



(RESEARCH ARTICLE)



Antibacterial activity of lactic acid bacteria isolated from traditionally fermented food against food pathogen

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GSC Advanced Research and Reviews, 2023, 15(02), 020–031

Publication history: Received on 05 January 2023; revised on 30 April 2023; accepted on 03 May 2023

Article DOI: <https://doi.org/10.30574/gscarr.2023.15.2.0036>

Abstract

Aim: To evaluate the antibacterial activity of lactic acid bacteria against selected food pathogens (*Escherichia coli* and *Staphylococcus aureus*).

Method: A total of twenty (20) traditionally fermented food samples were purchased from a market in Umuahia metropolis. Isolation and identification of lactic acid bacteria (LAB) were conducted using standard microbiological techniques. LAB were identified using standard morphological and biochemical tests. They were tested against food pathogens using the Agar well diffusion method.

Results: The isolated lactic acid bacteria include *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum* and were label as SP1, SP2, SP3, and SP4 respectively. The isolated LAB species had a considerable inhibitory effect on food borne pathogens. Cell free supernatant of four species were tested for antagonistic activity at 24hrs and 48hrs incubation time. Cell free supernatant from 48hrs broth of SP1 showed the highest zone of inhibition on *Escherichia coli* (28mm) while the cell free supernatant from 24hrs broth of SP4 showed the lowest zone of inhibition on *Staphylococcus aureus* (13 mm).

Conclusion: The antimicrobial activity increases with time. LAB demonstrated high antimicrobial properties against food borne pathogens. This potential can be employed as starters in food industry to inhibit food spoilage microbes and contaminants.

Keywords: Antibacterial activity; Lactic acid bacteria; traditionally fermented foods; food pathogens.

1. Introduction

Lactic acid bacteria are a group of gram-positive, non-spore-forming, and non-motile bacteria that produce lactic acid as the primary end product of glucose fermentation. The classification of lactic acid bacteria (LAB) is based on criteria such as cellular morphology, glucose fermentation, growth temperature range, and sugar utilization patterns. Four genera were traditionally recognized as LAB: *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. However, with the use of molecular biological methods, the number of genera included in this group has been expanded. (Von-Wright and Axelsson, 2012). The current taxonomic classification of LAB group is included in the phylum Firmicutes, class Bacilli, and order Lactobacillales. The genus *Lactobacillus* is divided into three groups based on their sugar fermentation profile: obligate homofermentative (such as *Lactobacillus acidophilus*, *Lactobacillus salivarius*), facultative

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heterofermentative (such as *Lactobacillus plantarum*, *Lactobacillus curvatus*), and obligate heterofermentative (such as *Lactobacillus fermentum*, *Lactobacillus reuteri*). (Buddhiman *et al.*, 2008).

The presence of harmful microorganisms in food results in spoilage, making the food product unappealing and unacceptable to consumers (Petruzzi *et al.*, 2017). According to Burkepile *et al.* (2006), Food spoilage is a metabolic process that alters the sensory characteristics of food, making it unappealing or unacceptable for human consumption. Although spoiled food may not pose a health risk as there are no pathogens or toxins present, changes in texture, smell, taste, or appearance make it unappealing. Some researchers have suggested that the unpleasant smells produced by microbes are used to repel larger animals and keep the food source for themselves.

The diverse range of foods available presents a challenge for microbiologists, engineers, and technologists to discover effective methods of preventing the entry of microorganisms, destroying those that do manage to enter, and halting the growth and activity of those that evade processing treatments (Rawat, 2015). A variety of microorganisms can cause food spoilage, including aerobic psychrotrophic bacteria, gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria are known to produce large amounts of extracellular enzymes that can cause recontamination of pasteurized fluid milk products, which greatly affects their shelf life. Fungal spoilage is characterized by the presence of a wide range of metabolic by-products, leading to off-odors and flavors, as well as visible changes in color or texture. Yeasts, coliforms, heterofermentative lactic acid bacteria, and spore-forming bacteria can also cause defects in cheeses. The spoilage of many foods can be slowed by reducing the pH through fermentation, adding acids or other preservatives, introducing desirable microflora, adding sugar or salt to reduce water activity, removing water, packaging to limit oxygen, and freezing. The type of spoilage microorganisms varies widely among dairy foods due to the selective effects of production, formulation, processing, packaging, storage, distribution, and handling practices (Martorell *et al.*, 2005).

