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ZMS designed the experiment. AAA and AYB supervised and approved the experiment. ZMS UBI, AYF and AIB performed the experiment. ZMS wrote the paper. MGS, RSD, MRJ and MM revised the paper. All authors read and approved the final version of the manuscript.

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Preservative Potential of Partially Purified Bacteriocin Produced by *Lactobacillus helveticus* in the Preservation of Tigernut Drink

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Abstract:

Lactic acid bacteria (LAB) produce bacteriocins as one of its primary products of fermentation and they are generally regarded as safe. The research was aimed to test the biopreservative potential of partially purified bacteriocin produce by *Lactobacillus helveticus.* A total of 20 samples of fermented cow milk were obtained from Wamakko Local Government, Sokoto. de Man, Rogosa and Sharpe (MRS) agar was used to isolate the Lactic acid bacteria and they are identified using Gram-staining, biochemical tests and further confirmed using Polymerase chain reaction (PCR) and Sanger sequencing methods. Agar well diffusion assay was used to determine the bacteriocin production of the identified LAB and the optimization of the bacteriocin activity at different pH and temperature. Biopresevative potential of bacteriocin in *kunun aya* was accessed by adding partially purified bacteriocin from *Lactobacillus helveticus* to the *kunun aya* and observed for six days. The LAB isolates identified were *L. Plantarum* (3), *Pediococcus* (4) and *L.casei* (1). The molecular method confirmed 2 species of LAB as *Lactobacillus plantarum* (2) and *Lactobacillus helveticus* (1). Screening for bacteriocin production shows that 2 strains of *L. plantarum* and strain of 1 *L.casei* have the potential to produce bacteriocin. The bacteriocin is active at different pH (2-10) and temperature 30, 50, 70, 90 and 100°C respectively. The assessment of bio-preservation in *kunun aya* shows that the shelf life of *kunun aya* was extended at 4°C for five days by addition of D5 (*Lactobacillus helveticus*) bacteriocin. Isolation of LAB in this fermented food implies that it contains potentially bacteriocin producing LAB which inhibit some food pathogens by extending the shelf life of the *kunun aya*.

Keywords: *Lactobacillus helveticus,* Biopreservative, Bacteriocin, Wamakko.

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INTRODUCTION

Traditional fermentation has long been used to preserve foods, while also improving their digestibility and increasing nutritional value through the fermentative actions of naturally occurring microorganisms, particularly lactic acid bacteria (LAB) (Kårlund *et al.,* 2020). LAB produces metabolites during fermentation including organic acid, bacteriocin, exopolysaccrides, amino acids and vitamins which increase development of taste in food and prevent spoilage, making them very helpful in a variety of applications, particularly dairy and food industries. The dairy industry benefits greatly from their metabolic activities. They predominantly produce lactic acid and other compounds like bacteriocins which possess antimicrobial activity against other microorganisms (Darbandi *et al.,* 2022). The antimicrobial activity of bacteriocins produced by LAB has been reported in foods like dairy products, meats, barley, sourdough, red wine and fermented vegetables (Joshi *et al.,* 2006).

Bacteriocin is a protein or peptide produced by bacteria that kills or inhibit the growth of other bacteria usually closely related species bacteriocins synthesized by lactic acid bacteria have been shown to suppress the growth of food spoilage organisms and pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, and *Clostridium perfringens* (Mokoena *et al.,* 2021). *Lactobacillus helveticus* is a Gram-positive, catalase-negative, non-spore-forming, rod-shaped bacterium that was previously known as *Thermobacterium helveticus Lb. helveticus* is mainly isolated from dairy sources such as cheeses and sour milks. *Lactobacillus helveticus* is used as a starter culture primarily in cheese manufactured at elevated cooking temperatures, such as hard and extra hard Italian cheese. In the majority of cases, it constitutes the primary component of natural whey starters. In addition to its role as a starter, it contributes to flavor development. In recent years, *Lb. helveticus* has been increasingly used as a flavor adjunct in cheeses such as Cheddar, where thermophilic *lactobacilli* are not traditionally included (Chelladhurai *et al.,* 2023). The investigation was aimed to test the biopreservative potential of partially purified bacteriocin produce by *Lactobacillus helveticus.*

MATERIALS AND METHODS

Collection of samples

Fermented cow milk was obtained from different hawkers at the markets of the selected areas (Arkilla, Dundaye, Kalambaina and Gidan Kara). A total of twenty samples were collected, five samples were collected from each area and packed in 50ml sterile bottles and placed in a sterile ice container. The samples were immediately transported to the laboratory in the Department of Microbiology, Usmanu Danfodiyo University Sokoto, Nigeria (UDUS).

