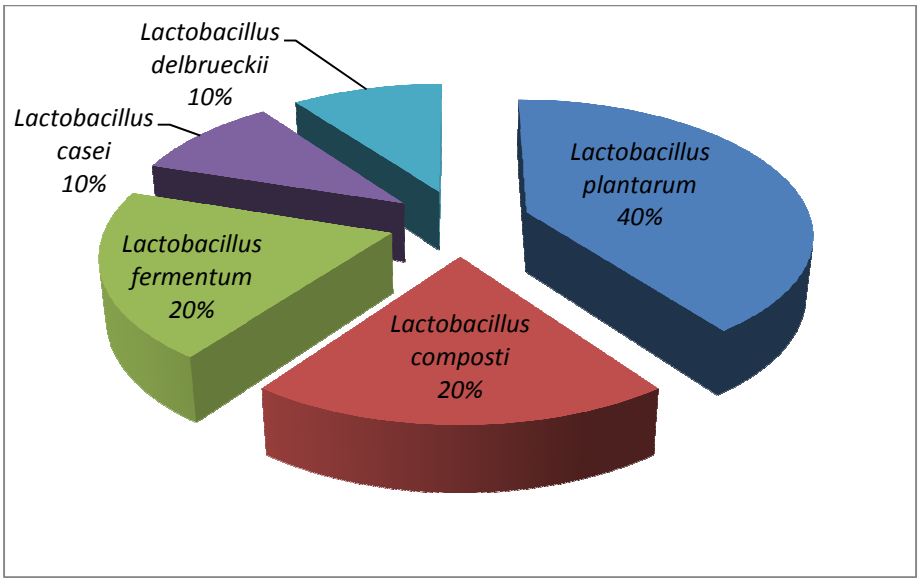


**Figure 1: Percentage Occurrence of the Lactic Acid Bacteria Isolates**

**Table 2: Exopolysaccharide Production of Lactic Acid Bacteria**

| Isolate designation | LAB isolates | Confirmatory EPS Production |
|---|---|---|
| A1 | *Lactobacillus plantarum* | + |
| A3 | *Lactobacillus composti* | + |
| A4 | *Lactobacillus fermentum* | + |
| A7 | *Lactobacillus fermentum* | 2+ |
| A8 | *Lactobacillus plantarum* | 3+ |
| B1 | *Lactobacillus delbrueckii* | 3+ |
| B2 | *Lactobacillus plantarum* | + |
| B3 | *Lactobacillus composti* | + |
| B5 | *Lactobacillus plantarum* | + |
| B6 | *Lactobacillus casei* | 2+ |

**Table 3: Production of Biogenic Amine by Lactic Acid Bacteria Isolates**

| Isolate code | Isolate | Amino acids Lysine | Tyrosine | Tryptophan | Histidine | Total amino acids decarboxylated |
|---|---|---|---|---|---|---|
| A1 | *Lactobacillus plantarum* | - | - | - | - | 0 |
| A3 | *Lactobacillus composti* | + | - | - | - | 1 |
| A4 | *Lactobacillus fermentum* | - | - | - | - | 0 |
| A7 | *Lactobacillus fermentum* | - | - | - | - | 0 |
| A8 | *Lactobacillus plantarum* | - | - | - | - | 0 |
| B1 | *Lactobacillus delbrueckii* | - | - | - | - | 0 |
| B2 | *Lactobacillus plantarum* | - | - | - | - | 0 |
| B3 | *Lactobacillus composti* | - | - | + | - | 1 |
| B5 | *Lactobacillus plantarum* | - | - | - | - | 0 |
| B6 | *Lactobacillus casei* | - | - | - | - | 0 |

