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The melibiase activity of lactic acid bacteria isolated from yellow *Ogi*: A fermented cereal gruel

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ABSTRACT: This study focuses on the isolation and identification of Lactic Acid Bacteria (LAB) from yellow *Ogi* samples within Ile- Ife community and their enzymatic potential. Yellow *Ogi* were collected randomly at five different locations in Ile-Ife and LAB were isolated using De Man Rogosa Sharpe (MRS) agar under anaerobic condition for forty-eight hours at 37°C. The isolates obtained were identified using standard physiological and biochemical methods. The mean microbial load of the samples was enumerated. The melibiase activities of the isolates were determined using DNSA method. The mean microbial load of LAB in the samples ranged from 2.1 x 10^5 to 2.8 x 10^5 cfu/g. The organisms were identified as *Lactobacillus plantarum* (45.5%), *Lactobacillus acidophilus* (36.3%) and *Lactobacillus pentosus* (18.2%). Melibiase activities of the isolates were determined using DNSA method, showing activity levels ranging from 0.125 mg/ml to 1.775mg/ml. These findings underscore the prevalence of LAB in fermented foods like *Ogi*, highlighting their role in improving the flavor, aroma, shelf life and organoleptic properties. Moreover, LAB demonstrate significant enzymatic capabilities in the breakdown of sugars, further emphasizing their importance in food fermentation processes.

Keywords: Fermentation, fermented cereal gruel, lactic acid bacteria, melibiase activity, microbiology, yellow *Ogi.*

INTRODUCTION

Ogi is an African fermented gruel or porridge brought about by the action of Lactic acid bacteria (LAB) and yeasts. It is conventionally prepared from maize, millet and sorghum. *Ogi* contributes significantly to the everyday diet of both rural and urban communities as it can be given as a weaning food for children and food for sick people since it is not heavy and digests easily (Afolayan *et al.,* 2010). *Ogi* is produced locally in homes on a small scale or for sale, and the characteristic of the fermented product may be determined by the expertise of the makers as learnt over the years (Chelule *et al.,* 2010).

Other African fermented foods comprise *fufu*, *garri*, *kefir*, *tofu* and drinks such as *nunu*, *palm wine*, *agadagidi* and *burukutu* which are all made by the metabolic actions of LAB such as *Lactobacillus plantarum*. It has been observed that LAB contribute significantly to flavour improvement, dietary, quality, and vitamin content of fermented foods. Some foods are made appropriate for intake through fermentation by LAB. At the same time, poisonous ingredients found in cassava such as cyanide are also eliminated by the fermentative activities of LAB and the enzymes they produce (Tadesse *et al.,* 2021).

Lactic acid bacteria comprise a group of bacteria whose morphologic and physiological attributes are similar. This group of bacteria also have identical metabolism pathway, and they are also relatively and closely related phylogenetically. They are Gram-positive bacilli or cocci, non-spore-forming, and incapable of producing catalase. They are categorized among relative or obligatory anaerobes, and they can withstand the acidic pH of the environment (Gupta *et al.,* 2018) and have the capability to ferment carbohydrates to yield lactic acid as their key

product and other products in decreased quantities (Mgomi *et al.,* 2023). LAB have been employed in food and feed fermentation procedures long before any understanding of the occurrence of the bacteria themselves. Old Egyptian murals indicated the production of spoilage several thousand years ago and the utilization of fermentation as a degree for the conservation of food has been present since historical times.

Throughout the last century, it was observed that LAB were accountable for the fermentation and the bio-preservative result that occurs in many food and feed developments. Bio-preservation describes the prolonged shelf life and improved protection of foods using the native or introduced microflora and their numerous antimicrobial products. For example, biopreservation via natural microflora, such as LAB, has been proposed in place of the usual application of chemical preservers. Microbiotas are safer, uphold dietary enrichment, and are good label additives (Sieuwerts *et al.,* 2018). LAB are universal and can be observed in several environments loaded with nutrients and they also exist naturally in numerous types of food products like meat and milk products and vegetables. They need carbohydrates as an essential supply of nutrients, but they also require to be provided with amino acids, vitamins, peptides and salts amongst others (Carr *et al.,* 2002). They are generally regarded as safe (GRAS) and possess unique features that makes them ideal for these applications (Khushboo *et al.,* 2023).

Principal preserving impact of LAB is attained because of the manufacturing of lactic acid which leads to reducing the pH which promptly inhibits several microorganisms. The antimicrobial activities of LAB strains are facilitated by the antimicrobial substances generated by these strains. These antimicrobials can be separated into three main groups: (a) peptidic or proteinaceous bacteriocins; (b) organic acids (butyric acetic acids and lactic acids); (c) other minor molecules, for example, diacetyl, hydrogen peroxide, acetaldehyde, acetoine, reuterin, and reutericyclin. These antimicrobials contribute immensely to the keeping abilities of this group of bacteria (Ibrahim *et al.,* 2021). *Lactobacillus plantarum* has also been identified in the reduction of anti-nutrients and unnecessary materials in food during the process of fermentation (Adeyemo and Onilude, 2013). *Lactobacillus acidophilus*, like other LAB, is also significant in the fermentation activities of various foods extending from dairy products to fruits to cereals and vegetables.

Antimicrobial peptides or bacteriocins are generated in the ribosomes by some strains of LAB which can be applied to target and inhibit the activities of other Gram-positive bacteria (Salvucci *et al.,* 2016). Although antimicrobial peptides occupy an inhibition limit lower than that of antibiotics (Sidhu and Nehra, 2021), bacteriocins formed by LAB have been detected to infiltrate the outer membrane of Gram-negative bacteria and to cause the deactivation of Gram-negative bacteria in combination with other favourable antimicrobial environmental crescendos, such as low temperature, organic acid and detergents (Alvarez-Sieiro *et al.,* 2016).

Fermentation occurs when LAB breaks down sugars and carbohydrates to form alcohol, lactic acid and carbon dioxide together with energy (Adenosine-triphosphate/ATP) release (Todar, 2012). These by-products are the source of the distinctive flavour of fermented foods and assist in conserving and improving their taste. Recent research has suggested an innovative means of employing LAB as probiotics or living organisms which upon consumption in numerous quantities, confers extra health gains beyond naturally elementary nutrition. The antimicrobial effects of *Lactobacillus* species include inhibitive functions against *Salmonella* and *Pseudomonas* and other pathogens and food poisoning constituents that can negatively impact animal well-being (Thanh *et al.,* 2010).

