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

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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 Owerri metropolis. Isolation and identification of lactic acid bacteria (LAB) were conducted using standard microbiological techniques. LAB was 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 *homoferementive* (such as *Lactobacillus*acidophilus, *Lactobacillus*salivarus) 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, lacticin, enterocin* and others) and bacteriocin-like inhibitory substances (BLIS) (Şanlibaba 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 Owerri, a town in the state of Imo, 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 powered 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 of 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 factors.

2.4. noculation 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 environmental for growing Lactobacilli.

2.5. Identification of Isolates

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

2.6. 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.

2.7. 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.

2.8. 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.

2.8.1. 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 clothing

2.8.2. 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 indicted by production of bubbles (Cheesbrough, 2006).

2.8.3. 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).

2.8.4. 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 bijou 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).

2.8.5. 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).

2.8.6. 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.

2.8.7. 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*.

2.9. 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.

2.10. Screening of lactic acid bacteria for antimicrobial activity

2.10.1. 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 was grown on nutrient agar medium followed by identification using conventional methods.

2.10.2. 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.

2.10.3. 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

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	*Lactobacillus fermentum*
Ugba	White, small, spherical, convex, entire edges.	Gram positive, rods in pairs and chains	*Lactobacillus acidiophilus*
Soy milk	Off white, Small, spherical, convex, entire edges.	Gram positive, small rods in chains	*Lactobacillus casei*
Ogi	Creamy, small, spherical convex, entire edges.	Gram positive bacilli	*Lactobacillus plantarum*

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* acidiophilus, *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 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	*Lactobacillus fermentum*
-	+	-	-	-	+	A	AG	AG	AG	AG	-	-	HM	*Lactobacillus acidiophilus*
-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	AG	HT	*Lactobacillus casei*
-	+	-	-	-	+	AG	AG	AG	AG	AG	AG	AG	HT	*Lactobacillus plantarum*

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	*Escherichia coli*
2.	Golden yellow; round; convex	Cocci, purple, grape-like clusters	+	+	-	+	-	-	A	A	A	A	*Staphylococcus aureus*

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)	
	Escherichia coli	***Staphylococcus aureus***
Lactobacillus fermentum (SP1)	19	16
Lactobacillus acidiophilus (SP2)	16	17
Lactobacillus casei (SP3)	15	16
Lactobacillus plantarum (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)**	
	Escherichia coli	***Staphylococcus aureus***
Lactobacillus fermentum (SP1)	28	27
Lactobacillus acidiophilus (SP2)	26	25
Lactobacillus casei (SP3)	25	26
Lactobacillus plantarum (SP4)	26	26

Key: 1= *Lactobacillus* fermentum, 2=*Lactobacillus* acidiophilus, 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* acidiophilus, 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* acidiophilus, 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* acidiophilus, 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 Methly red and nitrate reduction test, the results are similar with the findings of Elizete and Carlos (2005). The isolates *Lactobacillus* fermentum, *Lactobacillus acidiophilus*, *Lactobacillus* casei and *Lactobacillus* plantarum were labelled SP1, SP2, SP3, SP4 respectively. The isolates from this study were all Gram positive, this result compares favorably 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 Ghananian 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 result 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 compare favorably 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

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Disclosure of conflict of interest

There are no conflicting interests.

References

[1] Von-Wright, A. and Axelsson, L. (2012) Lactic Acid Bacteria: An Introduction. In: Lahtinen, S., Ouwehand, A. C., Salminen, S. and von-Wright, A., Eds., Lactic Acid Bacteria: Microbiological and Functional Aspects, 4th Edition, Tayor and Francis Group LLC, CRC Press, Boca Raton, pp. 1-16.

[2] Buddhiman, T., Jyoti, P. T., Ulrich, S., Charles, M., Michael, G. and Wilhelm, H. H., (2008). Phenotypic and genotypic identification of lactic acid bacteria isolated from ethnic fermented bamboo tender shoots of North East India. International Journal of Food Microbiology, 121:35-40.

[3] Petruzzi L, Corbo MR, Sinigaglia M and Bevilacqua A (2017). Microbial spoilage of foods: Fundamentals. In The Microbiological Quality of Food. Woodhead Publishing, pp. 1-21.

[4] Burkepile, D. E., Parker, John D., Woodson, C. B., Mills, H. J., Kubanek, J., Sobecky, P. A., and Hay, M. E. (2006). Chemically-mediated competition between microbes and animals: microbes as consumers in food webs. Ecology. 87:2821– 2831.