**Table 4: Antagonistic Activity of Lactic Acid Bacteria against Selected Indicator Organisms**

| Isolate code | LAB isolates | Test organisms *S. aureus* NCIB 8555 | *E. coli* NCIB 86 |
|---|---|---|---|
| A1 | *Lactobacillus plantarum* | 15mm | 13mm |
| A3 | *Lactobacillus composti* | 11mm | 12mm |
| A4 | *Lactobacillus fermentum* | 16mm | 14mm |
| A7 | *Lactobacillus fermentum* | 9mm | 9mm |
| A8 | *Lactobacillus plantarum* | 17mm | 15mm |
| B1 | *Lactobacillus delbrueckii* | 15mm | 12mm |
| B2 | *Lactobacillus plantarum* | 6mm | 7mm |
| B3 | *Lactobacillus composti* | 8mm | 6mm |
| B5 | *Lactobacillus plantarum* | 15mm | 11mm |
| B6 | *Lactobacillus casei* | 14mm | 14mm |

**Table 5: Production of Lactic Acid, Diacetyl and Hydrogen Peroxide by the Lactic Acid Bacteria Isolates**

| Isolate code | LAB identity | Amount of Lactic acid produced (mg/mL) | Amount of Diacetyl produced (mg/mL) | Amount of Hydrogen Peroxide produced (mg/mL) |
|---|---|---|---|---|
| A1 | *Lactobacillus plantarum* | *0.3471±0.0064$^{a\dagger}$ | 0.1729±0.0006$^{a}$ | 0.0005±0.0001$^{b}$ |
| A3 | *Lactobacillus composti* | 0.2838±0.0098$^{c}$ | 0.1508±0.0055$^{b}$ | 0.0006±0.0001$^{a}$ |
| A4 | *Lactobacillus fermentum* | 0.3319±0.0064$^{a}$ | 0.1903±0.0001$^{a}$ | 0.0003±0.0001$^{c}$ |
| A7 | *Lactobacillus fermentum* | 0.3211±0.0058$^{b}$ | 0.1953±0.0001$^{a}$ | 0.0005±0.0001$^{b}$ |
| A8 | *Lactobacillus plantarum* | 0.3718±0.0001$^{a}$ | 0.2100±0.0001$^{a}$ | 0.0008±0.0001$^{a}$ |
| B1 | *Lactobacillus delbrueckii* | 0.3692±0.0001$^{a}$ | 0.2000±0.0001$^{a}$ | 0.0007±0.0001$^{a}$ |
| B2 | *Lactobacillus plantarum* | 0.3064±0.0001$^{b}$ | 0.1481±0.0001$^{b}$ | 0.0005±0.0001$^{b}$ |
| B3 | *Lactobacillus composti* | 0.2725±0.0001$^{c}$ | 0.1617±0.0001$^{ab}$ | 0.0006±0.0001$^{a}$ |
| B5 | *Lactobacillus plantarum* | 0.3405±0.0001$^{a}$ | 0.1756±0.0001$^{a}$ | 0.0006±0.0001$^{a}$ |
| B6 | *Lactobacillus casei* | 0.3404±0.0001$^{a}$ | 0.1707±0.0001$^{a}$ | 0.0006±0.0001$^{a}$ |

***Values are the Means ± Standard Deviation where n = 3. †Values with different superscript letter within each column are significantly different (p< 0.05) using Duncan's Multiple Range Test**

**Table 6: Amylase Enzyme Production by the Lactic Acid Bacteria Isolates**

| Isolates code | Isolates identity | Enzymes Unit (mg/mL) |
|---|---|---|
| A1 | *Lactobacillus plantarum* | *0.750±0.010$^{c\dagger}$ |
| A3 | *Lactobacillus composti* | 0.700±0.010$^{c}$ |
| A4 | *Lactobacillus fermentum* | 0.750±0.010$^{c}$ |
| A7 | *Lactobacillus fermentum* | 0.800±0.010$^{b}$ |
| A8 | *Lactobacillus plantarum* | 1.250±0.010$^{a}$ |
| B1 | *Lactobacillus delbrueckii* | 1.200±0.010$^{b}$ |
| B2 | *Lactobacillus plantarum* | 0.900±0.010$^{b}$ |
| B3 | *Lactobacillus composti* | 0.950±0.010$^{b}$ |
| B5 | *Lactobacillus plantarum* | 0.900±0.010$^{b}$ |
| B6 | *Lactobacillus casei* | 0.750±0.010$^{c}$ |

