Isolation and Molecular Identification of Lactic Acid Bacteria from Fermented Maize Grain (Ogi) and their Antimicrobial Activities against Pathogenic Bacteria

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Abstract

An increase in the emergence of antibiotic resistant pathogen has led to the search for other alternative antimicrobial agents. Probiotics are described as live microorganisms which help in the maintenance of the health and well-being of the hosts by improving the intestinal microbial balance. Probiotics can be an excellent solution to treat many common food-borne diseases. Lactic Acid Bacteria (LAB) are known to possess many health benefits and are commonly used as probiotics. As they can cause inhibition of growth of food pathogens by the reduction of pH due to lactic acid production, hydrogen peroxide production and production of antimicrobial compounds such as bacteriocin. In this study, lactic acid bacteria (*Lactobacillus fermentum subsp. reuteri, Lactobacillus plantarum subsp. plantarum JCM 1149, Lactobacillus paracasei strain JCM 8130 and Lactobacillus brevis)* were isolated from Ogi and screened for antimicrobial activity against pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Proteus* sp., *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhi*. The LAB isolates showed inhibitory activity against majority of the pathogens. The largest zone of inhibition was produced by *L. plantarum* (18 mm) against *Pseudomonas aeruginosa.* The lowest zone was produced by *L. paracasei strain JCM 8130* against *Klebsiella pneumoniae* (5.5 mm*). L. brevis* showed no zones against *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus.* Also, there was no zones when *L. plantarum subsp. plantarum JCM 1149* was used against *Proteus* sp. This agree with several reports that the antimicrobial activity might be due to bacteriocin production, the production of lactic acid and hydrogen peroxide. In conclusion, LAB is an effective probiotic against food-borne diseases as they have antimicrobial properties but more sophisticated methods and equipment should be used to isolate and purify their antimicrobial products.

Key words: Lactic Acid, Antibiotics, Antagonistic, Probiotics, Bacteriocin, Inhibition

Introduction

The excessive application of antimicrobial agents and immunosuppressive therapy may alter the gut composition and adversely affect the gut microbiota. Consequently, the introduction of probiotic bacteria in the gastro-intestinal tract has recently become an effective option to ascertain a healthy microbial

equilibrium (Torah *et al.,* 2019). Beneficial microbes associate with the raw food (substrate) during fermentation and products that are of medical advantages such as enzymes and vitamins are produced. Moreover, the consumption of these beneficial microbes produces a protective effect on the gut environment. It is to be noted that probiotic impacts are strain-dependent, and hence, not all strains are effective for efficient fermentation and treatment of all diseases (Ahmed *et al.,* 2019).

Lactic acid bacteria (LAB) are prominent non-pathogenic bacteria that play a vital role in our day-to-day activities, from fermentation, preservation, production of foods and vitamins, to prevention of certain diseases and cancer due to their antimicrobial effects (Kalhoro *et al.,* 2019). The lactic acid bacteria are considered potential probiotic candidates and are diverse group of Gram-positive, non-spore-forming, catalase and cytochrome oxidase negative, and non-motile bacteria, which produce lactic acid as a product of fermentation (Rahmeh *et al.,* 2019). Lactic acid bacteria should meet these basic criteria before consideration as probiotics. They should be generally recognized as safe; they should be resistant to low pH and high bile concentration and survive in gastrointestinal fluids, they should have adhesion characteristics, they should have antibacterial characteristics against enteric pathogens, and they should survive and be viable during the processing and storage (Hassan *et al.,* 2020).

Ogi is a fermented cereal gruel processed from maize, although sorghum or millet can also be used as the substrate for fermentation (Hu *et al.,* 2017). It is considered as one of the most important weaning foods for infants in Nigeria, although it is also consumed by adults. Along the West African coastal region, the product is given other names such as Eko, Agidi, Akamu, Koko and Furah depending on the substrate used and the form in which it is eaten (Afolayan and Ayeni, 2017).

Lactic acid fermentation generally requires little or no heat and the process is cheap. Lactic acid bacteria exert strong antagonistic activity against many microorganisms including food spoilage organisms and disease-causing pathogens (Ayivi *et al.,* 2020). In addition, some strains may contribute to the preservation of fermented foods by producing bacteriocin. These microorganisms are one of the prominent bacteria that inhabit the gastrointestinal tract, and the importance of these non-pathogenic bacteria has recently been more noticed (Daranas *et al.,* 2019). Many species of Lactobacilli have been reported to have nutritional benefits, improve lactose utilization, have anti-cholesterol, anti-tumor activities, and protection against other diseases. Lactic acid bacteria have been used successfully, with few adverse effects, to treat various diarrheal illnesses, to prevent antibiotic associated diarrhea, to treat acute infantile diarrhea and recurrent *Clostridium difficile* disease (Pato *et al.,* 2019).

The antagonistic property of lactic acid bacteria is attributed to the lowered pH and production of other primary and secondary antimicrobial metabolites (Mathur *et al.,* 2017). The metabolites produced by the fermentation process, except the volatile ones, are kept in the foods and result in growth inhibition of food spoilage or poisoning bacteria and detoxification of noxious compounds of plant origin (Islam *et al.,* 2020). In the case of traditional foods and beverages fermented by lactic acid bacteria, the fact that the products arrest the survival and growth of pathogens is more related to the *in-situ* action of lactic acid bacteria and their metabolites as observed in fermented cereal gruels and fermented milk, and in the fermentations of kocho and awaze (Ye *et al.,* 2021).