Fermentation pathways in lactic acid bacteria

Lactic acid bacteria can be categorized based on the product of their fermentation in the hexose pathways. From sugars found in foods, hexose is the most frequently used substrate for LAB. Fermentation of glucose, which leads to the production of lactic acid under appropriate settings (surplus sugar and reduced access to oxygen) is described as Homolactic Fermentation i.e., in which their metabolic activities tentatively generate two molecules of Pyruvate from one molecule of glucose in the Embden-Meyerhof-Parnas's pathway. Intracellular redox stability is maintained by the oxidation of Nicotinamide adenine dinucleotide (NADH) which is alike to the breakdown of pyruvate to lactic acid. This activity results in the formation of two molecules of ATP for every single molecule of glucose taken in (Abbott *et al.,* 2009). Homolactic fermentation is distinct because it is one of the only respiration actions that does not release gas as its byproduct. LAB that utilize this pathway has the aldolase enzyme and can transform glucose nearly completely to lactic acid e.g., *Lactobacillus delbruckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus salivarius* subsp. *thermophilus*, and *Lactobacillus helveticus.*

Heterolactic fermentation exploits the pentose phosphate pathway, also described as the pentose phosphoketolase pathway. One molecule of glucose-6-phosphate is dehydrogenated to 6-phosphogluconate and subsequently decarboxylated to yield one mole of carbon dioxide. The ensuing pentose-5-phosphate is split into one more glyceraldehyde phosphate which is then degraded to lactate as in homolactic fermentation, with the acetyl phosphate broken down to give ethanol through the acetyl-CoA and acetaldehyde intermediates pathway. Theoretically, the end products (inclusive of ATP) are formed in similar amounts from the breakdown of one mole

of glucose. Heterolactic fermentation can be said to be an intermediary between lactic acid fermentation, and other kinds of fermentation, e.g. alcoholic fermentation (Sunano, 2015).

Acids such as lactic acid, acetic acid, formic acid and propionic acid are generally thought to carry out their antimicrobial effect by disrupting the conservation of cell membrane potential, averting active transport, reducing intracellular pH and hindering various metabolic purposes. They guarantee a very extensive approach to activity and impede both Gram-positive and Gram-negative bacteria inclusive of yeast and molds also. In addition to acids, starter strains can generate a sequence of complementary antimicrobial metabolites such as ethanol during their metabolism in the heterofermentative pathway (Todar, 2012). Hydrogen peroxide is produced during the aerobic development process and diacetyl is released from excess pyruvate pending from citrate. Hydrogen peroxide precisely has a strong oxidizing significance on membrane phospholipids and cellular proteins and is created by the action of enzymes like the flavo protein oxidoreductases NADH oxidase, NADH peroxidase and a-glycerophosphate oxidase (Todar, 2012).

Preparation of *Ogi*

The steps associated with the manufacturing of traditional yellow *Ogi* comprises washing of yellow maize grains, soaking for 3 days at room temperature (28 ± 2ºC) in clean water, wet-grinding, wet-sifting using a hand strainer or a muslin cloth with a pore size of around 300 µm then sedimentation or fermenting of filtrate for 1-3 days. Afterwards, the water on the top is discarded and the wet, clean sediment (*Ogi*) is acquired and stored for private use or sold to customers in its wet mode packed in small units in leaves or polypropene bags (Omemu and Omenike, 2010).

The wet fermented gruel is cooked and consumed as *Ogi, Akamu* and *Akassan* amongst the Yorubas, Ibos and Hausas in the west, east and northern Nigeria, respectively (Parveen and Hafiz, 2003). These fermented maize produces are normally eaten in apparently poverty-stricken areas throughout the underdeveloped countries. These products are gotten mostly from *Zea mays*, *Oryza Sativa, Sorghum Valgare* and *Tritiecum aestivum*. The making of these fermented products is frequently achieved by small-scale organisation performed by untrained female employees (Aminigo and Akingbala, 2004).

The conventional preparation of *Ogi* requires the activities of microorganisms such as LAB e.g. *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus acidophilus* and some yeast e.g. *Geotrichum sp*. Some other fungi and aerophilic bacteria may also be identified in the first 36 hours but will gradually vanish as the fermentation proceeds (Adeleke *et al.,* 2010). The amount of LAB and yeasts rises at a very speedy pace during the fermentation procedures. The conventional fermentation of *Ogi* does not demand the usage of a starter culture since the normal flora of the maize is responsible for the fermentation of the cereals (Ezeronye, 2003).

The pH of *Ogi* is usually 4.8, thus it is acidic, which encourages the prevention of the growth of pathogenic and unnecessary bacteria. Its decomposition, nonetheless, is aided by some external factors such as the temperature of storage. The shelf life of *Ogi* can be sustained by utilizing numerous methods such as freezing, refrigeration and drying (dehydration) which leads to decrease of the microbial load and subsequently spoilage (Amakoromo, 2011). This method of acidification is one of the most critical consequences caused by the growth of LAB in food. The pH may decrease to as low as 4.0, making it sufficiently low to inhibit the growth of most other microorganisms such as frequent pathogens, hence endowing these foods with lengthened shelf life.

Throughout the processing of cereals for *Ogi,* a high quantity of nutrient losses happen thus, various attempts have been presented to enhance the nutritious level of *Ogi* by enriching it with substances rich in protein. However, the nutritional enhancement of these fermented cereal gruels with foods rich in protein reduces their pasting thicknesses and occasionally influences their sensory properties unpleasantly. These characteristics are likely to affect consumer approval of the food (Osungbaro, 2009). The established fermentation procedures of products like *Ogi* can boost the microbial evolution of strains with exceptional technological and other significant favourable properties. In some communities in southwestern Nigeria, raw *Ogi* is regularly given to people having running stomach to lower the degree of stooling but the studied data for this statement is lacking (Aderiye *et al.,* 2007).

The dietary advantages obtained from *Ogi* have been studied greatly but the curative and protective impacts of *Ogi* and LAB, which are accountable for the organoleptic attributes and fermentation of *Ogi,* have been sparsely reported. LAB have been detected in the fermentation of *Ogi* and have been frequently obtained. The flow chart to produce *Ogi* is described in Figure 1.

Melibiase enzyme and its importance

Enzymes are macromolecular biological compounds which escalates or reduce the speed of a chemical reaction. At the start of the reaction, the initial constituents are called substrates, and these biocatalysts typically transform these constituents into several significant compounds which are called products. Nearly all the metabolic activities that happen in the cell need enzymes to speed up the rates quickly enough to maintain life (Stryer *et al.,* 2002).