***Values are the Means ± Standard Deviation where n = 3. †Values with different superscript letter within each column are significantly different (p< 0.05) using Duncan's Multiple Range Test**

**Table 7: Growth of the Lactic Acid Bacteria at Different pH**

| LAB Code | LAB Identity | pH 3 | pH 5 | pH 6 | pH 7 | pH 9 |
|---|---|---|---|---|---|---|
| A1 | *Lactobacillus plantarum* | *0.210±0.001$^{b\dagger}$ | 0.276±0.002$^{b}$ | 0.521±0.001$^{a}$ | 0.387±0.001$^{a}$ | 0.533±0.005$^{a}$ |
| A3 | *Lactobacillus composti* | 0.202±0.002$^{b}$ | 0.328±0.002$^{a}$ | 0.328±0.002$^{a}$ | 0.383±0.001$^{a}$ | 0.288±0.010$^{ab}$ |
| A4 | *Lactobacillus fermentum* | 0.171±0.001$^{b}$ | 0.210±0.000$^{b}$ | 0.390±0.002$^{a}$ | 0.370±0.001$^{a}$ | 0.165±0.001$^{b}$ |
| A7 | *Lactobacillus fermentum* | 0.354±0.001$^{c}$ | 0.164±0.001$^{c}$ | 0.756±0.001$^{c}$ | 1.513±0.017$^{a}$ | 1.119±0.007$^{b}$ |
| A8 | *Lactobacillus plantarum* | 0.3200±0.001$^{a}$ | 0.585±0.002$^{a}$ | 0.460±0.001$^{a}$ | 0.513±0.001$^{a}$ | 0.314±0.001$^{b}$ |
| B1 | *Lactobacillus delbrueckii* | 0.683±0.001$^{b}$ | 0.709±0.004$^{b}$ | 0.279±0.002$^{c}$ | 1.421±0.000$^{a}$ | 0.408±0.001$^{b}$ |
| B2 | *Lactobacillus plantarum* | 0.182±0.001$^{b}$ | 0.150±0.001$^{b}$ | 0.504±0.002$^{c}$ | 1.110±0.004$^{a}$ | 1.031±0.002$^{a}$ |
| B3 | *Lactobacillus composti* | 0.313±0.002$^{d}$ | 0.712±0.005$^{c}$ | 0.718±0.001$^{c}$ | 0.772±0.008$^{b}$ | 1.183±0.008$^{a}$ |
| B5 | *Lactobacillus plantarum* | 0.220±0.001$^{a}$ | 0.158±0.001$^{a}$ | 0.730±0.001$^{a}$ | 0.540±0.003$^{a}$ | 0.165±0.000$^{a}$ |
| B6 | *Lactobacillus casei* | 0.195±0.001$^{b}$ | 0.353±0.001$^{b}$ | 1.601±0.004$^{a}$ | 1.663±0.004$^{a}$ | 1.272±0.002$^{a}$ |

***Values are the Means ± Standard Deviation where n = 3. $^{\dagger}$Values with different superscript within each row are significantly different (p< 0.05) using Duncan's Multiple Range Test**

**Table 8: Growth of the Starter Culture at Different Time Interval**

| Isolate | 0 h | 24 h | 48 h | 72 h | 96 h | 120 h |
|---|---|---|---|---|---|---|
| *Lactobacillus plantarum* | *0.220±0.001$^{c\dagger}$ | 0.255±0.001$^{b}$ | 0.280±0.001$^{b}$ | 0.310±0.001$^{a}$ | 0.325±0.001$^{a}$ | 0.312±0.002$^{a}$ |
| *Lactobacillus delbrueckii* | 0.195±0.001$^{c}$ | 0.240±0.005$^{b}$ | 0.265±0.001$^{b}$ | 0.295±0.111$^{a}$ | 0.315±0001$^{a}$ | 0.301±0.103$^{a}$ |