Increased outbreaks of foodborne diseases in recent years along with antimicrobial resistance of pathogens against commercial antibiotics (Agriopolou *et al.,* 2020) demand greater interest and need for natural

alternative ways to control foodborne pathogens. Therefore, the present study was undertaken to characterize and identify the lactic acid bacteria isolated from the fermentation of Ogi and to evaluate their antimicrobial activities and bio-preservation potential against some enteric and food spoilage bacteria.

Materials and Methods

Materials used for the study are Autoclave, Incubator, Petri-dishes, Test-tubes, Wire loop, Digital weigh balance, Aluminum foil, Microscope, and Glass slide. The culture media and reagent used includes: Nutrient agar, MacConkey agar, Eosin methylene blue agar, Salmonella-Shigella agar, Mannitol salt agar, and Nutrient broth**,** Gram staining reagents (Crystal violets, Iodine, Safranin, Acetone), Distilled water, Normal saline, Glucose, Mannitol, Sucrose, Xylulose, Galactose, Sorbitol, Cellobiose, Ribose, Lactose, Phenyl red (indicator) and Sodium chloride.

Cleaning of Glasswares and Sterilization

All glass wares utilized for this work were thoroughly washed with detergent rinsed with distilled water, it was dried and sterilized in the hot air oven at a temperature of 160° C for 1 hour. Furthermore, all glassware, reagents and equipment used for each experiment were mentioned under each procedure as appropriate.

Sample Collection

Maize grains (*Zea mays L.*) were purchased from a local market in Oshodi, Lagos State, Nigeria. They were immediately processed and transported aseptically to the department of microbiology laboratory, University of Lagos, for further analysis.

Fermentation of Ogi

For the preparation of Ogi, the maize grains are steeped in a sterile plastic bucket for 2 days, followed by wet milling and sieving to remove bran, hulls and germs according to the method of Akinrele *et al*., 1970. The pomace is retained on the sieve and later discarded as animal feed while the filtrate is fermented for 96 hours (4 days) to yield Ogi, which is a sour, white starchy sediment.

Determination of pH

The pH of the fermenting liquor of maize grain samples was determined directly by using a pH meter and the changes in pH of fermenting samples were monitored daily for 4 days according to Anumudu *et al.,* 2018.

Determination of Titratable Acidity (TA)

In order to determine the titratable acidity (TA), 10 ml of the samples were transferred to a measuring flask and filled up to 50 ml with distilled water. After mixing, 10 ml of the diluted sample were titrated against 0.1 M sodium hydroxide (NaOH) using phenolphthalein as indicator until a pink colour appeared. Each ml of 0.1 M NaOH is equivalent to 90.08 mg of lactic acid (Nwachukwu *et al.,* 2019).

$$\text{TA of lactic acid (mg/ml)} = \frac{ml\,NaOH \times M\,.\,NaOH \times M\,.\,E\,.}{Volume\,of\;Sample\,Used}$$

Where:

ml NaOH = Volume of NaOH used,
M NaOH = Molarity of NaOH used,
M.E = Molar Equivalent Factor of Lactic Acid = 90.08 mg (Wakil and Ajayi, 2013).

Isolation of Lactic Acid Bacteria from Fermented Ogi

A 25 g sample of fermented Ogi was taken aseptically and transferred to sterile plastic bags and then homogenized in 225 mL of sterile buffered peptone water. Five-fold dilutions of the homogenates was prepared and inoculated on plates of deMan Rogosa and Sharpe agar (MRS agar), acidified with glacial acetic acid to pH 5.7 and incubated anaerobically for 48 hours at 37° C. After 48 hours of incubation, the colonies were carefully selected and streaked on bromocresol purple agar (BCP) and incubated for 24 hours. Lactic acid bacteria were selected based on yellow-coloured zones around the colonies on the BCP agar plates. Colonies were tested for Gram staining and were identified using the 16S rRNA gene sequencing.

Identification Of The Lactic Acid Bacteria Isolates By Sequencing Of 16s rRNA Gene

The total DNA of the isolated strains was extracted and purified using Wizard Genomics DNA purifications kit by Promega (Madison, USA). Genetic characterization of the lactic acid bacteria isolates was performed by polymerase chain reaction (PCR) targeted to the 16S rRNA gene using the set of primers, forward primer: BSF8N 5′-AGAGTTTGATCMTGGCTCAG-′3 and reverse primer: BSR534 5′-ATTACCGCGGCTGCTGGC-′3. PCR parameters were initial denaturation at 95 °C for 5 min, annealing at 54 °C for 2 minutes, extension at 72 °C for 5 minutes. Amplified 16S rRNA gene fragments were purified and sequenced using a DNA sequencing device (BigDye Terminator v3.1 Cycle Sequencing Kit). The obtained 16S rRNA gene sequences were matched with those from the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast) of the National Center for Biotechnology Information (NCBI) to identify nucleotide sequence matching with the reference sequences (Kumar *et al.,* 2018).