Lactic acid bacteria have been examined to produce

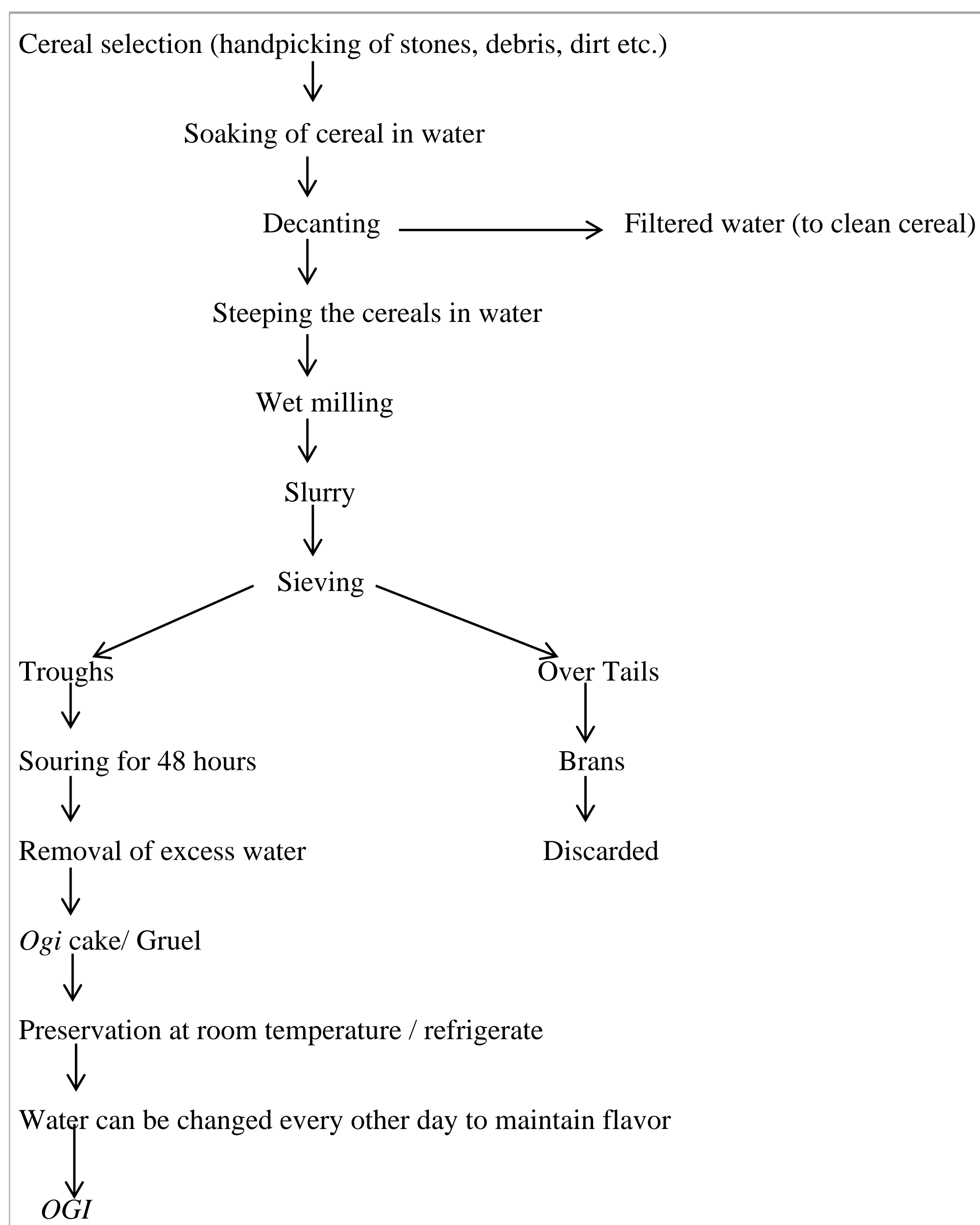


Figure 1. Flow chart for *Ogi* production (Omemu *et al.,* 2007).

various forms of enzymes which may influence the composition, processing, and organoleptic characteristics and the total attributes of foods and feeds. They make several hydrolytic enzymes in the gastrointestinal tract which can exercise prospective synergistic effects on assimilation and lessen indicators of abdominal malabsorption. In other words, these advantageous bacteria may be used as an alternate source to produce enzyme extracts that are competent for performing under environmental circumstances of fermentation (Tamang, 2011). Distinct species of *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Bifidobacterium* that associate with the fermentation of foods have been identified to produce carbohydrate reducing enzymes like glucosidases, xylanases and amylases (Patel *et al.,* 2012). Generally, LAB have been detected to yield a greater concentration of alpha and beta- galactosidase than *Bifidobacteria* (Alazzeh *et al.,* 2009).

Melibiase, also called Alpha-galactosidase (EC 3.2.1.33) catalyzes the hydrolysis of melibiose into galactose and glucose units (Zhou *et al.,* 2017). Alpha-galactosidase has been beneficially utilized in many areas

such as the feed, food, chemical, pulp, and medicinal industries. These applications involve upgrading the nutritional value in animal feed by the breakdown of sugars that are not readily metabolized, lessening the flatulence-triggering characteristics of soybean products, improving oil and gas retrieval by hydrolysis of proppant matrix, enzymatic discolorizing of softwood pulps, and transforming erythrocyte antigens from type B to type O (Kruskall *et al.,* 2000). Alpha-galactosidase is found in a diversity of organisms, for example in LAB such as *Lactobacillus acidophilus* (Farzadi *et al.,* 2011).

This study was carried out to isolate LAB from yellow *Ogi* samples collected randomly from the local producers in Ile-Ife community, to enumerate and characterize LAB in the samples collected, to assay the enzymatic activity of Lactic Acid Bacteria isolated from the *Ogi* samples in breaking down galactose by the production of melibiase enzyme.

METHODOLOGY

Sample collection

The freshly prepared samples of yellow *Ogi* used for this study were purchased from different sellers in Ile-Ife, Nigeria. Samples were immediately taken to the laboratory in sterile containers. One of the samples was obtained from a household in Ile-Ife, three of the samples were obtained randomly at different markets in Ile-Ife and the last sample was prepared in the laboratory from yellow maize that was obtained from the market.

Isolation of lactic acid bacteria

One gram of yellow *Ogi* sample was weighed into a test tube containing 9 ml of sterilized distilled water. Serial dilution was carried out by pipetting 1 ml of the resulting solution into the next test tube also containing 9 ml of sterilized distilled water. The process was repeated for the second test tube until the fifth test tube. 1 ml of the solution from the third and fifth test tubes with the dilution factor 10^{-3} and 10^{-5} was poured into a sterile petri dish. This was repeated for another set of petri dishes to serve as replicate samples. Freshly prepared media, De Man Rogosa Sharpe (MRS) agar, which has been cooled to about 45ºC was gently poured into each of the petri dishes very close to a flame from the Bunsen burner (aseptically). The MRS plates were incubated under anaerobic conditions at 37ºC for 48 hours (Awan and Rahman, 2005).

Enumeration and preservation of isolates

The plates were examined after 48 hours and colony forming units were enumerated. The colonies on the plates were examined and the description was recorded. The organisms were subcultured several times to obtain the pure cultures of the various isolates. The pure cultures of LAB were preserved on MRS agar slant. They were stored in the refrigerator at 4ºC for later identification. The isolates were subcultured every two weeks to maintain the viability of the organisms (Olutiola *et al.,* 1991).