The issue of food contamination, food poisoning, and food-borne illnesses is a significant public health concern in developing countries, resulting in an increased awareness of food safety and hygiene over time. Food safety is a major concern in public health due to the prevalence of food-borne diseases. Some examples of bacteria that cause serious cases of food poisoning include *Bacillus cereus*, *Salmonella typhimurium*, and *Escherichia coli*. *Bacillus cereus* is a known food-borne disease causing bacteria. Species of *Bacillus* and related genera have been a long-standing problem for food producers due to their resistant endospores. (Tewari, and Abdullah, 2015). *Bacillus cereus* is a significant cause of food poisoning in industrialized countries, it produces one emetic toxin and three different enterotoxins. *Escherichia coli* (*E. coli*) is another cause of food poisoning which can lead to mild to severe gastrointestinal illness. Some types of pathogenic *E. coli*, such as Shiga toxin-producing *E. coli*, can be life-threatening. Different types of *E. coli* tend to contaminate different types of foods and water. Consumers are becoming increasingly concerned about the safety of synthetic preservatives used in food, leading to a growing demand for natural alternatives as food preservatives. (Gyawali and Ibrahim 2014). Fermented foods are often linked to beneficial fermenting bacteria, such as lactic acid bacteria (LAB). LABs produce antibacterial substances, making them useful as probiotics and food preservatives. These bacteria also produce metabolites such as organic acids, hydrogen peroxide, diacetyl, and bacteriocin, which can have beneficial effects (Gálvez *et al.*, 2007).

Fermented foods containing LAB displaying antimicrobial activities can be used as natural bio preservatives, preventing or inhibiting the growth of pathogenic and spoilage bacteria and fungi. Additionally, these LABs can help preserve the nutritional quality of the food (Ammor *et al.*, 2006, Ahmadova *et al.*, 2013). In recent years, there has been a significant effort to identify, document, and analyze the diverse range of antagonistic compounds produced by LAB (Liu, 2003). The preservation effect of LAB is due to the production of active metabolites, such as organic acids (lactic, acetic, formic, propionic acids) that reduce the pH of the medium, and other substances like fatty acids, acetoin, hydrogen peroxide, diacetyl, antifungal compounds (propionate, phenyl-lactate, hydroxyphenyl-lactate, cyclic dipeptides and 3-hydroxy fatty acids), bacteriocins (nisin, reuterin, reutericyclin, pediocin, lactacin, enterocin and others) and bacteriocin-like inhibitory substances (BLIS) (Şanlıbaba and Güçer, 2015).

The industrialization of biotechnological food processing has elevated the economic significance of lactic acid bacteria (LAB) as they play a crucial role in the sensory and safety aspects of fermented foods. Additionally, there has been a growing global interest in the use of LAB due to their potential health benefits. (Thakur *et al.*, 2017). Lactic acid bacteria are commonly found in nature and are able to ferment food by consuming its nutrients and producing a range of substances including organic acids, aromatic compounds, and beneficial compounds. (O'Shea *et al.*, 2011). The aim of this study is to evaluate the antibacterial activity of lactic acid bacteria isolated from traditionally fermented food against selected food spoilage pathogens (*Escherichia coli* and *Staphylococcus aureus*).

2. Material and methods

2.1. Sources of materials

Samples of traditional fermented food (*Ogiri*, *Ugba*, Soymilk and *Ogi*) were purchased from local markets in Umuahia, a town in the state of Abia, Nigeria. The samples were carefully collected and packaged in a sterile nylon material to prevent contamination. They were then transported to a microbiology laboratory for further analysis. The samples were kept at a cool temperature between 4-8°C until they were ready to be used for further testing.

2.2. Media Preparation

The media used for the isolation were prepared according to the manufacturer's instruction by dissolving the required amount of the powder in a known volume of diluted water and autoclaved at 121 °C for 15mins. The media used were Nutrient agar, de Man Rogosa and Sharpe agar (MRS agar) and de Man Rogosa and Sharpe broth (MRS broth).