Identification Of Indicator Pathogenic Bacteria

The test organisms used for this study (*Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Proteus* sp.*, Klebsiella pneumoniae, Escherichia coli* and *Salmonella typhi*.) was collected from Lagos state drug quality control laboratory and all isolates were confirmed with standard microbiological tests.

Antimicrobial Activities of Cell-free Supernatant of Lactic Acid Bacteria Isolates

The lactic acid bacteria were grown in MRS broth overnight under micro-aerophilic condition at 37 °C for 24 hours to determine their ability to produce inhibitory substances. The LAB cultures were centrifuged at 12,000x g for 10 minutes at 4° C and the supernatant was decanted into sterile test tubes. The antimicrobial activity of the isolated LAB (cell free filtrate) against the bacteria isolates *(Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus* sp., *Candida albicans* and *Staphylococcus aureus*) was performed by the well diffusion assay. 0.1 mL of the supernatant was placed in 6 mm wells cut with a cork borer into cooled Mueller Hinton agar plates inoculated with 0.2 mL × 10^7 CFU/mL of the test pathogens, the supernatant was allowed to diffuse by leaving the plates at room temperature for 1 hours before incubation at 37° C for 24 hours. The antimicrobial activity was determined by measuring the clear zone around the wells.

Extraction of Crude Bacteriocins

Selected LAB isolates were grown in MRS broth at 37° C for 24 hours. Cell-free culture supernatant of each isolate was obtained by centrifugation at 3,000x g for 25 min. The cell-free supernatant was adjusted to pH 6.5 with 1 M NaOH to neutralize any effect of acidity. Inhibitory activity from hydrogen peroxide was eliminated by the addition of a 5 mg/ml catalase and subsequently filtered through sterilized 0.2 µm membrane filter (Onwuakor *et al.,* 2014).

Inhibitory Activity of Bacteriocin from Selected Isolates

The agar-well diffusion method was employed and 0.1 ml of test organisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Proteus* sp. *Klebsiella* sp. *Escherichia coli* and *Salmonella typhi*) were plated using spread plate method on Mueller Hinton agar (MHA) plates. Wells were cut into the agar with a sterile 6 mm diameter cork borer. A 100 µL partially purified bacteriocins of each potential producer species was placed into each well. The plates were then incubated at 30° C for 24 to 48 h after which they were examined for probable clearing zones (Bali *et al.,* 2011).

Effect of Storage Time on Crude Bacteriocin

The pH of cell free culture supernatant (CFCS) of the LAB isolates was adjusted and the effect of the organic acids and hydrogen peroxide (H_2O_2) was eliminated as stated earlier. 20 ml of the crude bacteriocin of LAB isolates was then stored at 37° C and 5ml of the crude bacteriocin was tested in well diffusion assay against the indicator organisms every 24 h for 4 days (96 h) using well diffusion method (Bali *et al.,* 2011).

Haemolytic Activity

Haemolytic activity is a determining factor for probiotic bacteria, and the absence of haemolytic activity indicates that the particular bacteria were none virulent. Bacterial culture was streaked on sheep blood agar and incubated at 37° C for 48 h. The zone formed around the colonies was observed, which is categorized as β-haemolysis (clear zone), α-haemolysis (green-hued zone), or γ-haemolysis (no zone).

Results

Four species of lactic acid bacteria were isolated from fermented maize grain during Ogi production. The lactic bacteria isolates were identified based on cultural characteristics on growth media, biochemical test, sugar fermentation and using the 16S rRNA gene sequencing technique. The lactic acid bacteria isolates were identified as; *Lactobacillus fermentum subsp. reuteri, Lactobacillus plantarum subsp. plantarum JCM 1149, Lactobacillus paracasei strain JCM 8130 and Lactobacillus brevis* (Table 6). During fermentation, the successive pH of the fermenting liquor was tested at 24 hours interval and recorded (Table 2). Also, the total titratable acidity was measured at an interval of 24 hours and recorded (Table 3). The cell-free supernatant of the lactic acid bacteria isolates was extracted and the antimicrobial activities were tested against various food spoilage and disease-causing microbes (Table 8).

Crude bacteriocin was also extracted from the lactic acid bacteria and its inhibitory effect was evaluated against the test organisms, also the effect of storage on the bacteriocin was carried out on the test organisms (Table 9). All the lactic acid bacteria isolated from this study were tested for their haemolytic properties by culturing on sheep blood agar and they all exhibited γ-haemolytic activity (no haemolysis). Absence of haemolytic activity confirm the non-virulent nature of the isolates which may be regarded as a selecting criterion for potential probiotic strains (Perez *et al.,* 2014).