Isolation and characterization of LAB

One milliliter (1ml) of fermented cow milk was mixed with nine (9ml) of peptone water to obtain a stock homogenate. One millimeter from the stock homogenate was transferred into sterile test tubes containing 9ml of sterile peptone water, and it was serially diluted to 10^{-3}, using a micropippet 0.1ml of the sample from 10^{-3} dilution factor were spread evenly on the MRS media and incubated at 30°C for 72 hours. Colonies were subcultured on Mann Rogosa and Sharp agar to obtain a pure culture. The pure isolates were stored in Mann Rogosa and Sharpe agar slant at 4°C in a refrigerator for further study. The fermentation ability of the isolated organisms was examined in different sugars. The activity of catalase enzyme was examined by adding the colonies of isolate with 3% hydrogen peroxide (H_2O_2) and the oxidase test was accomplished by using 1% solution of tetramethyl-p-phenylenediamine hydrochloride. According to biochemical test results, all the isolates belonged to *Lactobacillus spp*. in accordance with Bergey's Manual of Determinative Bacteriology (Bergey, 1994).

The LAB were characterized based on cell morphology, biochemical tests (Oyeleke and Manga, 2008), screening of the LAB isolates and molecular method such as PCR and Sangers sequencing.

Screening for LAB with potential antibacterial activity

Test bacteria

Pathogenic bacteria (*Staphylococcus aureus, Clostridium botulinum, Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes* and *Salmonella typhimurium* are collected at the Microbiology research laboratory UDUS. They are identified using biochemical test and there were subcultured on their respective selective media.

Fermentation

The LAB isolate was cultured in MRS broth and incubated at 37°C for 48 hours. Following incubation, the broth culture was centrifuged at 5,000 rpm for 10 minutes. The cells were pelleted, and the resulting supernatant was collected and used as crude bacteriocin.

Antibacterial activity of fermented broth

The bacterial isolate was spread on the entire agar surface then a hole with the diameter of 6mm was punched aseptically with a sterile cork borer, and a volume of 150µl of the cell free supernatant from the samples are introduced into the wells bored with corkborer. The plates were allowed to set for 1 hour for the antibacteria to diffuse. The plates were incubated at 37°C for 48 hours. After incubation the zone of inhibition were measured in millimeters and recorded (Ali *et al.,* 2016).

Molecular identification of Bacteriocins producing isolate

Polymerase chain reaction (PCR) was used for molecular identification. The isolates that show the antibacterial activity were selected for molecular confirmation.

DNA extraction

Colonies of overnight growth bacteria that shows zone of inhibition were used. The The colonies were transferred into a test tube containing 1 mL of distilled water and heated in a water bath for 10 minutes., they were centrifuged at 1000 rpm for five minutes. Five microliters of the supernatant was used for the PCR (Dashti *et al.,* 2009).

Amplification of extracted DNA

The 16S rRNA gene was amplified using the universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG3') and 1492R (5'TACGGTTACCTTGTTACGACTT-3'). PCR was carried out in final reaction volume of 25µl containing 10mg of extracted DNA, 0.4 µmol/L of each primer, 0.2 µmol/L dNTP, 2µmol/L $MgCl_2$, 2.5µl PCR reaction buffer and 1 U of Taq DNA polymerase. The thermal reaction condition was initial denaturation at 94°C for 5min, 40 cycles of denaturation at 94°C for 1 minute, annealing at 53°C for 30 second, extension at 72°C for 90 second followed by final extension step at 72°C for 5 minutes.

Agarose gel electrophoresis of PCR amplification

PCR products were analyzed by electrophoresis on a 1% agarose gel 1x TBE buffer, run at 100V for 45 minutes. A stock solution of 50x of TEA buffer in 1000ml of distilled water and a sufficient electrophoresis was prepared to cast gel and preparation of agarose was made. The sample DNA with 0.20 volumes of the desired 6x gel was slowly loaded using a disposable micropipette. The gel was stained with ethidium bromide at 1 µg/mL concentration and photographed using a gel documentation machine (Pace, 1997).

Sanger sequencing

The extracted purified PCR fragments were sequenced in the forward and reverse direction (Nimagen, Brilliant dye Terminator cycle sequencing kit V3.1, BRD3-100/1000) and purified (Zymo Research ZR-96 DNA

Sequencing clean up kit, catalogue No D4050). The purified fragments were analysed on the ABI 3500xl genetic analyser (applied Biosystems, Thermofisher scientific) for each reaction for every sample. Bio-edith sequence alignment editor version 7.2.5 was used to analyse. At the end of the reaction, the tubes were briefly centrifuged, and samples loaded onto the ABI 3500xl gene sequencer. The results were analyzed using MEGA software (version 6.0) and BLAST (NCBI), and the evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.96694155 was obtained. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per s ite. The analysis involved 10 nucleotide sequences. Evolutionary analyses were conducted in MEGA6.