Characterization and Identification of Isolates

For proper identification of the isolates, the cultural, morphological, biochemical, and physiological characterization including microscopic and macroscopic examination of the various isolates were carried out as described by (Sneath *et al.,* 2009). The morphological examination includes Gram's reaction, shape, colour, size, surface, edge, elevation, and pigmentation.

Gram's staining

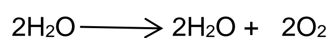
A heat-fixed smear from 18-24 hours old culture was stained with a crystal-violent solution for sixty seconds. The solution was then rinsed off. The slide was flooded with Gram's iodine solution for sixty seconds after which it was rinsed off and the slide was flooded with 95% alcohol until no more violet colour ran from it. It was later rinsed under gentle running tap water and counter-stained with safranin for sixty seconds. After this, it was again rinsed under gentle running tap water, blotted dry and examined under the microscope using an oil immersion objective at x100. Gram-positive cells appear purple retaining the purple colour of crystal violet-iodine complex, while Gram-negative cells appear red or pink (Cheesbrough, 2000).

Biochemical classification of bacterial isolates

Catalase test

A microscopic slide was placed inside the petri dish and a drop of sterile distilled water was introduced to it. A loopful of the test organism from an 18 to 24 hours colony was picked and placed on the microscopic slide. With the use of a pipette, 1 drop of 3% hydrogen peroxide was dropped on the test organism on the microscope slide. A positive reaction is evident by immediate effervescence (bubble formation). For negative a result, no bubble is formed.

The basic principle behind this test is to determine whether the organism can produce the enzyme catalase that hydrolyzes the hydrogen peroxide into water and oxygen (Chelikani *et al.,* 2004).



$$2H_2O \longrightarrow 2H_2O + 2O_2$$

Indole test

The organisms were inoculated into peptone water containing the amino acid tryptophan and incubated for five days anaerobically at 37ºC. A few drops of Kovac's reagent were added after incubation, the formation of a red or pink colour ring at the top indicates a positive test; the alcoholic layer concentrates the red colour. A negative reaction remains yellow. This test shows the ability of some bacteria to produce indole from the amino acid tryptophan by using the enzyme tryptophanase (Olutiola *et al.,* 1991).

Oxidase test

Whatman filter paper (No 1) was placed in a sterile petri dish and then soaked with a few drops of oxidase reagent (1% aqueous tetramethyl-p-phenylenediamine hydrogen chloride). A 24-hour-old colony of the organism was picked with the aid of a slide and used to touch the oxidase reagent on the filter paper. A positive oxidase test is indicated by the development of purple colouration within 5 to 10 seconds while a negative test is signified by a delayed reaction or no colour change. This test is aimed at detecting the presence or absence of certain oxides that will accelerate the transport of electrons between electron donors and a redox dye in the bacteria. An uninoculated tube serves as control for the experiment (Cowan and Steel, 2002).

Methyl red test

The test organism was inoculated into a glucose phosphate broth. It was incubated at 37ºC for 48 hours. A methyl red indicator was added after incubation. A positive test is indicated by the formation of red colouration while a negative test is evident by no colour change. An uninoculated tube serves as control for the experiment (Cowan and Steel, 2002).

Voges-Proskauer test

The organism was inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml alpha-naphthol was added to the test broth and shaken. 0.2 ml of 40% KOH was added to the broth and shaken. The tube was left for 15 minutes. The development of red colouration is taken as a positive test. An uninoculated tube serves as a control (Cowan and Steel, 2002).

Citrate utilization

Sterile Simmons citrate medium in test tubes was inoculated with 24-hour-old test isolates and incubated at 37ºC for 7 days. A positive result was indicated by a colour change from green to blue showing that the isolate utilized citrate while no colour change indicated a negative reaction. The test is based on the ability of the isolated bacteria to utilize citrate as the sole source of carbon (MacWilliams, 2009).

Growth and physiological studies

Growth in 6.5% NaCl

The medium used consists of 6.5% w/v NaCl in nutrient broth. The four-hour-old culture was aseptically transferred into the sterile tubes containing 6.5% NaCl broth. The incubation was done at 35-37ºC for 24 hours. This test was carried out to determine the ability of the test organism to grow in 6.5% NaCl. Turbidity of the broth after incubation showed a positive result while a negative result showed no turbidity after incubation (Forbes, 2007). Uninoculated tubes served as control.

Growth at different pH (3.9 and 9.4)

Hydrochloric acid (HCl) was prepared (0.1M) and was added to homogenized molten MRS agar, until a pH of 3.9 was obtained for the acidic medium while 0.1 M sodium hydroxide (NaOH) was added drop-wisely to homogenized molten MRS agar to raise the pH to 9.4. The mixtures were sterilized for 15 minutes at 121ºC and poured into plates after cooling to about 45ºC. The 24-hour-old lactic acid bacteria isolates were streaked on the solidified agar and incubated anaerobically at 37ºC for 48 hours. This test was carried out to determine the pH that favours the growth of lactic acid bacteria isolates (Adamberg *et al.,* 2003).

Growth at different temperatures (20ºC and 70ºC)

The MRS agar plates were streaked with 18-24 hours old cultures of lactic acid bacteria isolates. Set of the plates were incubated at a low temperature of about 20ºC while the other sets were incubated at a high temperature of 70ºC. This test was carried out to determine the temperature at which the lactic acid bacteria can grow. The uninoculated plates served as a control (Adamberg *et al.,* 2003).

Carbohydrate fermentation of sugars

The test organisms were introduced into the prepared medium of fermentable sugar containing 1% fermentable sugar, peptone water and bromocresol indicator. The media was sterilized at 121ºC for 10 minutes. The fermentable sugars used for this test are glucose, mannitol, trehalose, fructose, lactose, galactose, maltose,