Table 1: Cultural Characteristics of The Isolates from the Starch Gruel (Ogi)

Isolates	Form	Elevation	Colour	Surface
BOG-1	Rough	Raised	Cream	Jelly-like
BOG-2	Irregular	Raised	Gray-white	Smooth
BOG-3	Circular	Flat	Gray-white	Glistering
BOG-4	Circular	Flat	Cream	Glistering

Table 2: Changes in pH Value of the Fermenting Liquor during Maize Grain Fermentation

Fermentation Period (Hours)	Fermentation pH
0	6.6
24	6.2
48	5.1
72	4.6
96	4.2

Table 3: Changes in Titratable Acidity Produced by the LAB Isolate during Fermentation

Fermentation Period (Hours)	Titratable Acid (mg/L)
0	0.32
24	0.48
48	0.55
72	0.71
96	0.79

Table 4: Biochemical Tests Results of the Lactic Acid Bacteria (LAB) Isolates

Sample	Indole	MR	Catalase	Oxidase	Gram Stain/Shape	Endospore Test
BOG-1	-	-	-	-	+/rod	-
BOG-2	-	-	-	-	+/rod	-
BOG-3	-	-	-	-	+/rod	-
BOG-4	-	-	-	-	+/rod	-

+ = Positive, - = Negative, MR = Methyl Red Test

Table 5: Sugar Fermentation Test of the Lactic Acid Bacteria Isolates

Isolates	Xylose	Cellobiose	Glucose	Maltose	Ribose	Sorbitol	Fructose
L. plantarum	-	+	+	+	+	-	-
L. fermentum	+	+	+	+	+	+	+
L. paracasei	+	+	+	+	+	+	+
L. brevis	+	-	+	+	+	-	+

+ = Positive, - = Negative,
L. fermentum = *Lactobacillus fermentum subsp. reuteri*,
L. plantarum = *Lactobacillus plantarum subsp. plantarum JCM 1149*,
L. paracasei = *Lactobacillus paracasei strain JCM 8130*,
L. brevis = *Lactobacillus brevis*

Table 6: Genetic Characterization of the Isolates based on Sequencing of Partial 16S rRNA Sequences and BLAST Alignment

Sample	Similarity Index (%)	Homologous with
BOG-1	100	*Lactobacillus plantarum subsp. plantarum JCM 1149*
BOG-2	99.9	*Lactobacillus paracasei strain. JCM 8130*
BOG-3	99.9	*Lactobacillus brevis*
BOG-4	99.8	*Lactobacillus fermentum subsp. reuteri*

Table 7: Biochemical Confirmation of the Test Organism

Isolates	Gram Stain	Catalase	Indole	Coagulase	Methyl Red	Citrate
Escherichia coli	-	+	+	-	+	-
Klebsiella pneumoniae	-	+	-	-	+	+
Pseudomonas aeruginosa	-	+	-	-	-	+
Staphylococcus aureus	+	+	-	+	+	+
Proteus sp.	-	+	-	-	+	+
Salmonella typhi	-	+	-	+	+	-

+ = Positive, - = Negative

Table 8: Antimicrobial Activities of Cell-free Supernatant of LAB against Common Pathogens (mm)

Isolates	*L. plantarum*	*L. fermentum*	*L. brevis*	*L. paracasei*
Escherichia coli	9.5	8.5	NZ	5.5
Klebsiella pneumoniae	10	7.5	NZ	5.5
Pseudomonas aeruginosa	18	12	10	7.5
Staphylococcus aureus	7.5	NZ	NZ	9.0
Proteus sp.	NZ	7	15	9.5
Salmonella typhi	13.5	12.8	8.5	15
Candida albicans	8	9.5	6.8	11

NZ = No zone of inhibition,
L. fermentum = *Lactobacillus fermentum subsp. reuteri*,
L. plantarum = *Lactobacillus plantarum subsp. plantarum JCM 1149*,
L. paracasei = *Lactobacillus paracasei strain JCM 8130*,
L. brevis = *Lactobacillus brevis*

Table 9: Antimicrobial Activity of Crude Bacteriocin Extracted from Isolated LAB against Common Pathogens (mm)

Isolates	*L. plantarum*	*L. fermentum*	*L. brevis*	*L. paracasei*
Escherichia coli	7.4	6.5	NZ	NZ
Klebsiella pneumoniae	8.4	6.3	NZ	3.2
Pseudomonas aeruginosa	11.4	6.4	9.8	7.7
Staphylococcus aureus	7.6	NZ	NZ	7.1
Proteus sp.	NZ	5.4	10.7	9.7
Salmonella typhi	11.5	11.6	8.5	15
Candida albicans	3.8	9.5	NZ	4.2

NZ = No zone of inhibition,
L. fermentum = Lactobacillus fermentum subsp. reuteri,
L. plantarum = Lactobacillus plantarum subsp. plantarum JCM 1149,
L. paracasei = Lactobacillus paracasei strain JCM 8130,
L. brevis = Lactobacillus brevis

Table 10: Effect of Storage Time of Bacteriocin on its Inhibitory Properties (mm)

Bacteriocin Producing LAB	Bacteriocin Storage Time				
	0 Hours	**24 Hours**	**48 Hours**	**72 Hours**	**96 Hours**
Test Organisms: *Escherichia coli*					
L. plantarum	7.5	7.1	6.4	5.2	3.1
L. fermentum	6.4	6.3	5.8	2.8	NZ
L. brevis	NZ	NZ	NZ	NZ	NZ
L. paracasei	NZ	NZ	NZ	NZ	NZ
Test Organisms: *Klebsiella pneumoniae*					
L. plantarum	8.2	7.6	6.9	5.1	3.8
L. fermentum	6.4	5.5	3.2	NZ	NZ
L. brevis	NZ	NZ	NZ	NZ	NZ
L. paracasei	3.3	NZ	NZ	NZ	NZ
Test Organisms: *Pseudomonas aeruginosa*					
L. plantarum	11.4	10.4	9.6	8.9	8.4
L. fermentum	6.3	3.2	NZ	NZ	NZ
L. brevis	10.0	9.3	8.2	7.1	5.2
L. paracasei	7.7	6.5	5.9	5.1	3.4
Test Organisms: *Staphylococcus aureus*					
L. plantarum	7.6	7.4	7.4	3.2	NZ
L. fermentum	NZ	NZ	NZ	NZ	NZ