Extraction and partial purification of Bacteriocins

The supernatant was obtained by centrifuging at 5000rpm for 10 minutes it was used as crude Bacteriocins. The crude extract was precipitated with ammonium sulphate (40% saturation). The mixture was stirred for 2 hours using a magnetic stirrer and then centrifuged at 20,000rpm for 1 hour at 4°C. The precipitate was suspended again in 25ml of 0.05M potassium phosphate buffer (pH 7.0). Catalase (5mg) was added and the new precipitate was filtered and stored at 4°C for further use (Aly *et al.,* 2004).

Effect of pH on Bacteriocins Activity

Cell free supernatants of the LAB isolate was heated at different pH values (2.0- 7.0). The pH of samples is adjusted to pH 2, 3, 4, 5 and 6 with 0.1M hydrochloric acid (HCl) and pH 8, 9 and 10 with 1M Sodium hydroxide (NaOH) and subsequently incubated at ambient temperature for 1 hour, the activity of the sample against test organisms were monitored by agar well diffusion method by streaking the pathogenic organisms on Mueller Hinton agar, cell free supernatant of the isolate was poured into the holes bored with cork borer. The plates are allowed to set for the antibacterial to diffuse and then incubated at 37°C for 48 hours after which the plates are examined for clear zones of inhibition. The activity of the sample at pH 7.0 will be used as control (Okpara *et al.,* 2014).

Effect of temperature on Bacteriocins activity

The supernatants of the isolate were heated at different temperatures ranging from 40 to 80°C for 30 minutes and 100°C for 15 minutes, following heat treatment samples were cooled to ambient temperatures. Afterwards, the activities of the heat-treated samples were monitored by agar well diffusion method (Ali *et al.,* 2016).

Bio-preservation study of Tiger-nut drink

Preparation of Tiger-nut drink

Tiger-nut drink is traditionally prepared by thoroughly washing the tiger nuts to remove soil and dirt. After washing, the nuts are soaked for approximately 4–8 hours, after soaking, the nuts are ground together with coconut and date fruit to form a mash. During processing, cold water is added at a ratio of 3 liters of water to 1 kilogram of tiger nuts, after which the mixture is sieved. A measured quantity of sugar is then added based on the volume obtained.

Total viable count (TVC) of Tiger-nut drink

Tiger-nut drink sample (1ml) was mixed with 9ml of peptone water in a sterile beaker to obtain a stock homogenate. One millimeter of the sample from the stock culture was aseptically transferred into test tubes containing 9ml of peptone water, it was serially diluted to 10^{-6}, 0.1ml from 10^{-2} 10^{-4} 10^{-6} spread on Plate Count Agar (PCA) in duplicate then incubated aerobically at 37°C for 24 hours while for anaerobic counts, plates were incubated anaerobically at 37°C for 48 hours using a candle jar. Colonies were counted using a colony counter and results were recorded (Adamu, 2016).

Potential of BLAB Bacteriocins producing LAB to extend the shelf life of Tiger-nut drink

Partially purified bacteriocin was dissolve with 5ml deionize water, 1ml of bacteriocins was added to 19ml of the tiger-nut drink and the test tubes were covered with cotton wool and aluminum foil. The samples refrigerated in a refrigerator at 4°C and they were removed at 24hours interval to check for total viable count by spreading on plate count agar, tiger-nut drink without bacteriocins served as control (Ali *et al.,* 2016).

Statistical analysis

Data obtained from the experiments were analyzed descriptively, and the results were presented in tables and figures.

RESULTS

Biochemical characterization of the isolates

The biochemical test results shows that 8 LAB were isolated and they were phenotypically identified as *L. Plantarum* (3)*, Pediococcus* (4) and *L.casei* (1).

Antibacterial activity of the cell free supernatant of the LAB producing bacteriocin against test bacteria

The cell free supernatant of *L. plantarum* [sample number 3 collected from Dundaye (D3)] has the highest zone of inhibition on *Listeria monocytogenes* with (39mm) and the lowest zone of inhibition was observed on *P. aeruginosa* with 16mm by *L. plantarum* [sample number 2 collected from Wamakko (W2)], *Pediococcus* [sample number 5 collected from Dundaye (D5)] inhibit all the bacteria except *E. coli* while *L. plantarum* (W2) only inhibit the growth of *P. aeruginosa* and *S. aureus* with (16mm and 17mm) clear zone of inhibition respectively no zone of inhibition on *S. typhimurium, E. coli*, *L. monocytogenes* and *C. botulinum* (Table 1).

Table 1. Antibacterial Activity of the cell free supernatant of the bactriocin producing LAB against test bacteria.

