Data in Brief 52 (2024) 110021

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Dataset of 16S ribosomal DNA sequence-based identification of bacteriocinogenic lactic acid bacteria isolated from fermented food samples

Emmanuel Edoghogho Imade[a], Solomon Esharegoma Omonigho[a], Olubukola Oluranti Babalola[b,*], Ben Jesuorsemwen Enagbonma[b,c], Ozede Nicholas Igiehon[a], Abraham Goodness Ogofure[a]

[a] *Department of Microbiology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154, Benin City, Edo State, Nigeria*

[b] *Food Security and Safety Focus Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho 2735, South Africa*

[c] *Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154, Benin City, Edo State, Nigeria*

ARTICLE INFO

Article history:
Received 27 November 2023
Revised 4 December 2023
Accepted 27 December 2023
Available online 30 December 2023

Dataset link: Limosilactobacillus pontis strain EOINONO (Original data)
Dataset link: Limosilactobacillus pontis strain OGENONO (Original data)
Dataset link: Limosilactobacillus pontis strain OGENONO (Original data)
Dataset link: Limosilactobacillus pontis strain EEIKUNU (Original data)
Dataset link: Lactiplantibacillus plantarum strain MJIKUNU (Original data)

ABSTRACT

The dataset profiled in this research is built on sequencing of lactic acid bacteria 16S rDNA mined from Nono (N_4 and N_5), Kunu (K_4 and K_1) and Garri. The 16S rDNA sequences files are accessible under the data identification numbers: OK017047, OK017046, OK017044, OK017043, OK017045 at the GenBank database, NCBI. Taxonomic identification and phylogenetic tree analysis were done using the online BLAST (blastn) and MEGA11 software, respectively. The effect of the bacteriocin produced by these organisms on spoilage bacteria associated with salad was evaluated using an agar well diffusion assay. Limosilactobacillus pontis strain EOINONO, Limosilactobacillus pontis strain OGENONO, Limosilactobacillus pontis strain SEOGARI, Lactiplantibacillus plantarum strain MJIKUNU and Limosilactobacillus pontis strain EEIKUNU were the identified bacteriocinogenic organisms while Bacillus tequilensis strain SEOABACHA, Bacillus tequilensis strain EEIABACHA,

* Corresponding author.
E-mail address: olubukola.babalola@nwu.ac.za (O.O. Babalola).
Social media: @EmmanuelIm27805 (E.E. Imade)

https://doi.org/10.1016/j.dib.2023.110021

Keywords:
Abacha
Antimicrobial peptides
Food preservation
Lactic acid bacteria
Taxonomic identification

Achromobacter xylosoxidans strain IMABACHA and Achromobacter insolitus strain MJIABACHA were the identified spoilage organisms.

Specifications Table

Subject	Microbiology
Specific subject area	Exploring the antimicrobial potential of lactic acid bacterial bacteriocin against food spoilage organisms associated with African salad
Data format	Raw and analyzed
Type of data	Table, Chart and Figure
Data collection	Lactic acid bacteria (LAB) were obtained from fermented food samples (*Garri*, *Kunu*, and *Nono*), while spoilage microorganisms were obtained from stale African salad. The isolation of LAB involved the inoculation of 0.1 ml of serially diluted fermented food samples onto De Mann, Rogosa, and Sharpe agar (MRS) using the pour plate technique. Subsequently, the cultures were incubated under anaerobic conditions at 37°C for 48 - 72 hours. In a similar fashion, 0.1 ml of serially diluted stale African salad sample was inoculated into nutrient agar supplemented with nystatin. After gently swirling the plates, they were allowed to solidify at room temperature. Triplicate plates were prepared and subsequently incubated at 37°C for 24 - 48 hours.
Data source location	Institution: Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
Data accessibility	Repository name: GenBank database, NCBI Data identification numbers: OK017047, OK017046, OK017044, OK017043, OK017045 Direct URL to data: https://www.ncbi.nlm.nih.gov/nuccore/OK017047 https://www.ncbi.nlm.nih.gov/nuccore/OK017046.1/ https://www.ncbi.nlm.nih.gov/nuccore/OK017044 https://www.ncbi.nlm.nih.gov/nuccore/OK017043 https://www.ncbi.nlm.nih.gov/nuccore/OK017045

1. Value of the Data

- Lately, there has been a shift from the use of chemical preservatives or therapeutic agents to natural inhibitory substances such as bacteriocin to curb the menace of bacterial infection and food spoilage.
- Bacteriocin has been chosen as a preferred antibacterial candidate to replace chemicals and antibiotics in the future because of its proteinaceous nature and relatively lower toxicity.
- On evaluating the potential of the isolated LAB to produce bacteriocin that could control food spoilage bacteria associated with Abacha, 4 of them presented remarkable inhibitory ability.
- These LAB are promising candidates for the establishment of active starting cultures with high biopreservation potential for Abacha or other foods.