2.3. Serial Dilution

Five grams of each sample were weighed and added into 45 ml of 0.9% physiological saline. After homogenization, ten-fold serial dilutions of the samples were prepared by taking 1ml of 100% stock solution into 9ml of distilled water using sterile needle and syringe the dilutions were made up to 10⁵ dilution factor.

2.4. Inoculation and Incubation of Culture Media

The 0.1 mL from each dilution was then cultured aseptically into MRS (deMan Rogosa and Sharpe) agar (Guessas and Kihal, 2004) using pour plate technique, all plates were then incubated at 37 °C for 24-48 hours in aerobic condition to provide an optimal environment for growing *Lactobacilli*.

2.5. Identification of Isolates

The identification of isolates was carried out in two phases; morphological Identification and biochemical characterization.

2.5.1. Morphological Identification

This was done by examining the isolates microscopically for cellular morphology. Day-old cultures of the bacteria isolates were gram-stained, and their color (purple or pink), shape (cocci or rods), and arrangement (singles, pairs, chains, or clusters) were observed and recorded.

Gram staining

Using a sterile loop, a light suspension of organism in sterile distilled water was prepared on a clean microscope slide. The film was air-dried and heat-fixed by passing the slide twice through a gas flame. The slide was then allowed to cool. The slide was placed on a staining rack, flooded with crystal violet solution, and left for 60sec before washing off with running tap water. The slide was again flooded with Lugol's iodine solution and left for 60sec before washing off with running tap water. To decolorize, acetone was run over the film and washed off immediately with running tap water. The film was flooded with safranin solution and left for 1 min before washing off with running tap water. The film on the slide was allowed to air-dry. A drop of immersion oil was then placed on the film, and it was examined under the microscope using the ×100 oil immersion lens. Dark purple indicated Gram-positive reaction and pink indicated Gram-negative reaction. The shapes and arrangement of the cells were also recorded.

2.5.2. Biochemical identification

Conventional biochemical tests were carried out on the bacterial isolates for further identification such as catalase test, oxidase test, motility test and sugar fermentation test.

Coagulase test

This test was carried out to differentiate between the pathogenic *Staphylococcus* from nonpathogenic *Staphylococcus*. A drop of water was placed on a slide and a pure culture was then emulsified with the drop of water on the slide to obtain a suspension. A drop of blood plasma was then mixed with the suspension on the slide and it was immediately observed for agglutination, a positive test indicates that the plasma has undergone clotting.

Catalase test (slide method)

A drop of 3% aqueous solution of hydrogen peroxide was placed on a clean slide. A small amount of the bacteria under test was placed on the hydrogen peroxide drop using a glass rod. Positive results were indicated by production of bubbles (Cheesbrough, 2006).

Oxidase test

A piece of filter paper in a Petri dish was moistened with 2–3 drops of Kovac's oxidase reagent (1% tetramethyl-*p*-phenylenediamine). Using a wire loop, a colony of the test organism was transferred to the filter paper and rubbed on the moistened area. Purple coloration within 30 sec indicated the production of cytochrome *c* oxidase (Cheesbrough, 2006).

Indole test

This test was carried out to determine the ability of bacteria to break down tryptophan to indole by the enzyme tryptophanase. The test organism was inoculated in a bijoux bottle containing 3 ml of sterile tryptone water and incubated at 35–37 °C for 48 h. Indole was tested for by adding 0.5 ml (5 drops) of Kovac's reagent (isoamyl alcohol; para-dimethyl aminobenzaldehyde; concentrated hydrochloric acid) and shaking gently. A red color in the surface layer within 10 min indicated a positive reaction while a yellow color indicated a negative reaction (Cheesbrough, 2006).

Sugar Fermentation Test

A twenty four hours old culture was stabbed into a sterile triple sugar iron agar slant (TSI) in a test tube and incubated at 37 °C for 24 hours. It was then observed for glucose, lactose, sucrose and gas production, in a positive test for glucose was indicated by redness of the bottom of the test tube, while in lactose the media appeared yellow (Cheesbrough, 2006).

Methyl red test

This test was carried out to identify enteric bacteria based on their pattern of glucose metabolism (mixed acid fermenters are positive to this test). The bacterium was inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37 °C for 48 h. The pH of the medium was tested by the addition of five drops of methyl red reagent. The tube was gently rolled between the palms to disperse the methyl red reagent. Development of red color was taken as positive and yellow as negative.