Bacteria	Zone of inhibition (mm)				
	D3(150µl)	D5(150µl)	W2(150µl)	Positive control(5µg/ml of oflaxacin)	Negative control (MRS broth)
Pseudomonas aeruginosa	35	26	16	30	0
Staphylococcus aureus	27	23	17	33	0
Escherichia coli	0	0	0	18	0
Salmonella typhimurium	36	32	0	30	0
Listeria monocytogenes	39	31	0	36	0
Clostridium botulinum	0	31	0	26	0

Key: D3: sample number 3 collected from Dundaye, D5 is sample number 5 collected from Dundaye, W2 is sample number 2 collected from Wammakko.

Molecular identification of Bacteriocin producing LAB

Using conventional PCR 16s-r rDNA target region was amplified and sequenced; the obtained sequenced were entered into the Basic local alignment tool BLAST in the national center for biotechnology information NCBI data to compare with known organism. The isolates were identified as *Lactobacillus helveticus* (1) and *Lactobacillus plantarum* (2) (Figures 1 and 2).

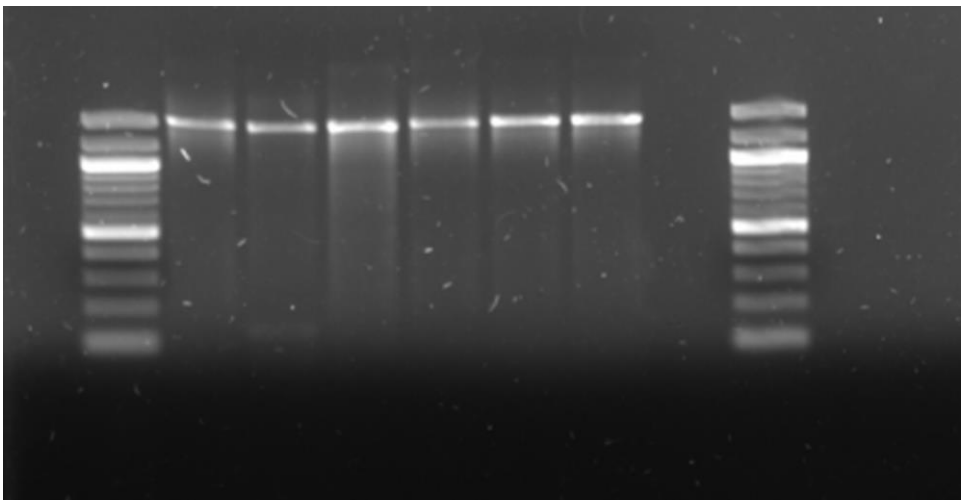

Fig. 1. Agarose Gel Image Showing 16S amplicons 1t 1465-bp.

Keys: M= Ladder, NC=Negative Control

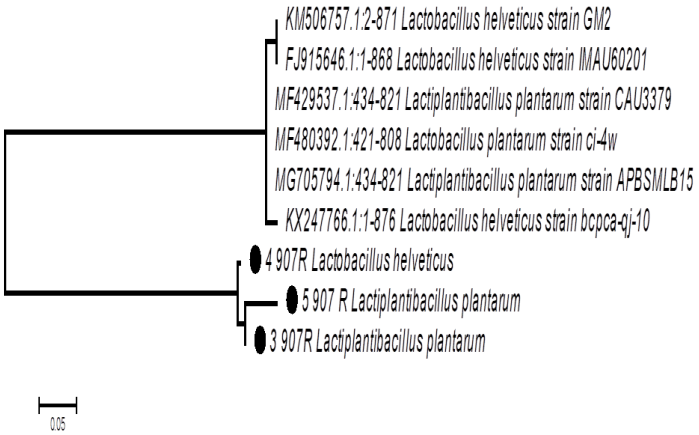

Fig. 2. Evolutionary relationships of bacteriocin producing LAB isolated genera with a known species from NCBI gene Bank.

The phylogenetic history was reconstructed using the Maximum Likelihood approach applied to the sequence data (Tamura and Nei, 1993). The tree exhibiting the highest log likelihood (-208.9809) is shown. The initial tree(s) for the heuristic search were automatically generated using the Maximum Composite Likelihood (MCL) method. The topology with the highest log likelihood value was then selected. The tree is drawn to scale, with branch lengths representing the number of substitutions per site The analysis involved 9 nucleotide sequences. All positions with gaps or missing data were removed. A total of 73 positions were included in the final dataset (Tamura *et al.,* 2013).

Effect of temperature on Bacteriocin activity

Figure 3 showed D5 bacteriocin are also active after heating at different temperature range, with the highest activity notice on *Listeria monocytogens* at 70 °C and *Pseudomonas aeruginosa* has the least activity at 30 °C.