2. Background

Microbiological food deterioration is caused by the contamination and proliferation of microorganisms which produce enzymes that alter the physicochemical properties of the food and render the food unpalatable [1]. Traditional preservatives like salt, sulfur dioxide, and sugar

have historically combated this issue, enhancing public health and safeguarding the food supply. While beneficial, some common chemical preservatives trigger adverse immunological reactions, disrupting gut microbiota and contributing to metabolic and neurological disorders. Unlike chemical additives, bacteriocins selectively target pathogens, leaving the gut microbiota unharmed and fostering a healthier microbial environment. Lactic acid bacteria (LAB) are the most common group of bacteria that produce bacteriocin. They are abundantly present in the gut as symbiont [2], they are also present in decayed plants and animal matter, fecal substances and in fermented foods such as *Garri, Kunu* and *Nono* [3]. The data was collected to identify the lactic acid bacteria present in *Garri, Kunu* and *N*ono that can produce antimicrobial polypeptides. The antimicrobial potency of the polypeptides was evaluated against spoilage organisms associated with African salad.

3. Data Description

The raw dataset contained 16S rDNA sequences of lactic acid bacteria isolated from Nono, Kunu and Garri. The pH, mean viable LAB counts (cfu/ml) and LAB isolated from the fermented food samples is depicted in Table 1 while the antibacterial efficacy of the LAB bacteriocin obtained from *Kunu* (K_4 and K_1) as well as *Nono* (N_4 and N_5) against the spoilage organisms isolated from *Abacha* is presented in Fig. 1. The phylogenetic tree constructed by the neighbor-joining method showing the position of the bacteriocin producing LAB and related species based on 16S rRNA gene sequences, *Achromobacter xylosoxidans* (BHU-AV1) is represented by Fig. 2 while the phylogenetic tree constructed by the neighbour-joining method showing the position of spoilage bacteria isolated from *Abacha* and related species based on 16S rRNA gene sequences is represented by Fig. 3.

Table 1
pH, mean viable LAB counts (cfu/ml) and LAB isolated from the fermented food samples.

Food Sample	pH	Mean LAB count ($x10^4$ cfu/ml)	Isolated LAB	Accession Numbers
Nono	3.0^a	4.00 ± 0.46^b	*Limosilactobacillus pontis* strain EOINONO and *Limosilactobacillus pontis* strain OGENONO	OK017047 OK017046
Garri	4.6^b	0.09 ± 0.02^a	*Limosilactobacillus pontis* strain SEOGARRI	OK017044
Kunu	3.9^{ab}	3.67 ± 0.74^b	*Lactiplantibacillus plantarum* strain MJIKUNU, *Limosilactobacillus pontis* strain EEIKUNU	OK017043 OK017045

Key: Same alphabets in superscript across column indicates no significant difference ($p > 0.05$).