[5] Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research, 5(4):47-56.

[6] Martorell, P., Fernández-Espinar, M. T., and Querol, A. (2005). Molecular monitoring of spoilage yeasts during the production of candied fruit nougats to determine food contamination sources. International journal of food microbiology, 101(3), 293–302.

[7] Tewari, A., and Abdullah, S. (2015). Bacillus cereus food poisoning: international and Indian perspective. Journal of Food Science and Technology, 52(5):2500-2511. Gyawali, R. and Ibrahim, S. A. (2014). Natural products as antimicrobial agents, Food Control, 46: 412-429.

[8] Gálvez, A., Abriouel, H., López, R. L., and Ben Omar, N. (2007). Bacteriocin-based strategies for food biopreservation. International journal of food microbiology, 120(1-2), 51–70.

[9] Ammor, S., Tauveron, G., Dufour, E. and Chevallier, I. (2006). Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale facility 1- Screening and characterization of the antimicrobial compounds. Food Control, 17:454-461.

[10] Ahmadova, A., Todorov, S. D., Hadji-Sfaxi, I., Choiset, Y., Rabesona, H., Messaoudi, S., Kuliyev, A., de Melo-Franco, B. D. G., Chobert, J. M. and Haertle, T. (2013). Antimicrobial and antifungal activities of *Lactobacillus* curvatus strain isolated from homemade Azerbaijani cheese, Anaerobe, 20:42-47

[11] Liu, S. Q. (2003). Practical implications of lactate and pyruvate metabolism by lactic acid bacteria in food and beverage fermentations. International journal of food microbiology, 83(2), 115–131.

[12] Şanlıbaba, P., and Güçer, Y. (2015). Antimicrobial activity of lactic acid bacteria. Agriculture and Food, 3:451-457.

[13] Thakur, M. Deshpande, H. W. and Bhate, M. A. (2017). Isolation and Identification of Lactic Acid Bacteria and their Exploration in Non-Dairy Probiotic Drink.International Journal of Current Microbiology and Applied Science 6(4): 1023- 1030.

[14] O'Shea, E. F., Cotter, P. D. Stanton, C. Ross, R. P. and Hill, C. 2011. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: Bacteriocins and conjugated linoleic acid. International Journal of Food Microbiology, 152(3): 189-205.

[15] Guessas, B., and Kihal, M. (2004). Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats' milk. African Journal of Biotechnology, 3(6): 339- 342.

[16] Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries Part 2, Second Edition, Cambridge University Press; pp 62-70.

[17] Ida M. M. Y., Ina, S. M. Y., Ghazali, A. R., Hing, H. L. and Nor, F. R. (2017).Identification and characterization of the lactic acid bacteria isolated from Malaysian fermented fish (Pekasam). International Food Research Journal, 24:868-875.

[18] Jamuna, M. and Jeevaratnam, K. (2004). Isolation and partial characterization of bacteriocins from Pediococcus species," Applied Microbiology and Biotechnology, 65(4)433–439.

[19] Chen, H. and Hoover, D. G. (2003). Bacteriocins and their food applications. Comprehensive Reviews in Food Science and Food Safety. 2:83-97.

[20] Elizete, D. F. R. P. and Carlos, R. S. (2005) Biochemical characterization and identification of probiotic *Lactobacillus* for swine. B.CEPPA Curitiba, 23: 299-310.

[21] Mezaini, A., Chihib, N. E., Bouras, A. D., Nedjar-Arroume, N., and Hornez, J. P. (2009). Antibacterial activity of some lactic acid bacteria isolated from an Algerian dairy product. Journal of environmental and public health, 2009, 678495.

[22] Mugula, J. K., Nnko, S. A., Narvhus, J. A., and Sørhaug, T. (2003). Microbiological and fermentation characteristics of togwa, a Tanzanian fermented food. International journal of food microbiology, 80(3), 187–199.

[23] Asmahan, A. A. and Muna, M. M. (2009). Isolation, Characterization and Identification of Lactic Acid Bacteria from Fermented Sorghum Dough Used in Sudanese Kisra Preparation. Pakistan Journal of Nutrition, 8: 1814-1818.

[24] Adegoke, G. O. and Babalola, A. K. (1988). Characteristics of micro-organisms of importance in the fermentation of fufu and ogi-two Nigerian foods. Journal of Applied Bacteriology, 65(6): 449-453.