Effect of pH on Bacteriocin activity

Figure 4 showed that D5 bacteriocin is active at all the pH values and on all the test organisms except *Escherichia coli* with the highest activity noted at pH 7 on *S. typhimurium.*

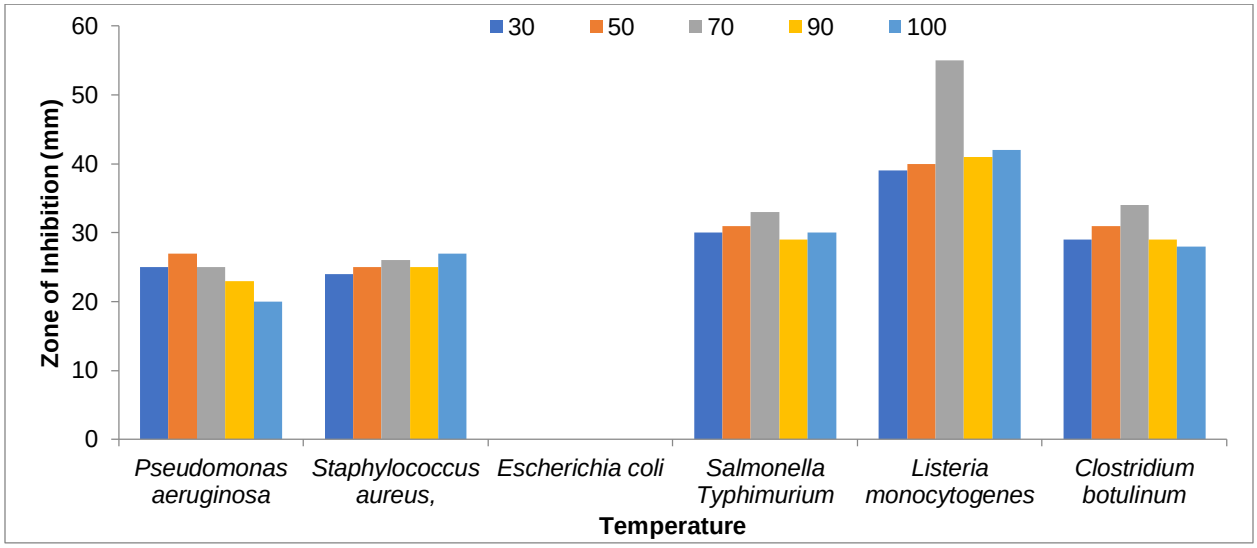

Fig. 3. Effect of Temperature on D5 Bacteriocin activity.

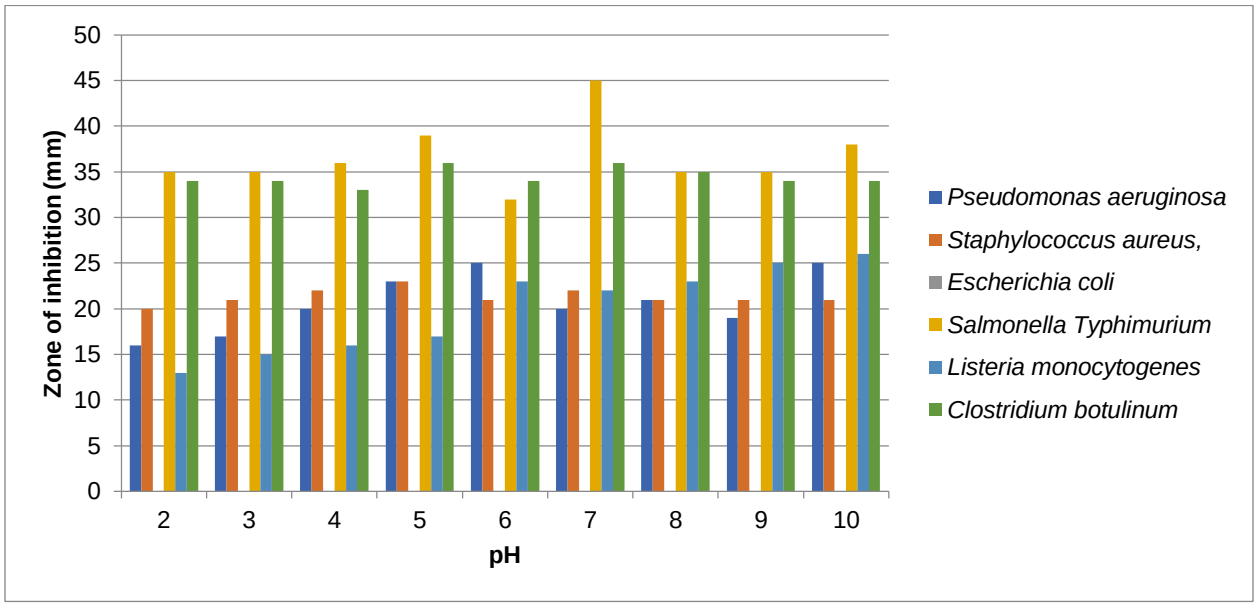

Fig. 4. Effect of pH on D5 Bacteriocin activity.