Citrate utilization test

This test was carried out to differentiate the enteric bacteria. Bacterial colonies from fresh (18-to 24-h-old) plates were picked up with wire loop, inoculated onto a slope of Simmons citrate agar and incubated overnight at 37 °C. A change of medium from green to blue indicated a positive reaction, that is, the organism has the ability to utilize citrate as sole source of carbon and energy. Positive control: *Klebsiella pneumonia*, Negative control: *Escherichia coli*.

2.6. Purification and preservation of isolates

The resulting colonies from the culture plates were purified by sub-culturing on a freshly prepared de Man Rogosa and Sharpe agar (MRS agar). The MRS agar were incubated at 35 °C for 24hrs. The purified isolates were kept on agar slants as stock cultures under refrigeration temperature. The isolates were sub cultured and transferred unto fresh agar slants on interval.

2.7. Screening of lactic acid bacteria for antimicrobial activity

2.7.1. Test Food Pathogen (Bacteria)

Escherichia coli and *Staphylococcus aureus* isolated from food samples obtained from Oevent clinical, diagnostic and research laboratory were used as test organisms for evaluating the antimicrobial activity of lactic acid bacteria. The pathogenic bacterial culture were grown on nutrient agar medium followed by identification using conventional methods.

2.7.2. Preparation of Cell free supernatant

Selected LAB isolates were sub-cultured in MRS broth at 37 °C for 24 hrs and 48hrs. The culture was spun at 5000 rpm for 15 minutes to obtain cell free supernatant. The spun culture was filtered using 0.22 µm millipore membrane. The

cell free supernatant (CFS) obtained was treated with 4M of NaOH in order to adjust the pH to 6.5 to inactivate antimicrobial activity of organic acids. The cell free supernatant (CFS) was kept in the refrigerator at 4°C for subsequent assay.

2.7.3. Cell free supernatant Inhibitory Activity Test

The antimicrobial activity of the Cell-free supernatant was determined using the agar well diffusion method, as described by Ida *et al.* (2017). The zone of inhibition of the crude extracts was determined using this method. Briefly, the test bacteria strains (*Staphylococcus aureus* and *Escherichia coli*) were standardized using a 0.5 McFarland solution and were spread on the surface of nutrient agar using a sterile cotton swab. Wells were created in the Petri plates of nutrient agar using a sterile cork borer and were labeled with numbers. A sterile micropipette was used to dispense 100µl of the cell-free supernatant into each well. The nutrient agar plates were then incubated at 37 °C for 24 hours. After incubation, the inhibition zone around the wells was measured using a ruler.

3. Results and discussion

Table 1 shows the cultural and morphological characteristics of lactic acid bacteria isolated from various traditionally fermented food. Based on their Gram reaction, shapes and cell arrangement all the isolates were Gram positive rods arranged in pairs or chains. Based on their cultural characteristics all the colonies of the isolates appeared creamy to white, small, spherical and convex with an entire edge.

Table 1 Cultural and morphological characteristics of lactic acid bacteria isolate from various traditionally fermented food

Isolation source	Colony Morphology	Microscopy	Suspected organism
Ogiri	Creamy, small, spherical convex, entire edges.	Gram positive, Long slender rods in chains	<i>Lactobacillus fermentum</i>
Ugba	White, small, spherical, convex, entire edges.	Gram positive, rods in pairs and chains	<i>Lactobacillus acidophilus</i>
Soy milk	Off white, Small, spherical, convex, entire edges.	Gram positive, small rods in chains	<i>Lactobacillus casei</i>
Ogi	Creamy, small, spherical convex, entire edges.	Gram positive bacilli	<i>Lactobacillus plantarum</i>

Table 2 shows the biochemical characteristics of the lactic acid bacteria isolated from traditionally fermented food. The details of the bacteria identified were *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus casei*. The Isolated bacterial from traditionally fermented food samples (Ugba, Ogi, Ogiri and Soy milk) were identified by several biochemical tests such as Indole, Methyl Red, Citrate, Catalase, Motility, Nitrate reduction, CO₂ Production and sugar fermentation test as the most special tests to identify them.