***Values are the Means ± Standard Deviation where n = 3. †Values with different superscript within each row are significantly different (p< 0.05) using Duncan's Multiple Range Test**

## DISCUSSION

Occurrence of *Lactobacillus plantarum* 4(40%), *L. composti* 2(20%), *L. fermentum* 2(20%), *L. casei* 1(10%) and *L. delbrueckii* 1(10%) in this study is in accordance with the work of Adeyemo (2014) and Wakil *and* Onilude (2012) who isolated similar organism from *Ogi*. These results were also in accordance with the work of Olukoya *et al.* (2003) in which *L. plantarum* is the most predominant LAB isolated from the study. The percentage occurrence of LAB isolates in this studies range from 10% to 40%. It was observed that *Lactobacillus plantarum* has highest percentage occurrence while *Lactobacillus casei* and *Lactobaccillus delbrueckii* has the least percentage occurrence of 10%. This result corroborates the findings of Adeyemo and Onilude (2013). The growth of the LAB isolates at pH 3.9 agrees with the work of Cotter and Hill (2003) that attributed such growth to the relative ATPase activities of microorganisms at different pH especially at low pH. The ability of LAB to grow at low pH contributed to the prophylactic effects of these organisms and enhanced the probiotic advantages of LAB as a result of which they are able to survive in the stomach where the pH is as low as 1.5. They also inhibit the growth and multiplication of pathogenic organisms in fermented foods (Omafuvbe and Enyioha, 2011).

The formation of exopolysaccharide in food has been reported to be good since it can function as viscosifying agents, stabilizers, emulsifiers, gelling agents or water binding agents and may play important role in the texture of the product (De Vuyst *et al.,* 2001). Exopolysaccharide production is an important property of LAB (Omafuvbe and Enyioha, 2011). All the LAB isolates tested for EPS production gave positive results in varying degree. This is similar to the work of Adebayo-Tayo and Onilude (2008) and Ishola and Adebayo-Tayo (2012).

The LAB strains were able to inhibit the selected indicator organisms in varying degrees with *L. plantarum* showing the highest zone of inhibition. This was similar to the report of Tadesse *et al*. (2005) who observed varying degrees of inhibition of various food borne pathogens by cell-free filtrates of LAB. The ability to inhibit other organisms is due to the fact that LAB produces antimicrobial substances such as diacetyl, hydrogen peroxide and lactic acid which are injurious to the indicator organisms depending on the concentration or quantity produced. These substances serve as competitive advantage to LAB when in mixed culture especially during fermentation and hence the dominance of LAB during fermentation of cereals. Wakil and Osamwonyi (2012) indicated that LAB isolates showing antimicrobial activity were discovered to produce antimicrobial substances like lactic acid which brings about reduction of pH of the fermentation medium (Ogunbanwo, 2005).

All the isolates produced amylase enzymes in varying concentration with *L. plantarum* producing the highest concentration (1.250mg/mL) which is in accordance with the work done by Adeyemo (2014). This property confers to the isolates a good technological property. This is in agreement with the work of Galvez *et al*. (2007).

## CONCLUSION

The study has revealed that Lactic acid fermentation at household level is a natural process brought about by lactic acid bacteria present in the raw food, or those derived from a starter culture. *Lactobaccillus plantarum* and *Lactobaccillus delbrueckii* were selected as appropriate starter culture based on their technological properties. The ability of selected starter to grow at low pH contributed to the prophylactic effects of these organisms and enhanced the probiotic advantages of lactic acid bacteria. The study concluded that *Lactobaccillus plantarum* and *Lactobaccillus delbrueckii* are potential starter culture and can be used for the production of traditional weaning blends for infants.

## RECOMMENDATION

The results of these studies underlined that lactic acid bacteria are potential starter culture. Hence, the introduction of appropriate starter culture techniques should be adopted as major steps towards improving the safety, quality and security of traditional production of weaning food.

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