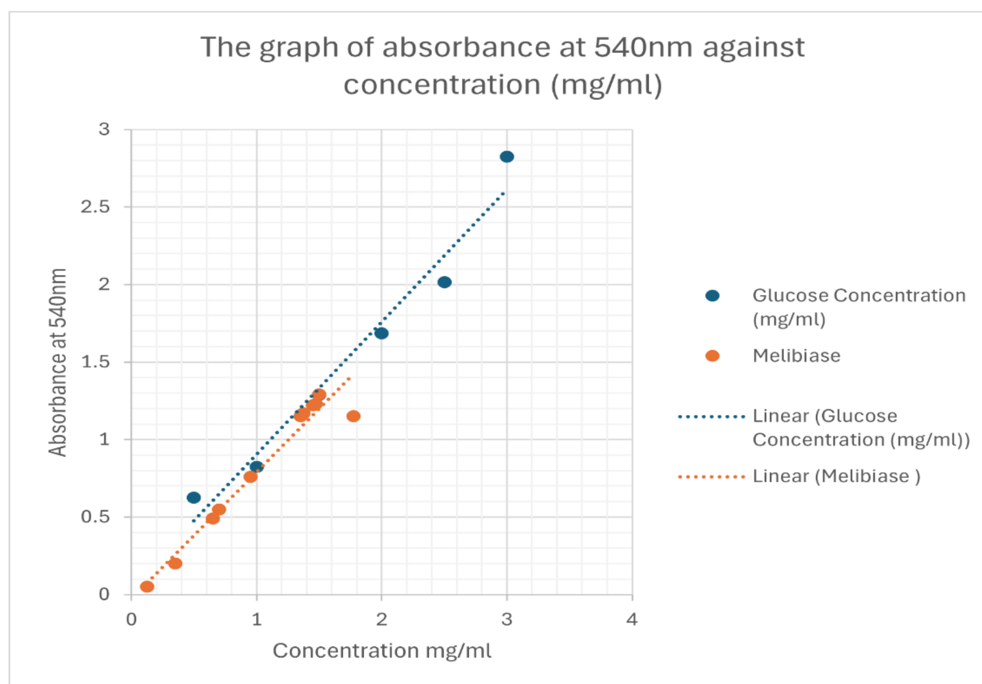


Figure 2. The graph of absorbance at 540nm against concentration (mg/ml).

inositol and raffinose. The tubes were then incubated for 5 to 7 days at 37ºC. Acid production is indicated by colour change from purple to yellow and this signifies a positive result. Gas production in the Durham tube indicates a positive result for gas production. This test was carried out to detect the ability of the bacterial isolates to break down the sugars to give acid and gas. Uninoculated tubes serve as a control experiment (Cowan and Steel, 2002).

Medium preparation for enzyme assay

The medium used was MRS- Broth in which glucose was substituted with an equivalent amount (w/v) of the substrate (galactose). The medium was dissolved and dispensed into different test tubes. It was sterilized at 121ºC for fifteen minutes and allowed to cool. The isolates were then inoculated into it and incubated at 30ºC for forty-eight hours (Awan and Rahman, 2005).

Enzyme

The cell broth was centrifuged at 10,000 rpm for fifteen minutes. The cell-free culture supernatant was labelled as the crude enzyme. This was used to assay for melibiase.

Melibiase assay

The melibiase activities of the organisms were determined by using the DNSA reagent method of Bernfield (1955) as modified by Giraud *et al.* (1991). The supernatant was added to 1 ml of the substrate containing 1.2% (w/v) substrate (galactose) in 0.1M phosphate buffer, pH 6.0. The enzyme-substrate mixture was incubated at 30ºC for 10 minutes. The reaction was stopped by the addition of DNSA reagent which contains NaOH. The amount of reducing sugar produced was estimated according to Bernfield (1955) with 3, 5- Dinitrosalicyclic acid (DNSA). 1 ml of DNSA was added to the filtrate-substrate reaction mixture and heated in a boiling water bath at 100ºC for ten minutes and then cooled with distilled water. The absorbance was measured at 540 nm using a spectrophotometer. 1 ml of uninoculated blank was used to set the spectrophotometer at zero. 1 melibiase unit is the amount of enzyme in 1 ml of the filtrate which releases 1 mg of reducing sugar from glucose from 1% starch solution in one hour at 30ºC.

Glucose standard curve

Glucose was prepared in concentrations of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/ml (Table 1). It was measured on a spectrophotometer at a wavelength of 550 nm. The values obtained were used to construct a standard curve (Figure 2).

RESULTS

The total viable count of lactic acid bacteria cells isolated

Table 1. Absorbance at 540 nm.

Absorbance at 540 nm	Glucose concentration (mg/ml)
2.826	3.0
2.014	2.5
1.684	2.0
1.288	1.5
0.825	1.0
0.624	0.5

Table 2. The Mean microbial load of the isolates from *Ogi* (CFU/g).

Samples	TBC
Sample 1	2.1×10^5
Sample 2	-
Sample 3	2.5×10^5
Sample 4	2.3×10^5
Sample 5	2.8×10^5

Key: TBC= Total Bacteria Count.

Table 3. The cultural and morphological characteristics of lactic acid bacteria isolated from *Ogi.*

Isolates	Colour	Shape	Size	Edges	Surface	Elevation	Margin
F1	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F2	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F3	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F4	Cream	Circular	Medium	Smooth	Wet	Low convex	Entire
F5	Cream	Circular	Medium	Smooth	Wet	Low convex	Entire
F6	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F7	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F8	Cream	Circular	Medium	Smooth	Wet	Low convex	Entire
F9	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F10	Cream	Circular	Small	Smooth	Wet	Low convex	Entire
F11	Cream	Circular	Small	Smooth	Wet	Low convex	Entire

from different samples was shown in Table 2 and this ranged between 2.1×10^5 and 2.8×10^5. It was observed that Sample 1 had the lowest Total Bacteria Count while Sample 5 had the highest Total Lactic Acid Bacteria Count.

The cultural and morphological characteristics of lactic acid bacteria isolated from the samples are shown in Table 3. It was observed that all the lactic acid bacteria were round; all the eleven isolates were creamy in colour. Also, the size ranges from small to medium but most of the isolates were small.

The biochemical characteristics of the lactic acid bacteria isolated from yellow *Ogi* samples are shown in Table 4. The lactic acid bacteria isolates were observed to give negative results to tests such as catalase, oxidase, citrate, indole, Methyl red and Voges Proskauer test.

The percentage occurrence of LAB isolated from the yellow *Ogi* samples analyzed can be seen in Table 5. The melibiase assay activity 540 nm produced by the lactic acid bacteria isolates in their breakdown of galactose and the melibiase concentration obtained is shown in Table 6.

DISCUSSION

In this study, the samples of *Ogi* used were basically of three groups. The first, third and fourth samples were the samples purchased from the market while the second sample was collected from a house in Ile-Ife and the fifth sample was prepared in the laboratory, the maize sample was fermented for five days. The results obtained show

Table 4. Biochemical characteristics of LAB isolated from yellow *Ogi* samples.