Table 3 shows the cultural, morphological and biochemical characteristic of the test food pathogen. Isolated food pathogens were identified by morphological characteristics, pigmentation on media, microscopy and several biochemical tests such as Gram stain, Citrate utilization, Voges-Proskauer (VP) test, Methyl Red (MR) test, Indole production, Oxidase, Catalase, Coagulase and sugar fermentation test as the most special tests to identify them. The two test food pathogens were confirmed to be *Escherichia coli* and *Staphylococcus aureus*.

Table 2 Biochemical characteristics of the lactic acid bacteria isolates using conventional method

Indole	Methyl Red	Citrate	Catalase	Motility	Nitrate reduction	Glucose	Galactose	Sucrose	Maltose	Fructose	Mannitol	Arabinose	CO ₂ Production	Suspected organism
-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	AG	HT	<i>Lactobacillus fermentum</i>
-	+	-	-	-	+	A	AG	AG	AG	AG	-	-	HM	<i>Lactobacillus acidophilus</i>
-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	AG	HT	<i>Lactobacillus casei</i>
-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	AG	HT	<i>Lactobacillus plantarum</i>

Key: A= Acid, AG= Acid and Gas, HM = Homofermentative, HT = Heterofermentative, - = Negative, + = Positive.

Table 3 Cultural, Morphological and Biochemical characteristics of test bacteria using conventional method

S/N	Cc	GSR	Ca	Co	Ox	MR	Ind	Mot	Glu	Lac	Suc	Man	Organism
1.	Pink; round; raised	Red rods, singles	+	-	-	+	+	+	AG	AG	AG	AG	<i>Escherichia coli</i>
2.	Golden yellow; round; convex	Cocci, purple, grape-like clusters	+	+	-	+	-	-	A	A	A	A	<i>Staphylococcus aureus</i>

Keys: Cc= Cultural characteristics, GSR=Gram stain reaction, Ca=Catalase, Co= Coagulase, Ox= oxidase, Mot=Motility, Ind=Indole, MR=Methyl Red, Glu=Glucose, Lac=Lactose, Suc=Sucrose, Man=Mannitol, A=Acid; G = Gas; AG = Acid and Gas; + = Positive; - = Negative

Table 4 Antibacterial activity of cell free supernatant from 24hrs broth culture of Lactic acid bacteria against food pathogens

Isolates	Zone of inhibition(mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
<i>Lactobacillus fermentum</i> (SP1)	19	16
<i>Lactobacillus acidophilus</i> (SP2)	16	17
<i>Lactobacillus casei</i> (SP3)	15	16
<i>Lactobacillus plantarum</i> (SP4)	20	13

Table 4 shows the antibacterial activity of the cell-free supernatant obtained from 24hrs broth culture of lactic acid bacteria (LAB) against food pathogens. Against *Escherichia coli*, the inhibition zone measured in millimeters varied from 15 to 20. The LAB isolate with the sample code SP4 showed the largest inhibition zone (20 mm) against *Escherichia coli*, followed by LAB isolate with the sample code of the SP1 showing 19 mm inhibition zone. SP3 showed the smallest inhibition zone (15mm). While against *Staphylococcus aureus*, the inhibition zone in millimeter ranges from 13-17. The LAB isolate with the sample code SP2 showed the largest inhibition zone of 17 mm, followed by LAB Isolates with the sample code of the SP1 and SP3 showing 16 mm inhibition zone each. SP4 showed the smallest inhibition zone (13mm).

Table 5 shows the antibacterial activity of the cell-free supernatant obtained from 48hrs broth culture of lactic acid bacteria against food pathogens. The inhibition zone against *Escherichia coli*, measured in millimeters ranged from 25 to 28. The LAB isolate with the sample code SP1 showed the largest inhibition zone (28 mm) against *Escherichia coli*, followed by LAB Isolates with the sample code of the SP2 and SP4 showing 26mm inhibition zone. SP3 showed the smallest inhibition zone (25mm). While against *Staphylococcus aureus*, the inhibition zone in millimeter ranges from 25-27. The LAB isolate with the sample code SP1 showed the largest inhibition zone of 27 mm, followed by LAB Isolates with the sample code of the SP3 and SP4 showing 26 mm inhibition zone each. SP3 showed the smallest inhibition zone (25mm).