Potential of Bacteriocins Producing LAB to Extend the Shelf Life of Tiger-nut Drink

Bacterial load of *Kununaya* sample inoculated with bacteriocins (Figure 5). The shelf life of *Kununaya* (control, *Kununaya* without bacteriocin) stored at refrigeration tempreture of 4 °C was 2 days, while the shelf life of the *Kununaya* sample inoculated with D5 stored at 4 °C the shelf life was extended for 5 days while spoilage occurred on the sixth day with 305 colonies.

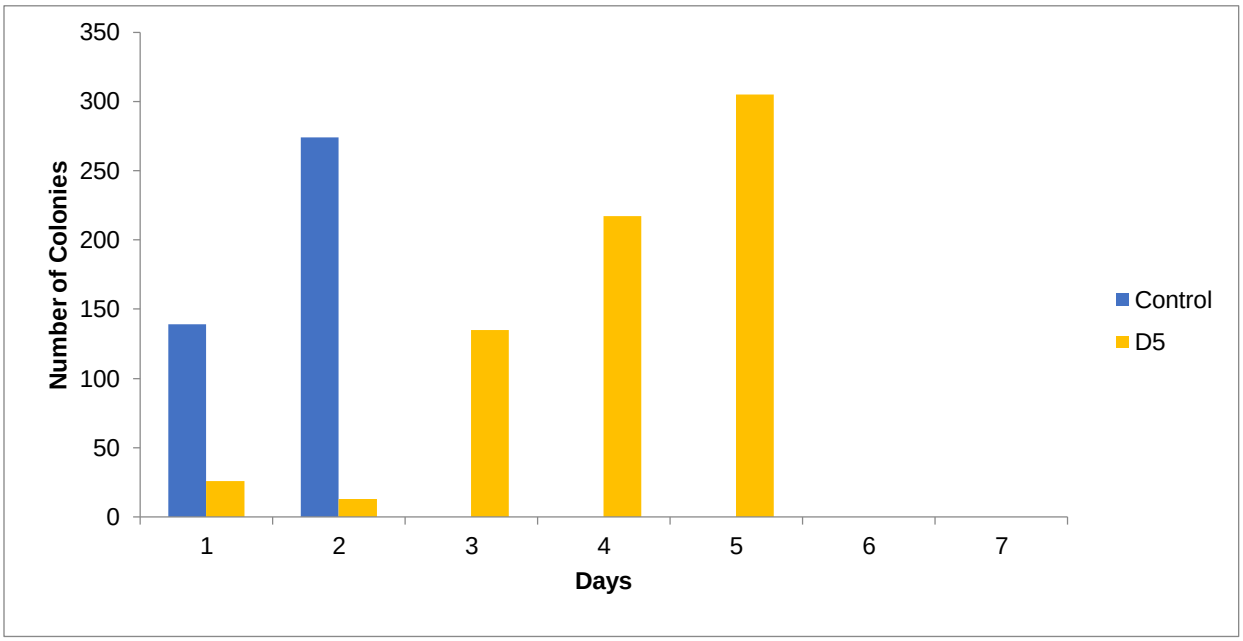

Fig. 5. Bacterial Load of *Kununaya* Sample Inoculated with Bacteriocins.

DISCUSSION

Lactic acid bacteria are naturally present in all fermented foods like cereal, milk, vegetables, meat, and fish they are also natural inhabitants of dairy products like yoghurt and cheese (Bintsis, 2018; Iqbal *et al.,* 2019; Ashaolu and Reale, 2020).

From the 20 samples collected 8 LAB are isolated and these were tested for antimicrobial effect but only 3 showed zone of inhibition against test bacteria. Formation of different clear zones of inhibition on the test bacteria surrounding the wells by agar well diffusion assay may be due to the production of various antimicrobial compounds like bacteriocins. This agreed with previous report by Labiou et al. (2005) that demonstrated an inhibitory effect against the bacteria indicates the possession of antibacterial activity, such as that of bacteriocins. However, the zone of inhibition observed in this work range from 16-39mm, it was greater than that observed by Babatunde et al. (2014), who observed a zone of inhibition ranging from 9-14mm. This may be due to high concentration of bacteriocin in the supernatant, the specific activity and potency of the bacteriocin can also influence the size of the inhibition zone. The highest zone of inhibition of 39mm was observed on *Listeria monocytogenes* by D3 bacteriocin. Strongest activity observed on *L.monocytogene* by D3 bacteriocin showed a broad inhibitory effect on food spoilage bacteria except *Escherichia coli* and *Clostridium botulinium*. Out of the 8 LAB, 3 exhibited bacteriocin production.