S/N	Isolates code	Gram's reaction	Cell morphology	Catalase test	Oxidase test	Methyl red test	Voges Proskauer test	Indole test	Citrate utilization test	Growth at 6.5% NaCl	Growth at 3.9	Growth at 9.4	Growth at (70ºC)	Growth at (20ºC)	Glucose	Mannitol	Trehalose	Fructose	Inositol	Lactose	Galactose	Maltose	Raffinose	Presumptive Identity
1	F1		R	_	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	*Lactobacillus acidophilus*
2	F2	+	R	_	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
3	F3	+	R	_	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
4	F4	+	R	_	-	-	-	-	-	-	+	+	-	+	+G	+	-	+	+	+	+	+	+	*Lactobacillus pentosus*
5	F5	+	R	_	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
6	F6	+	R	_	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
7	F7	+	R	_	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	*Lactobacillus plantarum*
8	F8	+	R	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	*Lactobacillus acidophilus*
9	F9	+	R	-	-	-	-	-	-	-	+	+	-	+	+G	+	-	+	+	+	+	+	+	*Lactobacillus pentosus*
10	F10	+	R	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	*Lactobacillus acidophilus*
11	F11	+	R	-	-	-	-	-	-	-	+	+	-	+	+	-	+	+	-	+	+	+	+	*Lactobacillus acidophilus*

Keys: R= Rod; + = positive; - = negative; G = gas production.

that the fifth sample of *Ogi* had the highest microbial load (2.8×10^5 cfu/g). This could be because the fermentation of the *Ogi* sample was prolonged by an additional three days and was prepared in the laboratory under strict hygienic conditions as reported by Adeyemo and Onilude, (2013). Furthermore, the total viable count of lactic acid bacteria in the first, third and fourth samples were lower than that of the fifth sample (2.1×10^5, 2.5×10^5, 2.3×10^5 CFU/g respectively). This observation might likely be because most sellers of these products are often eager and, in haste, to sell their products to the final consumers even when such products are not fully fermented as reported by Adeyemo and Onilude, (2013).

According to a study carried out by N'zi *et al.* (2021), it was discovered that a food fermentation process that has LAB is conventionally based on spontaneous fermentation. Lactobacilli put forth strong antagonistic impacts against many microorganisms, including spoilage organisms and pathogens found in food. Therefore, the absence of the actions of LAB in the processing of *Ogi* can bring about unfavourable consequences because pathogenic and resistant spores might be existing in *Ogi* and there will not be any lactic acid to inactivate their effects.

The isolated organisms were identified as *Lactobacillus plantarum* (45.5%), *Lactobacillus acidophilus* (36.3%) and *Lactobacillus pentosus* (18.2%). This report agrees with the study carried out by De Vuyst *et al*. (2007) who stated that lactic acid bacteria contribute to the conservation of foods by producing antimicrobial agents like bacteriocin which are considered as natural food stabilizers. *Lactobacillus plantarum* had the highest percentage occurrence (45.5%) and this is because these lactic acid bacteria occur in large numbers and confer qualities like extended shelf life, aroma and make the product safe for consumption (Ayivi *et al.,* 2020).

Ogi, as a product of the activities of LAB is a fermented food product with a long shelf life. This may likely be the reason why the Nigerian *Ogi* is always obtained fresh even though most of the local sellers of *Ogi* in the Ile-Ife community hardly store the product in a refrigerator and this is due to their ability to survive at low temperatures (30ºC)

Table 5. The percentage (%) occurrence of lactic acid bacteria isolated from Yellow *Ogi.*

Organism	Frequency	Percentage (%) occurrence
Lactobacillus plantarum	5	45.5
Lactobacillus acidophilus	4	36.3
Lactobacillus pentosus	2	18.2

Table 6. The melibiase activity (absorbance and concentration) of lactic acid bacteria.

Isolates	Absorbance at 540 nm	Mellibiase concentration (mg/ml)
F1	0.550	0.700
F2	0.050	0.125
F3	1.170	1.375
F4	0.200	0.350
F5	1.230	1.475
F6	0.760	0.950
F7	1.290	1.500
F8	1.150	1.775
F9	1.220	1.450
F10	0.490	0.650
F11	1.150	1.350

and produce lactic acid as reported by Adamberg *et al.* (2003). The ability of LAB to grow at a low pH of 3.9 contributes to the prophylactic effect of lactic acid bacteria in *Ogi* and had been studied and observed in the past (Gibson and Fuller, 2000). It was discovered that the health benefits of *Ogi* are primarily due to the ability of LAB to survive in the human gastrointestinal tract. The Lactic Acid Bacteria commonly observed and identified in the production of *Ogi* could survive in the stomach as reported by Gibson and Fuller (2000) and this is because they can survive both the stomach acid (pH as low as 1.5) and bile acids (pH as low as 2.0) and because they can adhere to the intestinal gastrointestinal tract thus inhibiting pathogenic organisms by secreting lactic acid in the stomach. This agrees with the physiological studies of the LAB that were identified in this work.

The presence of *L. acidophilus* has been reported by several researchers as contributing to immune response development. This is achieved when these microorganisms colonize the intestinal surface mucus layer since they can then affect the intestinal immune system by displacing enteric pathogens, providing antioxidants and anti-mutagens and possibly carrying out other effects through signalling. The intake of lactic acid bacteria which influences multiple systems were elegantly shown for *Lactobacillus* GG using microarray analysis (Di Caro *et al.,* 2005). The ability of lactic acid bacteria to be used as probiotics and induce the stimulation of the immune system makes this group of bacteria very important for most fermentation processes (Li *et al.,* 2020). *Lactobacillus acidophilus* as obtained in this work is a natural flora of the mammalian gastrointestinal system. This species of lactic acid bacteria is of considerable medical and industrial interest because *Lactobacillus acidophilus* is known to play a very important role in human health and nutrition. After all, it influences the intestinal flora as reported by Gao *et al.* (2022).

Several researches have shown that microbial pathogens such as enterotoxigenic *Escherichia coli*, *Shigella flexneri*, *Salmonella typhimurium* and *Bacillus cereus* are adversely affected when present in traditional fermented foods (Obadina *et al.,* 2006). Some of the antimicrobial properties exhibited by these fermented foods may be due to the low pH of the food as well as metabolic compounds produced by microorganisms such as LAB involved in the fermentation process. LAB are known to secrete antimicrobial substances mainly in the form of organic acids and metabolic compounds. This is a simple and safe method that is still used in many undeveloped countries.

The LAB isolated were all able to grow on glucose as their carbon and energy source while they were all unable to utilize citrate as their sole source of carbon and they were all negative to both catalase and oxidase tests as reported by Ismail *et al.* (2019). In addition, the variation in the result obtained under the three circumstances indicates the need for the local producers of *Ogi* to give more inclination to the fabrication of better-quality products by aligning with the standard microbiological requirement to produce fermented cereals as stated in the work of Omemu *et al*. (2007).