L. brevis	NZ	NZ	NZ	NZ	NZ
L. paracasei	7.2	7.1	6.3	4.1	3.2
Test Organisms: *Proteus* sp.					
L. plantarum	NZ	NZ	NZ	NZ	NZ
L. fermentum	5.5	3.9	NZ	NZ	NZ
L. brevis	10.5	9.3	8.1	5.5	3.4
L. paracasei	9.8	7.5	5.1	3.6	NZ
Test Organisms: *Salmonella typhi*					
L. plantarum	11.5	10.4	8.8	6.3	4.1
L. fermentum	11.7	10.2	9.3	7.6	5.1
L. brevis	8.5	7.2	5.3	3.2	NZ
L. paracasei	15.0	13.4	11.8	8.2	5.8
Test Organisms: *Candida albicans*					
L. plantarum	3.9	NZ	NZ	NZ	NZ
L. fermentum	9.5	5.6	3.1	NZ	NZ
L. brevis	NZ	NZ	NZ	NZ	NZ
L. paracasei	4.0	NZ	NZ	NZ	NZ

NZ = No Zone Of Inhibition,
L. fermentum = Lactobacillus fermentum subsp. reuteri,
L. plantarum = Lactobacillus plantarum subsp. plantarum JCM 1149,
L. paracasei = Lactobacillus paracasei strain JCM 8130,
L. brevis = Lactobacillus brevis

Figure 1: Changes in pH Values of the Fermenting Maize Grain

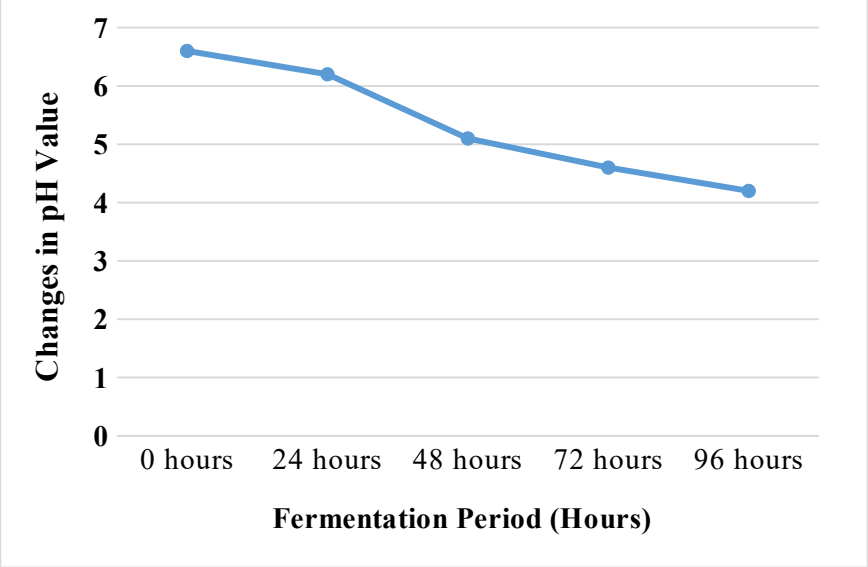


Figure 2: Changes in Total Titratable Acidity during 96-hours of Fermentation of Maize Grains

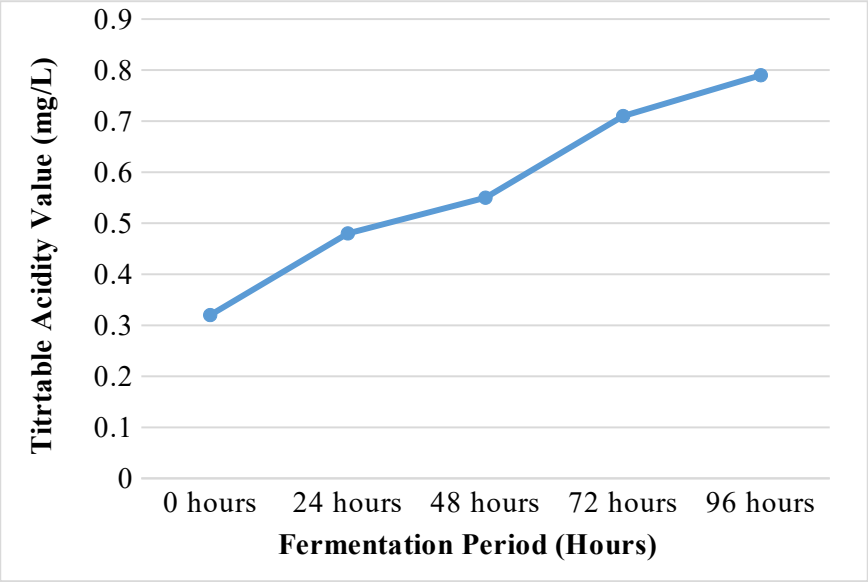


Figure 3: Antimicrobial Activities of the Cell-free Supernatant of the Lactic Acid Isolates (*Lactobacillus fermentum subsp. reuteri*, *Lactobacillus plantarum subsp. plantarum JCM 1149*, *Lactobacillus paracasei strain JCM 8130* and *Lactobacillus brevis*) against the Test Organisms. The Diameter of Zone of Inhibitions are Measured in Millimeters (mm).