Using 16S rRNA gene sequencing, 2 strains of *Lactobacillus plantarum* and 1 *Lactobacillus helveticus* were identified. Similarly, von Mollendorff et al. (2006) observed that bacterial metabolites of *L.plantarum* displayed broad-spectrum inhibitory activity against various pathogens, such as *Salmonella typhymurium, Escherichia coli*, *Clostridium perferinges* and other Gram-positive bacteria such as *Bacillus cereus, Staphylococcus aureus, Streptococcus pneumonia* and *Enterococcus faecum.* Moghadam et al. (2010) also reported that metabolites of *L. plantarum* had broad inhibitory spectrum against *Escherichia coli, Listeria monocytogenes, S. Typhymurium* and Vancomycin resistant enterococci (VRE) due to

the presence of 2 classes of bacteriocins, plantaricinEf and plantaricin W. This might be due to the class of bacteriocin it belongs or probably due to the activity of Plantaricin synthesized by the organism. Many studies have demonstrated that antimicrobial substances from *Lactobacillus* spp can combat *Salmonella* and other harmful pathogens involved in animal health disorders and food poisoning.

Bacteriocin was stable at different pH range (pH 2.0 to 10.0) at room temperature. Similar observations on pH stability of bacteriocin have been reported by Hartnett et al. (2002) and Shehane and Sizemore (2002). The stability of these compounds across a range of pH values is significant, as it is likely to improve the safety and preservation of such foods. Stability of LAB at low pH ranges justifies its use in fermented foods like *Kununzaki*, yoghurt, *Nono*, *Wara* and locust bean. Most of the fermented foods are acidic, and acidity inhibits the growth of pathogenic organism thereby enhancing safety of fermented foods. The inhibitory compound is considerably heat stable surviving treatment at 100°C for 15 min. Thermostability of bacteriocins have been reported earlier and are attributed to their small molecular sizes (Grinstead and Barefoot, 1992; Shehane and Sizemore, 2002). The heat stability of bacteriocins is a key factor in their application within food systems, particularly when they are used in combination with a multiple-hurdle approach to food preservation however, the tolerance of bacteriocins to heat may be dependent on factors such as the level of purification, pH and other protective components (Grinstead and Barefoot, 1992) and these must be considered in designing their applications for in food. From this study it indicates that the bacteriocin are active at different temperature range. Low, moderate and high temperature tolerance by the LAB isolates indicates that foods that harbour Lactic acid bacteria can be used as refrigerated or frozen foods or high temperature or even at room temperature in order to prevent the growth of pathogenic and spoilage psychrotophic organisms.

The isolation of lactic acid bacteria in this fermented food implies that they contain potentially bacteriocin producing Lactic acid bacteria which inhibit some food pathogens thereby making the beverage suitable for consumption. Similar result was reported by Babatunde et al. (2014) and Hassan et al. (2020). Lactic acid bacteria have a long history of use in a variety of cereal fermentation with predominance of *Lactobacillus plantarum* which had earlier been reported in Nigerian Ogi or pap a spontaneous fermented maize product (Oranusi *et al.,* 2003) and traditional Pakistani yoghurt in Pakistan (Hassan *et al.,* 2020)

Bacteriocin producing *Lactobacillus helveticus* and *Lactiplantibacillus plantarum* in fermented milk (*nono*) is an indication that this strain is present in various fermented foods and are being selected for application in the preservation of food. Thermal stability observed by the bacteriocin produced by *L. helveticus* likely belongs to the Class II bacteriocin family.

The extension of the shelf life of *Kununaya* and the difference observed in the shelf-life extension days of *Kununaya* sample might be due to the presence of bacteriocin. The shelf-life extension of *Kununaya* sample inoculated with D5 bacteriocin might be as a result of bacteriocin that was inoculated and *Lactobacillus helveticus* had the potential for the production of bacteriocin.

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CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest related to this work.

REFERENCES

Adamu, A.G., 2016. Biopreservation of milk using Bacteriocins producing lactic acid bacteria isolated from Kunun Zaki in Zaria, Kaduna State, Nigeria. Ahmadu Bello University, Zaria, Nigeria. (Doctoral dissertation).

Ali, M.S., Begum, A., Uddin, M.F., Ahmed, S., 2016. Biopreservative effect of bacteriocin in fruit juice preservation. Asian-Australas. J. Biosci. Biotechnol., 1(3): 435-440.

Aly,S., Cheik, A.T.,Quattara, C., Bassole, I., Traore, A., 2004. Antimicrobial activities of Lactic acid bacteria strains isolated from Burkina Faso fermented milk. Pak. J. Nutr., 3(3): 174-179.

Ashaolu, T.J., Reale, A., 2020. A holistic review on Euro-Asian lactic acid bacteria fermented cereals and vegetables. Microorganisms, 8(8): 1176. https://doi.org/10.3390/microorganisms8081176

Babatunde, D.A., Oladejo, P.O., Murtala, A.A., Christiana, J., 2014. Identification of Lactic acid bacteria isolated from selected Nigerian Foods and comparism of their Bacteriocin activities. Int. J. Pharm. Clin. Res., 6(1): 20-26.