The results obtained from the melibiase activities by the

lactic acid bacteria isolates differ from one another and this observation might be likely because the identified isolates differ in the rate at which they produce the melibiase enzyme during metabolism, they break down the sugar and convert it to reducing sugars. These strains can be used as starter cultures with predictable characteristics and contribute to the development of small-scale and commercial production of fermented food with stable consistent quality. This is in accordance with the report of Kostinek *et al*. (2007). The ability of the isolated LAB to produce the enzyme melibiase and break down sugars as seen in this research is in accordance with the research carried out by Farzadi *et al*. (2011), that Alpha-galactosidase is found in a variety of organisms, for example in lactic acid bacteria such as *Lactobacillus acidophilus.*

Conclusion

Ogi is a very common food given to young infants in Africa due to the nutritional benefits derived from consuming it. Various microorganisms are involved in the fermentation process of *Ogi* as a result producing various enzymes that are very good for infants. Much emphasis has been laid in the past on the positive effects of lactic acid bacteria present in foods and how they have proven to be beneficial and of great use to humankind in the time past. However, there are still quite numerous potentials of this group of bacteria yet to be tapped. Despite the unhygienic wet-milling and wet-sieving processes involved in the preparation of *Ogi* traditionally, the low pH of the fermented products would be responsible for making them safe for consumption. The isolation of lactic acid bacteria from different samples of yellow *Ogi* as carried out in this research has affirmed that lactic acid bacteria are indeed responsible for the fermentation of cereal gruel *Ogi* and that they can be isolated from such products.

CONFLICT OF INTEREST

The authors declare that they did not have any conflict of interest.

REFERENCES

Abbott, D. A., Van Den Brink, J., Minneboo, I. M., Pronk, J. T., & Van Maris, A. J. (2009). Anaerobic homolactate fermentation with Saccharomyces cerevisiae results in depletion of ATP and impaired metabolic activity. *FEMS Yeast Research*, *9*(3), 349-357.

Adamberg, K., Kask, S., Laht, T. M., & Paalme, T. (2003). The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. *International Journal of Food Microbiology*, *85*(1-2), 171-183.

Adeleke, R. O., Abiodun, O. A., & Ayansina, A. D. (2010). Physiochemical properties of commercial local fermented foods. *Pakistan Journal of Nutrition, 9*, 853-855.

Aderiye, J. B., Laleye, S. A., & Odeyemi, A. T. (2007). Hypolipidemic potential of potential of *Lactobacillus* and *Streptococcus* sp from some Nigeria fermented foods. *Research Journal of Microbiology, 2*(6), 538-544.

Adeyemo, S. M., & Onilude, A. A. (2013). Enzymatic reduction of anti-nutritional factors in fermenting soybeans by Lactobacillus plantarum isolates from fermenting cereals. *Nigerian Food Journal*, *31*(2), 84-90.

Afolayan, M. O., Afolayan, M., & Abuah, J. N. (2010). An investigation into sorghum based ogi (Ogi-Baba) storage characteristics. *Advance Journal of Food Science and Technology*, *2*(1), 72-78.

Alazzeh, A. Y., Ibrahim, S. A., Song, D., Shahbazi, A., & AbuGhazaleh, A. A. (2009). Screening for α-and β-galactosidases in Lactobacillus reuteri compared to different strains of bifidobacteria. *Milchwissenschaft*, *64*(4), 434-437.

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., & Kuipers, O. P. (2016). Bacteriocins of lactic acid bacteria: extending the family. *Applied Microbiology and Biotechnology*, *100*, 2939-2951.

Amakoromo, E. R. (2011). *Indigenous fermented foods of Nigeria: Processing, composition and Improvement*. University of Port Harcourt Press, P.H, Nigeria. Pp. 57-65.

Aminigo, E. R., & Akingbala, J. O. (2004). Nutritive composition and sensory properties of *Ogi* fortified with Okra seed meal. *Journal of Applied Sciences and Environmental Management, 8*(2), 23-28.

Awan, J. A., & Rahman, S. U. (2005). *Microbiology manual*. Unitech Communications, Faisalabad, Pakistan, Pp. 49-51.

Ayivi, R. D., Gyawali, R., Krastanov, A., Aljaloud, S. O., Worku, M., Tahergorabi, R., Silva, R. C. D., & Ibrahim, S. A. (2020). Lactic acid bacteria: Food safety and human health applications. *Dairy*, *1*(3), 202-232.

Bernfield, P. (1955). *Amylase, alpha and beta in Methods in Enzymology*. In: Colowick, S. P., & Kaplan, N. O. (eds.), Academic Press. New York. Pp. 149-158.

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: A literature survey. *Critical Reviews in Microbiology*, *28*(4), 281-370.

Cheesbrough, M. (2000). *District laboratory practice in tropical countries*. Cambridge University Press, Cambridge. Pp. 97-182.

Chelikani, P., Fita, I., & Loewen, P. C. (2004). Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences*, *61*, 192-208.

Chelule, P. K., Mbongwa, H. P., Carries, S., & Gqaleni, N. (2010). Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. *Food Chemistry*, *122*(3), 656-661.

Cowan, S. T., & Steel, K. J. (2002). Manual for the identification of medical bacteria. 2nd edition, Cambridge University Press, Cambridge. Pp. 51-120.

De Vuyst, L., & Frederick, L. (2007). Bacteriocin from LAB: Production, purification and food application. *Journal of Molecular Microbiology and Biotechnol*ogy, *13*, 194-199.

Di Caro, S., Tao, H., Grillo, A., Elia, C., Gasbarrini, G., Sepulveda, A. R., & Gasbarrini, A. (2005). Effects of Lactobacillus GG on genes expression pattern in small bowel mucosa. *Digestive and Liver Disease*, *37*(5), 320-329.

Ezeronye, O. U. (2003). Fermentation kinetics and removal of off-

odour in cassava fufu. *Nigerian Journal of Environmental and Applied Biol*ogy, 4, 1-4.

Farzadi, M., Khatami, S., Mousavi, M., & Amirmozafari, N. (2011). Purification and characterization of α-galactosidase from Lactobacillus acidofillus. *African Journal of Biotechnology*, *10*(10), 1873-1879.

Forbes, B. A. (2007). Bailey and Scott's Diagnostic Microbiology, twelfth edition. C.V. Mosby Company, St. Louis. Pp. 1103-1112.

Gao, H., Li, X., Chen, X., Hai, D., Wei, C., Zhang, L., & Li, P. (2022). The functional roles of Lactobacillus acidophilus in different physiological and pathological processes. *Journal of Microbiology and Biotechnology*, *32*(10), 1226-1233.

Gibson, G. R., & Fuller, R. (2000). Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *The Journal of Nutrition*, *130*(2), 391S-395S.

Giraud, E., Brauman, A., Keleke, S., Lelong, B., & Raimbault, M. (1991). Isolation and physiological studies of an amylolytic strain of *Lactobacillus plantarum. Applied Microbiology and Biotechnology,* 36, 379-382.

Gupta, R., Jeevaratnam, K., & Fatima, A. (2018). Lactic acid bacteria: Probiotic characteristic, selection criteria, and its role in human health. *Journal of Emerging Technologies and Innovative Res*earch, *5*(10), 411-424.