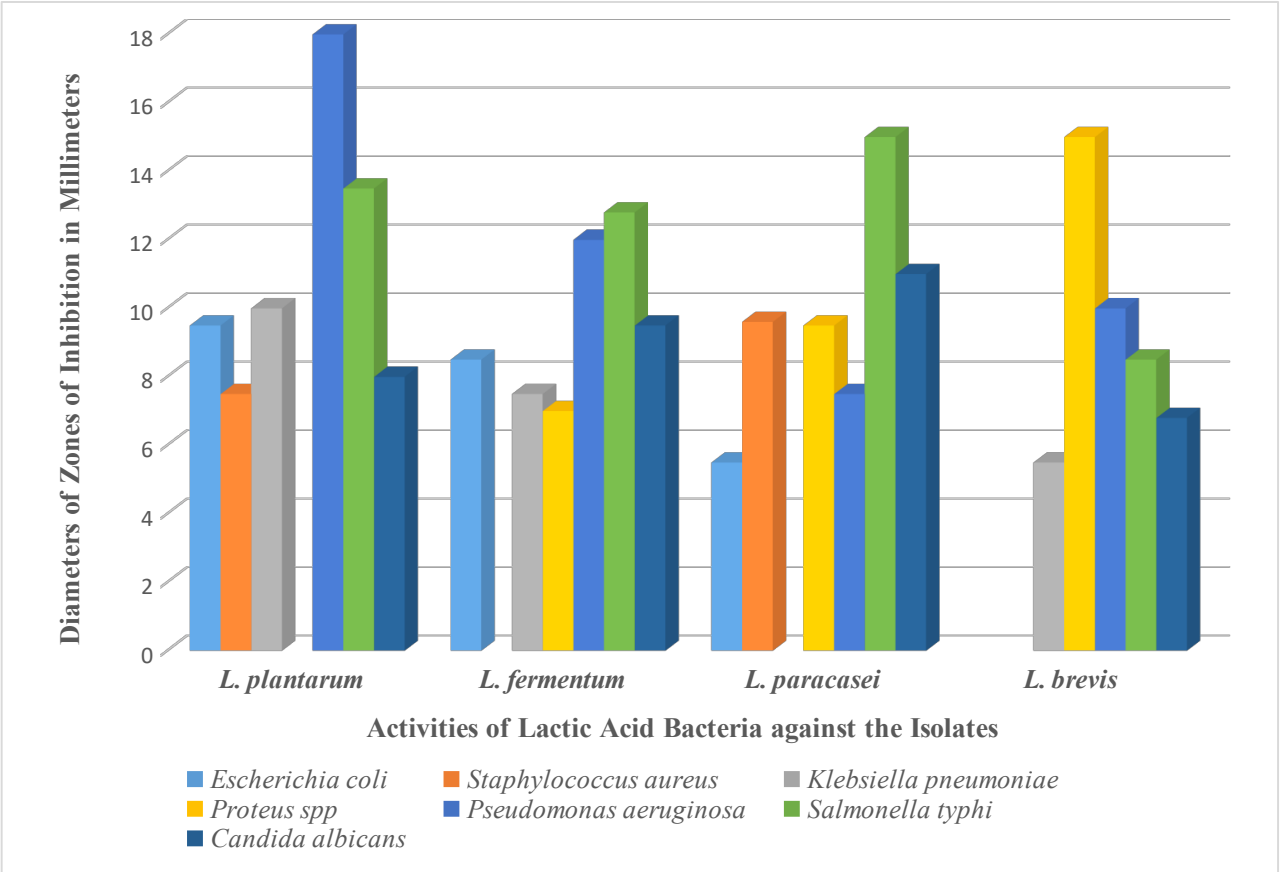
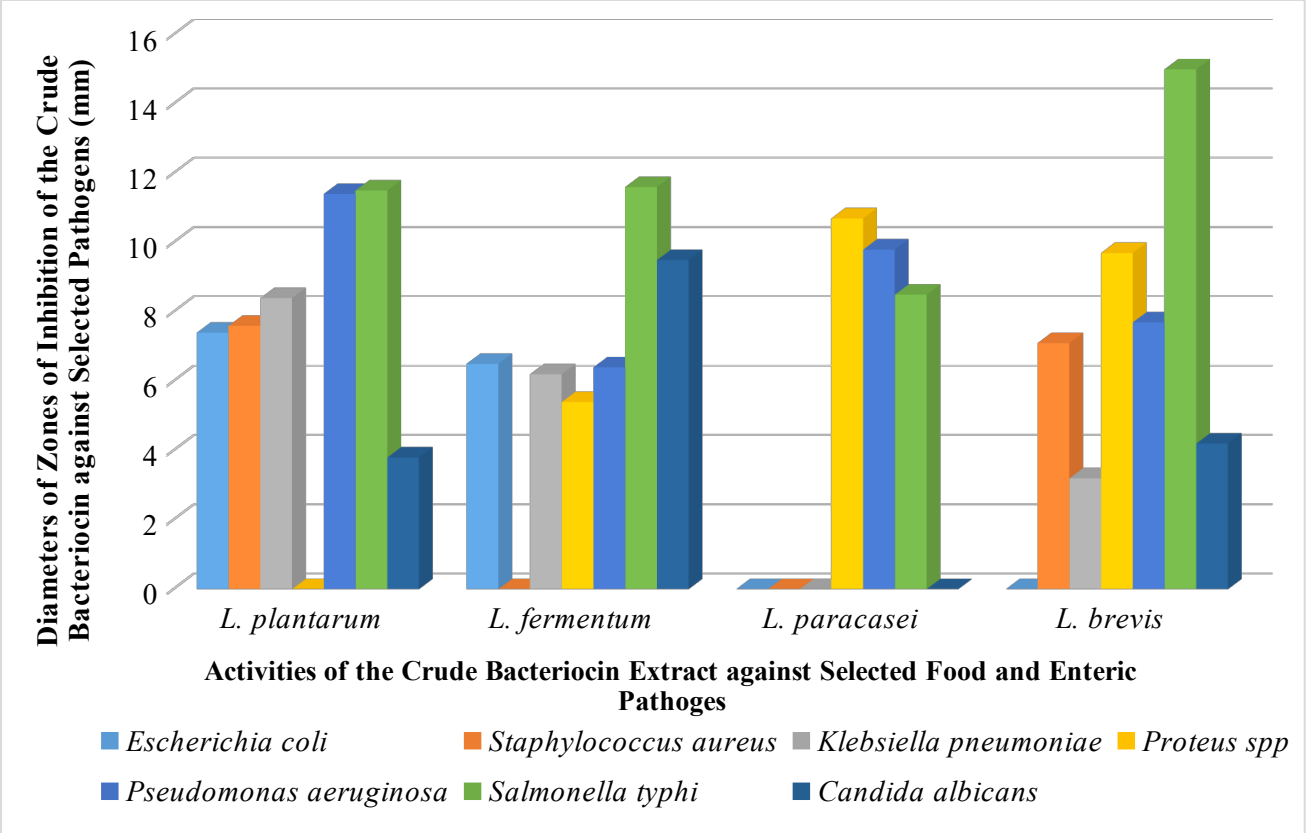