Bergey, D.H., 1994. *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.

Bintsis, T., 2018. Lactic acid bacteria: their applications in foods. J. Bacteriol. Mycol., 6(2): 89-94.

Chelladhurai, K., Ayyash, M., Turner, M.S., Kamal-Eldin, A., 2023. Lactobacillus helveticus: Health effects, current applications, and future trends in dairy fermentation. Trends Food Sci. Technol., 136: 159-168.

Darbandi, A., Asadi, A., Mahdizade Ari, M., Ohadi, E., Talebi, M., Halaj Zadeh, M., Kakanj, M., 2022. Bacteriocins:properties and potential use as antimicrobials. J. Clin. Lab. Anal., 36(1): e24093. https://doi.org/10.1002/jcla.24093

Dashti, A.A., Jadaon, M.M., Abdulsamad, A.M., Dashti, H.M., 2009. Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques. Kuwait Med. J., 41(2): 117-122.

Grinstead, D.A., Barefoot, S.F., 1992. Jenseniin G, a heat-stable bacteriocin produced by *Propionibacterium jensenii* P126. Appl. Environ. Microbiol., 58(1): 215-20.

Hartnett, D.J., Vaughan, A., Van Sinderen, D., 2002. Anti-microbial producing lactic acid bacteria isolated from raw Barley and sorghum. J. Inst. Brew., 108(2): 169-177.

Hassan, M.U., Nayab, H., Rehman, T.U., Williamson, M.P., Haq, K.U., Shafi, N., Shafique, F., 2020. Characterisation of bacteriocins produced by *Lactobacillus* spp. isolated from the traditional Pakistani yoghurt and their antimicrobial activity against common foodborne pathogens. BioMed Res. Int., 2020(1): 8281623. https://doi.org/10.1155/2020/8281623

Iqbal, M.N., Ashraf, A., Iqbal, I., 2019. Lactic Acid Bacteria from Dairy Products: Probiotic Potential and Biotherapeutic Effects. PSM Microbiol., 4(4): 88-90.

Joshi, V.K., Sharma, S., Rana, N.S., 2006. Production, purification, stability and efficacy of bacteriocin from isolates of natural lactic acid fermentation of vegetables. Food Technol. Biotechnol., 44(3): 435-439.

Kårlund, A., Gómez-Gallego, C., Korhonen, J., Palo-Oja, O.M., El-Nezami, H., Kolehmainen, M., 2020. Harnessing microbes for sustainable development: Food fermentation as a tool for improving the nutritional quality of alternative protein sources. Nutr., 12(4): 1-26.

Labio, E.D., Del Rosario, D.B., Strasser, S.I., McCaughan, G.W., Crawford, B.A., 2007. Effect of ascites on bone density measurement in cirrhosis. J. Clin. Densitom., 10(4): 391-4.

Moghadam, M.S., Foo, H.L., Leow, T.C., Rahim, R.A., Loh, T.C., 2010. Novel

bacteriocinogenic *Lactobacillus plantarum* strains and their differentiation by sequence analysis of 16S rDNA, 16S-23S and 23S-5S intergenic spacer regions and randomly amplified polymorphic DNA analysis. Food Technol. Biotechnol., 48(4): 476-483.

Mokoena, M.P., Omatola, C.A., Olaniran, A.O., 2021. Applications of lactic acid bacteria and their bacteriocins against food spoilage microorganisms and foodborne pathogens. Molecules., 26(22): 1-13.

Okpara, A.N., Okolo, B.N., Ugwuanyi, J.O., 2014. Antimicrobial activity of Lactic acid bacteria isolated form akamu and kununzaki (cereal based nonalcoholic beverages) in Nigeria. Afr. J. Biotechnol., 13(24): 2977-2984.

Oranusi, S.U., Umoh, V.J., Kwaga, J.K.P., 2003. Hazards and critical control points of kunun-zaki, a non-alcoholic beverage in Northern Nigeria. Food Microbiol., 20(1): 127-132.

Oyeleke, S.B., Manga, S.B., 2008. Essentials of laboratory practicals in microbiology. Minna, Nigeria: Tobest Publishers. 36-75.

Pace, N.R., 1997. A molecular view of microbial diversity and the biosphere. Science, 276(5313): 734-740.

Shehane, S.D., Sizemore, R.K., 2002. Isolation and preliminary characterization of bacteriocins produced by *Vibrio vulnificus*. J. Appl. Microbiol., 92: 322-328.

Tamura K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512-526.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30(12): 2725-9.

von Mollendorff, J.W., Todorov, S.D., Dicks, L.M., 2006. Comparison of bacteriocins produced by lactic-acid bacteria isolated from boza, a cereal-based fermented beverage from the Balkan Peninsula. Curr. Microbiol., 53(3): 209-16.