Table 5 Antibacterial activity of cell free supernatant from 48hrs broth culture of Lactic acid bacteria against food pathogens

Isolates	Zone of inhibition (mm)	
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
<i>Lactobacillus fermentum</i> (SP1)	28	27
<i>Lactobacillus acidophilus</i> (SP2)	26	25
<i>Lactobacillus casei</i> (SP3)	25	26
<i>Lactobacillus plantarum</i> (SP4)	26	26



Key: 1= *Lactobacillus fermentum*, 2=*Lactobacillus acidophilus*, 3=*Lactobacillus casei*, 4=*Lactobacillus plantarum*

Figure 1 Clear inhibition zone of cell free supernatant from 24hrs broth of *Lactobacillus* isolates against of *Escherichia coli*



Keys: 1= *Lactobacillus fermentum*, 2=*Lactobacillus acidophilus*, 3=*Lactobacillus casei*, 4=*Lactobacillus plantarum*

Figure 2 Clear inhibition zone of cell free supernatant from 24hrs broth of *Lactobacillus* isolates against of *Staphylococcus aureus*



Keys: 1= *Lactobacillus fermentum*, 2=*Lactobacillus acidophilus*, 3=*Lactobacillus casei*, 4=*Lactobacillus plantarum*.

Figure 3 Clear inhibition zone of cell free supernatant from 48hrs broth of *Lactobacillus* isolates against of *Escherichia coli*



Keys: 1= *Lactobacillus fermentum*, 2=*Lactobacillus acidophilus*, 3=*Lactobacillus casei*, 4=*Lactobacillus plantarum*

Figure 4 Clear inhibition zone of cell free supernatant of 48hrs broth of *Lactobacillus* isolates against of *Staphylococcus aureus*

4. Discussion

Probiotics, such as *Lactobacillus*, *Bifidobacterium*, and *Streptococcus* spp., have been shown to inhibit the growth of various intestinal pathogens in humans. Bacteriocins produced by lactic acid bacteria are valuable for preserving food and enhancing food safety (Jamuna and Jeevaratnam, 2004; Chen and Hoover, 2003). This study evaluates the antibacterial activity of lactic acid bacteria (LAB) isolated from traditionally fermented food against selected food pathogens. A total of four (4) bacteria isolates comprising of 4 different species from the same genera were isolated from traditionally fermented food (*Ogiri*, *Ugba*, Soy milk and *Ogi*). Based on morphological characteristics four (4) isolates were identified as *Lactobacillus* spp. Oxidase, catalase and IMViC test of selected isolates also identified them as *Lactobacillus* spp. All of the isolates were Indole, Citrate, and Catalase negative but positive to Methyl red and nitrate reduction test, the results are similar with the findings of Elizete and Carlos (2005). The isolates *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum* were labelled SP1, SP2, SP3, SP4 respectively. The isolates from this study were all Gram positive, this results compares favourably with results of Mezaini *et al.* (2009), who evaluated the antibacterial activity of some lactic acid bacteria isolated from an Algerian dairy product and isolated only Gram positive organisms including *Streptococcus thermophiles*, *Streptococcus cremoris*, *Lactococcus diacetylactis* and *Lactococcus lactis*. Mugula *et al.* (2003) isolated *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus fermentum*, *Pediococcus pentosaceus* from togwa a Tanzanian fermented food. Similarly, Asmahan and Muna (2009), isolated *Lactobacillus fermentum*, *Lactobacillus amylovorus* and *Lactobacillus brevis* from fermented sorghum dough in Sudan. The lactic acid bacteria identified in traditionally fermented food in this current study have been reported in other fermented foods. *L. fermentum* and *L. brevis* have been suggested to be the predominating microorganisms during fermentation of *fufu* and *ogi*, two Nigerian foods (Adegoke and Babalola, 1988), *kisra* a Sudanese sorghum fermented flat bread (Mohammed *et al.*, 1991; Abd-Elmoniem *et al.*, 1994), *kenkey*, a Ghanaian fermented maize dough (Halm *et al.*, 1993).