Figure 4: The Inhibitory Effect of the Crude Bacteriocin Extracted from the Lactic Acid Bacteria (*Lactobacillus fermentum subsp. reuteri*, *Lactobacillus plantarum subsp. plantarum JCM 1149*, *Lactobacillus paracasei strain JCM 8130 and Lactobacillus brevis*) Isolates and the Diameters of Zones of Inhibition Measured in Millimeter (mm)



Discussion

The present study was designed to identify the bacteria found in fermented maize grain (Ogi) and to explore its antimicrobial and bio-preservation potential. The isolates were found to be Gram-negative bacilli, non-spore forming, and tested negative for catalase and oxidase (Table 4). 16S rRNA sequence of the isolate showed 98–99% similarity to *Lactobacillus fermentum subsp. reuteri, Lactobacillus plantarum subsp. plantarum JCM 1149, Lactobacillus paracasei strain JCM 8130* and *Lactobacillus brevis* as recorded in the GenBank. The isolates were able to ferment majority of the tested sugars (Table 5), which is found to be similar to the studies of Yadav *et al.*, (2016). The isolates were able to utilize different types of carbohydrates such as glucose, sucrose, xylose, fructose, ribose, cellobiose, and maltose, indicating their ability to grow in varied habitats (Table 5). The cultural, morphological, and biochemical characteristics of isolates showed resemblance with other reported lactic acid bacteria, which were isolated from a wide variety of fermented foods (Nwachukwu *et al.,* 2019).

However, fermentation caused a general decrease in pH from 6.6 to 4.20 after 96 hours (Table 2) whereas titratable acidity increased from 0.32 to 0.79 during 96 hours fermentation (Table 3). The decrease in the pH values towards acidity was due to fermentation by the lactic acid bacteria (Abegaz, 2007). According to Inyang and Idoko (2006), an increase in acidity during fermentation was because of the accelerated growth rate of lactic acid bacteria. The amount of acid produced during fermentation increased exponentially with decrease in pH is in agreement with the findings of Nwachukwu *et al.,* (2019) which indicated an increase in titratable acidity with a reduction in pH during fermentation of maize for *Ogi*.

The pathogens used for this study (*Escherichia coli*, *Klebsiella pneumoniae Staphylococcus aureus, Proteus* sp. *Candida albicans, Pseudomonas aeruginosa and Salmonella typhi*) varies in their sensitivity to different antimicrobial agents. As the results indicate, the diameters of the inhibition zones vary between 5.5 mm to 18 mm (Table 8). This revealed that the lactic acid bacteria produced inhibitory substances against the organism. According to Kumar and Murugalatha, 2012, the inhibition is scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Similar study was carried out in Morocco by Khargwal *et al.,* 2014 who studied the activity of lactic acid bacteria on some Gram positive and negative pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 14 mm to 28 mm (Prabhurajeshwar, *et al.,* 2017).