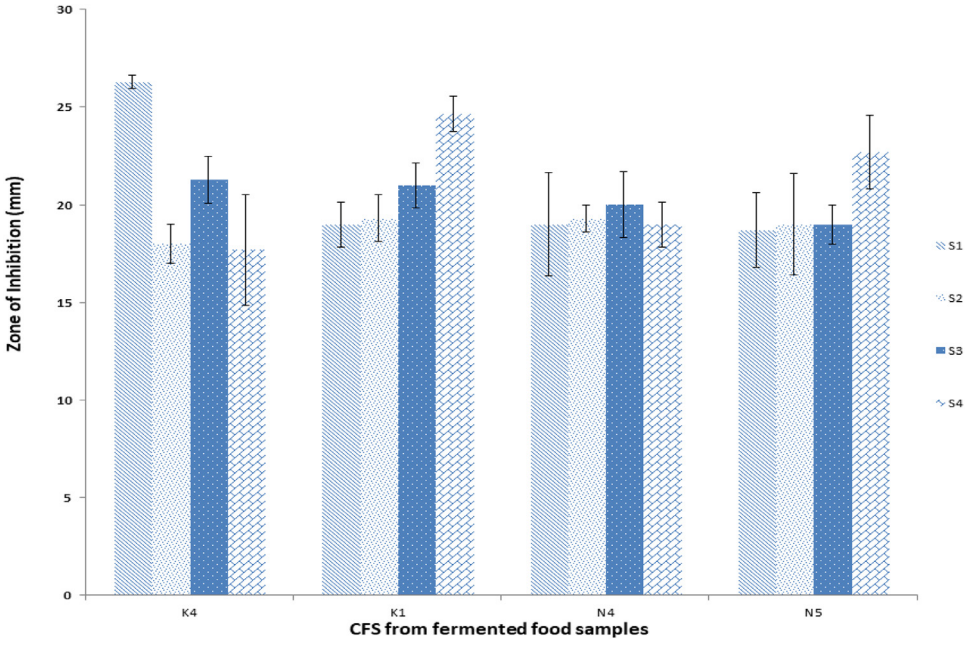

Fig. 1. Agar well diffusion assay using bacteriocin against spoilage organisms associated with salad

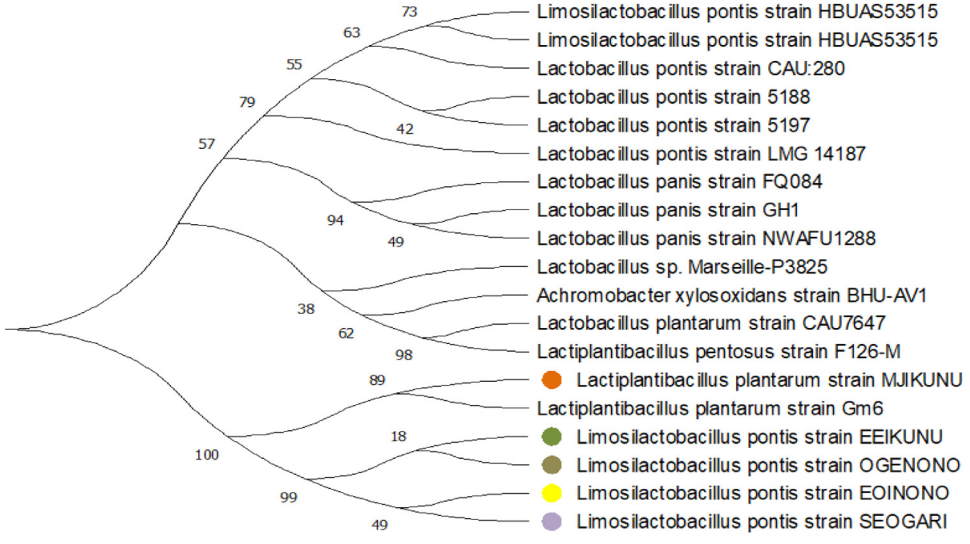

Fig. 2. Phylogenetic tree constructed by the neighbor-joining method showing the position of the bacteriocin producing LAB and related species based on 16S rRNA gene sequences, *Achromobacter xylosoxidans* (BHU-AV1) was used as an outgroup.

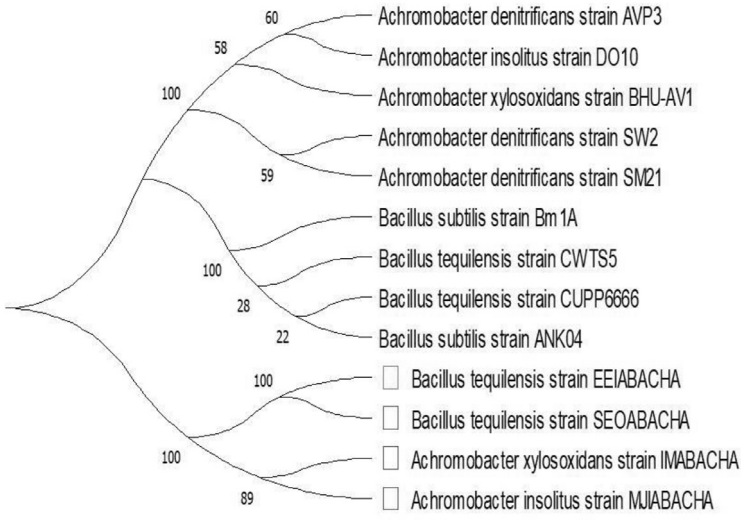

Fig. 3. Phylogenetic tree constructed by the neighbour-joining method showing the position of spoilage bacteria isolated from *Abacha* and related species based on 16S rRNA gene sequences

4. Experimental Design, Materials and Methods

4.1. Isolation and identification of lactic acid bacteria

Fermented food samples, including Garri, Kunu, and Nono, were procured from local vendors specifically for this research. These samples were promptly transported to the laboratory in sterile, airtight containers.