[25] Mohammed, S. I., Steenson L. R. and Kirleis, A.W. (1991). Isolation and characterization of microorganisms associated with the traditional sorghum fermentation for production of Sudanese kisra. Applied Environmental Microbiology, 57(9): 2529-2533.

[26] Abd-Elmoniem, O., Halifax E. and Abdullahi, H. E. T. (1994). Effect of fermentation on protein fraction and tannin content of low and high tannin cultivars of sorghum. Journal of Applied Bacteriology, 49: 265-269.

[27] Halm, M., Lillie, A. Sorensen A. K. and Jakobsen, M. (1993). Microbiological and aromatic characteristics of fermented maize doughs for kenkey production in Ghana. International Journal of Food Microbiology and Biotechnology, 12: 531-536.

[28] Dal-Bello F., Clarke, C. I., Ryan, L. A. M., Ulmer, H., Schober, T. J., Ström, K., Sjögren, J., van-Sinderen, D., Schnürer,J. and Arendt, E. K. (2007). Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus* plantarum FST 1.7. Journal of Cereal Sciences. 45(3): 309-318

[29] Rodríguez, N., Salgado, J. M., Solana, R. R., Cortés, S. and Domínguez, J. M. (2012) Biotechnological production of cell-free extract obtained by fermentation of cheese whey and phenylpyruvic acid using *Lactobacillus* plantarum and antimicrobial effect against pathogen bacteria. New Biotechnology, 29: 59-65.

[30] Chowdhury, A., Hossain, M. N., Mostazir, N. J., Fakruddin, M, Billah M. M. and Ahmed M. M. (2012) Screening of *Lactobacillus* spp. from Buffalo Yoghurt for Probiotic and Antibacterial Activity. Journal of Bacteriology and Parasitology, 3(8):1- 5.

[31] Adeyemo, S. M., Agun, T. F. and Ogunlusi, E. D. (2018). Antimicrobial Activity of Lactic Acid Bacteria Isolated from 'Pupuru': An African Fermented Staple against Food Borne-Pathogens. Journal of Molecular Biology and Biotechnology, 3(1:5):1- 6.

[32] Ivanova, I., Miteva, V., Stefanova T., Pantev, A., Budakov, I. Danova, S. Moncheva, P., Nikolova, I., Dousset, X. and Boyaval, P. (1998). Characterization of a bacteriocin produced by Streptococcus thermophilus 81," International Journal of Food Microbiology, 42(3):147–158.

[33] Atta, H. I., Gimba, A. and Bamgbose. T. (2014). Antimicrobial activity of lactic acid bacteria isolated from garri on Escherichia coli strains isolated from clinical and environmental samples. Agricultural Science and Technology, 12(4):357-363.

[34] Ren, D., Zhu, J., Gong, S., Liu, H., and Yu, H. (2018). Antimicrobial Characteristics of Lactic Acid Bacteria Isolated from Homemade Fermented Foods. BioMed research international, 8:1-9

[35] Kurniatia, T. H., Rahayu, S., Nathania, I. R. B. and Sukmawati, D. (2021). Antibacterial Activity of Lactic Acid Bacteria Isolated from Oncom, a Traditional Indonesian Fermented Food. AIP Conference Proceedings 2331.

[36] Sari, M., Suryanto, D. and Yurnaliza. (2018), Antimicrobial activity of lactic acid bacteria isolated from bekasam against *Staphylococcus aureus* ATCC 25923, Escherichia coli ATCC 25922, and Salmonella sp. IOP Conf. Series: Earth and Environmental Science, 130:1-7.

[37] Gu, S. B., Zhao, L. N., Wu, Y., Li, S. C., Sun, J. R., Huang, J. F., and Li, D. D. (2015). Potential probiotic attributes of a new strain of Bacillus coagulans CGMCC 9951 isolated from healthy piglet feces. World journal of microbiology and biotechnology, 31(6), 851–863.

[38] Kaskokiene, V., Stankevičius, M., Bimbiraitė-Survilienė, K., Naujokaitytė, G., Šernienė, L., Mulkytė, K., Malakauskas, M., and Maruška, A. (2017). Current state of purification, isolation and analysis of bacteriocins produced by lactic acid bacteria. Applied microbiology and biotechnology, 101(4), 1323–1335.