The cell-free supernatant of selected lactic acid bacteria has antagonistic activities against majority of organisms used in this work. The largest zone of inhibition was produced by *Lactobacillus plantarum subsp. plantarum JCM 1149* (18 mm) against *Pseudomonas aeruginosa.* The lowest zone was produced by *Lactobacillus paracasei strain JCM 8130* against *Klebsiella pneumoniae* (5.5 mm). *Lactobacillus brevis* showed activity against *Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus.* Also, there was activity when *Lactobacillus plantarum subsp. plantarum JCM 1149* was evaluated against *Proteus s*p. The isolated lactic acid bacteria produced antimicrobial compounds to varying degree, the increase in the production of lactic acid with time have been attributed to lowered pH which permit the growth of lactic acid bacteria. The antimicrobial potential of lactic acid is due to undissociated form of acid which enters the membrane and liberate hydrogen ion in the neutral cytoplasm, thereby leading to inhibition of vital cell functions. The inhibitory effect of hydrogen peroxide produced by lactic acid bacteria has also been reported (Khargwal *et al.,* 2014).

Bacteriocin production as well as its activity seemed to be influenced by some factors such as the incubation conditions or storage time. This present study also indicates the strong bio-preservation potential of these bacteriocins which may be used to extend the shelf life of food and also implies that bacteriocin obtained from lactic acid bacteria isolates will be effective against both Gram positive and Gram-negative bacteria such as *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* with maximum inhibitory activities at the first 48 hours of extraction. The potency of crude bacteriocin by these isolates after storage was affected as different zones of inhibition were observed against the indicator organisms at varied storage time (hours) (Table 10). Maximum activity was noted at the first 48-hour storage period ranging from 3.2 mm to 15.0 mm against the food spoilage and disease-causing microorganisms. There was observable reduction in bacteriocin activities as storage time is prolonged. This result totally conforms with the findings of Onwuakor *et al.,* (2014) who observed reduction of crude bacteriocin activities as incubation time increased. Tulini *et al.,* (2011) observed a reduction of the bacteriocin production at 96 hours when compared with the control incubation (24 hours). This present result is in complete agreement with Elayaraja *et al.,* (2014), who reported that the highest bacteriocin activity of *Lactobacillus tucceti* CECT 5920 was recorded within the first 1 to 3 days against *Staphylococcus aureus* NCTC 8325 and *Escherichia coli* 0157:H7. Among the lactic acid bacteria, there has been great interest in *Lactobacillus plantarum*, due to the potential applications of the microorganisms as a starter bacterium for a variety of fermented foods. It has also been demonstrated from this study that LAB has a high potential for the treatment of bacterial infection and food storage. The bacteriocin produced by *L. plantarum subsp. plantarum JCM 1149* and *L. fermentum subsp. reuteri* exhibited a wide spectrum of inhibition compared to the bacteriocin produced by *L. brevis.* The potential of these

bacteriocins to inhibit pathogens such as *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus, Proteus* sp., and *Pseudomonas aeruginosa* makes it of crucial interest especially in processed foods where there is risk of food pathogens. For instance, *L. plantarum* has been shown to produce plantaricin. Zoumpopoulou *et al*., (2018) demonstrated that *L. paracasei* possessed some anti-listerial properties. In this study, both species were isolated from Ogi. The entire lactic acid bacteria identified in this study are known probiotics. The association of pH, metabolites responsible for inhibitory activities and the probiotics potentials of the isolated lactic acid bacteria call for more research. This will promote the recommendation of the bacteria as probiotics, especially in the management of diarrhoea. Hypothetically, the anti-diarrhoeal activities of raw Ogi might be associated with the lactic acid bacteria contents. The result of our studies also indicates the non-pathogenic nature of the lactic acid bacteria isolates as evidenced by gamma-(γ)-haemolysis in sheep blood agar plates. Negative results in haemolysis assay have been previously reported in many strains of *Lactobacillus* sp. (Nwachukwu *et al.,* 2019).

Conclusion

Bioactive compounds from microorganisms especially lactic acid bacteria are a source of bio-preservative agents. Also, the growing rate of antibiotic resistance and side effect of chemical and synthetic preservatives has been a global health challenge, therefore there is a need to explore alternative remedy from microbes and other natural products. Metabolites produced by lactic acid bacteria can be of probiotics and bio-preservative benefits. Lactic acid bacteria exhibit numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids, and other compounds, such as ethanol, H_2O_2, diacetyl and bacteriocins. Bacteriocins has been reported to be effective against food-borne pathogens and many other gram-positive spoilage microorganisms, and have attracted considerable interest for use as natural food preservatives in recent years.

Each antimicrobial compound produced during fermentation provides an additional hurdle for pathogens and spoilage bacteria to overcome before they can proliferate in a food or beverage, from time of manufacture to time of consumption. The antibiotic producing lactic acid bacteria may be used as protective cultures to improve the microbial safety of foods. The results of these study underline the crucial role that antimicrobial compounds extracted from lactic acid bacteria may play in food industry so as to improve food safety, it can also serve as source of novel antimicrobial agents and natural bio-preservative compound in food industries.

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Conflict Of Interest

The authors declare no conflicts of interest.

Ethics Statement

The study did not include any human subjects and animal experiments.

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