In this current study, the inhibitory effect of the cell-free supernatant of each of the four (4) isolates was evaluated. The Lactic acid bacteria (LAB) strains isolated were tested against food pathogens and the zones of inhibition were observed. All isolated LAB showed high zones of inhibition on the test Gram positive and Gram negative bacterium (*Escherichia coli* and *Staphylococcus aureus*) from food sources. The report of these findings is similar to the work reported by Dal Bello *et al.* (2007) and Rodríguez *et al.* (2012), who reported that antimicrobial compounds such as phenyl-lactic acid

and lactic acid were effective against many Gram-negative and Gram-positive pathogenic bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecalis*.

In this present study, All the *Lactobacillus* isolates showed to inhibit the test organisms used in this study though the inhibition zone vary in diameter. The result showed that, Cell free supernatant from 48hrs broth of SP1 showed the highest zone of inhibition on *Escherichia coli* (28mm) while the cell free supernatant from 24hrs broth of SP4 showed the lowest zone of inhibition on *Staphylococcus aureus* (13 mm). From the results of this study, it was observed that the zone of inhibition increased with increase in incubation time, hence, it can be said that the production of antibacterial substances by lactic acid bacteria increases with time. This results is supported by the work Chowdhury *et al.* (2012), who in their study on Buffalo yoghurt for probiotic and antibacterial activity evaluated the amount of organic acid produced by lactic acid bacteria at 37 °C after 24hrs, 48hrs and 72hrs of incubation. Their experiment indicates that organic acid production increased with the incubation time. From the results of their study, highest acidity (1.8%) was observed after 72 hrs. incubation at 37 °C for *Lactobacillus* spp. isolated from Bogra. On the other hand, other probiotic bacteria isolated from yoghurt of Dhaka showed the acid (2.12%), Jhenidah region also showed the acid (2.07%) and acid (1.98%) value after 72 hrs. incubation. However, the production of this metabolites decreases with longer fermentation time (Adeyemo *et al.* 2018). According to Ivanova *et al.* (1998), Growth and bacteriocin production profiles showed that the maximal bacteriocin production was measured by the end of the late log phase, the level of production remained at a steady state during the stationary phase; similar results were obtained by Mezaini *et al.* (2009).

Other noble researchers have reported varying range of inhibition by lactic acid bacteria against different pathogens. Adeyemo *et al.* (2018), tested four species of LAB for antagonistic activity namely *L. plantarum*, *L. acidophilus*, *L. brevis* and *L. casei*, and all showed zones of inhibition. For *P. aeruginosa* (20 mm, 15mm, 19 mm and 4.5 mm respectively) and *S. aureus* (22mm, 16 mm, 19 mm and 5 mm); Atta *et al.* (2020), recorded a *Lactobacillus plantarum* inhibition zone range of 22mm and 19mm against *E. coli*. Their results compares favourably with the results of this present study. However, Ren *et al.*, (2018); Kurniatia *et al.*, (2021); Sari *et al.* (2018); Gu *et al.* (2015) and Kaskokiene *et al.* (2017) reported lower zone of inhibition which disagrees with the findings of this current study. The dissimilarity in results might be due to source and antibiotic susceptibility pattern of the test organism.

5. Conclusion

These *in-vitro* studies indicated that Lactic acid bacteria produce antibacterial compounds. The ability of these LAB strains to produce antimicrobial compounds suggests that they could be used as a source of new preservatives in the food industry. Given the growing importance of LAB as an alternative to antibiotics, understanding the antimicrobial activity of specific LAB species, such as *L. plantarum* and *L. acidophilus*, is particularly important.

The antimicrobial activity of *L. plantarum* and *L. acidophilus* shows that it can be used as a food preservative to reduce contaminants in food. There are three mechanisms that could explain the antimicrobial activity of LAB especially *L. plantarum* and *L. acidophilus*; the production of bacteriocins; the yield of organic acids and other inhibitory substances such as ethanol, carbon dioxide and hydrogen peroxide; and the competition for nutrients. These cannot be overemphasized.

Compliance with ethical standards

Acknowledgments

We gratefully acknowledge Dr. E. O. Nwankwo and the technical personnel of the Laboratory section of the Department of Microbiology at Michael Okpara University of Agriculture Umudike for their assistance.

Disclosure of conflict of interest

There are no conflicting interests.

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