Ibrahim, S. A., Ayivi, R. D., Zimmerman, T., Siddiqui, S. A., Altemimi, A. B., Fidan, H., Esatbeyoglu, T., & Bakhshayesh, R. V. (2021). Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods*, *10*(12), 3131.

Inyang, C. U., & Idoko, C. A. (2006). Assessment of the quality of ogi made from malted millet. *African Journal of Biotechnology*, *5*(22), 2334-2337.

Ismail, Y. S., Yulvizar, C., & Mazhitov, B. (2018, March). Characterization of lactic acid bacteria from local cow s milk kefir. In *IOP Conference Series: Earth and Environmental Science* (Vol. 130, p. 012019). IOP Publishing.

Khushboo, Karnwal, A., & Malik, T. (2023). Characterization and selection of probiotic Lactic Acid Bacteria from different dietary sources for development of functional foods. *Frontiers in Microbiol*ogy, *14*, 1170725.

Kostinek, M., Specht, I., Edward, V.A., Pinto, C., Egounlety, M., Sossa, C., Mbugua, S., Dortu, C., Thonart, P., Taljaard, L., Mengu, M., Franz, C.M., & Holzapfel, W.H. (2007). Characterisation and biochemical properties of predominant lactic acid bacteria from fermenting cassava for selection as starter cultures. *International Journal of Food Microbiology, 114*, 342-351.

Kruskall, M. S., AuBuchon, J. P., Anthony, K. Y., Herschel, L., Pickard, C., Biehl, R., Horowitz, M., Brambilla, D.J., & Popovsky M.A. (2000). Transfusion to blood group A and O patients of group B RBCs that have been enzymatically converted to group O. *Transfusion, 40*(11), 1290-1298.

Li, M., Wang, Y., Cui, H., Li, Y., Sun, Y., & Qiu, H. J. (2020). Characterization of lactic acid bacteria isolated from the gastrointestinal tract of a wild boar as potential probiotics. *Frontiers in Veterinary Science*, *7*, 49.

Macwilliams, R. T. (2009). *Citrate test protocols. Microbe Library*. American Society of Microbiology. Pp. 78-84.

Mgomi, F. C., Yang, Y. R., Cheng, G., & Yang, Z. Q. (2023). Lactic acid bacteria biofilms and their antimicrobial potential against pathogenic microorganisms. *Biofilm*, *5*, 100118.

N'zi, K. P., Adingra, K. M. D., N'guessan, K. F., Attchelouwa, C. K., & Tano, K. (2021). Effect of spontaneous fermentation time on physicochemical, nutrient, anti-nutrient and microbiological composition of Lima Bean (*Phaseolus lunatus*) flour. *Journal of Applied Biosciences*, *162*(1), 16707-16725.

Obadina, A. O., Oyewole, O. B., Sanni, L. O., & Tomlins, K. I. (2006). Bio-preservative activities of Lactobacillus plantarum strains in fermenting Casssava '*fufu'*. *African Journal of Biotechnology*, *5*(8), 620-623.

Olutiola, P. O., Famurewa, O., & Sonntag, H. G. (1991). An introduction to general microbiology: A practical approach. Heidelberger Velagsaustalg and Duckerei. GmbH Heidelberg. Pp. 157-175.

Omemu, A. M., & Omeike, S. O. (2010). Microbiological hazard and critical control points identification during household preparation of cooked ogi used as weaning food. *International Food Research Journal*, *17*, 257-266.

Omemu, A. M., Oyewole, O. B., & Bankole, M. O. (2007). Significance of yeasts in fermentation of maize for *ogi* production. *Food Microbiol*ogy, *20*, 127-132.

Osungbaro, T. O. (2009). Physical and nutritive properties of fermented cereal foods. *African Journal of Food Science*, *3*(2), 023-027.

Parveen, S., & Hafiz, F. (2003). Fermented cereal from indigenous raw material. *Pakistan Journal of Nutrition, 2*(5), 289-291.

Patel, A., Lindström, C., Patel, A., Prajapati, J. B., & Holst, O. (2012). Probiotic properties of exopolysaccharide producing lactic acid bacteria isolated from vegetables and traditional Indian fermented foods. *International Journal of Fermented Foods*, *1*(1), 87-101.

Salvucci, E., LeBlanc, J. G., & Pérez, G. (2016). Technological properties of lactic acid bacteria isolated from raw cereal material. *LWT*, *70*, 185-191.

Sidhu, P. K., & Nehra, K. (2021). Bacteriocins of lactic acid bacteria as potent antimicrobial peptides against food pathogens (Chapter 8). *Biomimetics*, IntechOpen.

Sieuwerts, S., Bron, P. A., & Smid, E. J. (2018). Mutually stimulating interactions between Lactic Acid Bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *LWT*, *90*, 201-206.

Sneath, P. H., Mair, N. S., Sharpe, M. E., & Holt, J.G. (2009). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins. Pp. 125-367

Stryer, L., Berg, J. M., & Tymoczko, J. L. (2002). Biochemistry (5th edition). San Francisco: W.H. Freeman. Pp. 123-246.

Sunano, Y. (2015). Procedure of brewing alcohol as a staple food: case study of the fermented cereal liquor "Parshot" as a staple food in Dirashe Special Woreda, Southern Ethiopia. *Food Science & Nutrition*, *4*(4), 544-554.

Tadesse, T., Kebede, B., Yimer, D., Workie, M., Tibebu, T., Abera, S., Tilahun, A., Alemu, M., Daba, T., Eshetu, A., & Dida, G. (2021). Screening and characterization of potential cyanide tolerating lactic acid bacteria from cassava pulp juice. International Journal of Biology and Pharmacy Research Updates, *1*(1), 26-31

Tamang, J. P. (2011). Prospects of Asian fermented foods in global market. 11th ASEAN Food Conference Proceeding, Bangkok, Thailand. Pp. 1308-1315.

Thanh, N. T., Chwen, L. T., Foo, H. L., Hair-Bejo, M., & Kasim, A. B. (2010). Inhibitory activity of metabolites produced by strains of *Lactobacillus plantarum* isolated from Malaysian

fermented food. *International Journal of Probiotics & Prebiotics*, *5*(1), 37-44.

Todar, K. (2012). Lactic acid bacteria. The good, the bad and the deadly. Todar's Online Textbook of Bacteriology. Pp. 1-5.

Zhou, Y., Zhu, Y., Dai, L., Men, Y., Wu, J., Zhang, J., & Sun, Y. (2017). Efficiency analysis and mechanism Insight of that whole-cell biocatalytic production of melibiose from raffinose with Saccharomyces cerevisiae. *Applied Biochemistry and Biotechnology*, *181*, 407-423.