4.2. Determination of the pH of the fermented food

The pH of the fermented food samples (*Garri, Kunu* and *Nono*) was measured with a pH meter (WPA pH Meter, India) after standardization using pH 4, 10 and 7 buffers (BDH England). The standardized pH probe was inserted into a 100 ml beaker containing 50 mls of sample and the values were recorded when the reading of the pH meter was stable. The required 50 ml for liquid samples was measured directly, while 10 g of *Garri* was homogenized in distilled water [4].

4.3. Isolation and characterization of LAB in fermented food samples using cultural methods

The study involved the isolation and characterization of 49 lactic acid bacteria (LAB) from the fermented food samples. A total of 49 replicates were isolated and identified from the fermented food samples. Isolation of LAB was done by inoculating 0.1 ml of serially diluted food samples in De Mann, Rogosa and Sharpe agar (MRS) using pour plate technique while cultures were incubated under anaerobic condition at 37°C for 48–72 h [5]. Distinct colonies in terms of shape, color, and size were selected at random and purified using MRS. Preliminary screening was conducted on the pure colonies using Gram staining, catalase test and microscopic observations. Other biochemical tests, namely: sugar fermentation, citrate, urease, indole and coagulase tests, were performed as delineated by Bergey's manual systematic bacteriology to further

characterize the isolates [4,6]. Gram-positive and catalase negative isolates were routinely propagated in MRS agar supplemented with glycerol (20% v/v) and stored at -20°C. Working cultures were sub-cultured 24 h before use.

4.4. Bacteriocin Assay

Putative bacteriocin producing LAB were cultured in MRS broth for 18 – 24 h at 37°C. For 15 min, the broth cultures were centrifuged at a speed of 10,000 rpm (Hettich EBA 85 Tutthugen centrifuge, made in Germany) to obtain culture extract. The study considered potential confounding factors, such as the presence of organic acids and hydrogen peroxide. The inhibitory effect of organic acid that could be present in the extract/supernatant was eliminated by adjusting the pH to 7.0 with 1M NaOH while the effect of hydrogen peroxide was eliminated by treating the supernatant with Catalase enzyme [35]. Afterwards, the liquid portion was passed through a membrane filtration unit equipped with a millipore filter with a pore size of 0.2 µm to ensure sterilization. The resulting filtrate was then utilized in an agar well diffusion assay. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, were used as test organisms [7].

4.4.1. Preparation of stock solution

To create the stock solution of the spoiled salad, a 10 g sample of the salad was weighed and then homogenized with 90 ml of sterile distilled water using a sterilized laboratory mortar and pestle. Subsequently, a ten-fold serial dilution was performed by transferring 1.0 ml of the stock solution into a test tube containing 9.0 ml of sterile distilled water to achieve a 10^{-1} dilution [8]. Further dilutions were then made to obtain a 10^{-3} dilution.

4.4.2. Enumeration of microorganisms

The pour plate method was used for enumeration. An aliquot of 1.0 ml of each dilution was transferred into sterile Petri dishes. About 18–20 ml of molten nutrient agar at 45°C amended with nystatin to prevent the growth of fungi was poured into the plates containing sample. The plates were swirled gently and allowed to solidify at room temperature. Triplicate plates were plated and incubated at 37°C for 24–48 h. After incubation, the number of discrete colonies were counted and recorded in cfu/g [5].

4.4.3. Characterization and identification of bacterial isolates

Using cultural, morphological, and biochemical features, all bacterial isolates were characterized and identified. Molecular studies were also done on these isolates to identify them, following the protocol described in section 3.8.

4.5. Control of spoilage organisms using LAB bacteriocin

McFarland Standard barium sulphate solution with a turbidity of 0.5 was prepared by mixing 9.95 mL of 1% H_2SO_4 and 0.05 mL 1% $BaCl_2$ [9]. The mixture was shaken properly and aliquoted into a McCartney bottle. A test suspension was prepared by taking inoculum from pure culture of bacteria isolated from spoilt African salad. This was inoculated into sterile distilled water in McCartney bottle, the turbidity of the test suspension was compared with that of the McFarland standard. With the aid of a sterile pipette tip, 0.1 ml of the bacterial suspension was introduced onto the center of the agar, while sterile glass spreader was used to spread the sample evenly over the surface of the agar. The mixture was left undisturbed for a duration of 1 hour. Following this, wells with an approximate diameter of 6 mm were aseptically created using a sterile cork borer. A volume of 1 ml of the neutralized cell-free supernatant (NCFS) was then carefully added to the well using sterile pipette tips. The plates were subsequently incubated at 37°C for

a period of 24 hours. After incubation, the diameter of the zone of inhibition was assessed by placing a transparent meter ruler against the back of the Petri plate, and the measurement was recorded and expressed to the nearest millimeter [10]. Inhibition was indicated as positive when the inhibition halo around the well was more than 2 mm [11,12].

4.5.1. Genomic DNA extraction

The Genomic DNA of the bacteriocinogenic LAB was extracted from overnight cultures following the procedure described by MicroSeq® 500 DNA bacterial isolation kits. The amplification of LAB culture was performed using different primer sets. For the 1507R primer set, the PCR protocol involved initial denaturation at 94°C for 2 min, followed by denaturation at 94°C for 30 s, annealing at 59°C for 30 s, extension at 72°C for 1 minute and 30 s, and a final step at 72°C for 5 min. A total of 36 cycles were conducted. Similarly, for the 27F and 1492R primer set, the PCR protocol included initial denaturation at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 53°C for 30 s, extension at 72°C for 1 minute and 30 s, and a final step at 72°C for 5 min. An optimized PCR was also performed for isolates that showed non-specific products using the 10F and 1507R primer set. The optimized protocol involved denaturation at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 61°C for 30 s, extension at 72°C for 1 min and 30 s, and a final step at 72°C for 5 min. A total of 36 cycles were run and centrifuged at 5000 rpm for 10 min at 4°C. To obtain pure pellets, the supernatant was discarded while the pellets were washed with buffer (pH 8) and centrifuged again under the same condition. Man Ultra Sample Preparation Reagent (200 µL) was added. This was vortexed for 30 sec and then heated to 100°C in a water bath for 10 min. The tubes were cooled and centrifuged at 16000 x g for 3 min at 4°C. The supernatant containing the extracted DNA was transferred into a new micro centrifuge tube for subsequent use.

4.5.2. Polymerase chain reaction (PCR)

Amplification of the extracted DNA was achieved by performing a polymerase chain reaction in a thermal cycler. Two sets of PCR were run using two sets of primers; 10F (AGTTTGATCATGGCTCAGATTG) and 1507R (TACCTTGTTACGACTTCACCCCAG) as well as 27F (GAGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) which spans nearly full-length of 16S rRNA gene. The PCRs were run with (illustra TM) puRe Taq Ready-To-Go PCR beads. The concentrations of each primer used were 1 µl per 100 ml reaction (14). The 93 PCR amplification was performed in a thermal cycler machine (BioRAD PTC 200, USA) under 94 standard cycling conditions. The PCR was performed at 95 ◦C for 1 min, 35 cycles, with each cy- 95 cle consisting of 95 ◦C for 30 s, 65 ◦C for 30 s, 72 ◦C for 30 s, and finally 72 ◦C for 10 min. The 96 PCR products were stored at −20 ◦C. Then, 2 µl of PCR product was examined by electrophoresis 97 on 1 % agarose gel in TAE buffer. Then, the generated PCR products were cut and sent to Apical 98 Scientific Snd Bhd (First Base Laboratories) for further PCR purification and DNA sequencing.

4.5.3. Agarose gel electrophoresis

To check the results obtained from the PCR, 5 µl of each product were electrophoresed on 1.5% (w/v) agarose gel for 45 min at 110 volts. To observe the pattern of DNA bands, the gels were subjected to staining in ethidium bromide (EtBr) for a duration of 10 min. Subsequently, the gels were de-stained in clean water for 20 min before being examined under ultraviolet (UV) light using a transilluminator.

4.5.4. Sequencing of PCR amplicons

After gel electrophoresis, the left-over amplicons were transferred from PCR tubes into correctly labelled sterile Eppendorf tubes. The tubes were maintained at −20°C in freezer boxes before transporting them for sequencing. The amplicons were purified using a DNA purification kit and thereafter sequenced with the Sanger method by Inqaba biotech company using the 27F 16S rRNA primer [13].

4.5.5. Sequence analysis and identification of isolates

The 16S rRNA sequences obtained were analyzed using the Basic Local Alignment Search Tool (BLAST) [14] version 2.6.0+ tool of the NCBI (National Center for Biotechnology Information) database. The 16S rRNA sequences database and optimization for highly similar sequences (megaBLAST) were selected. Low-quality nucleotide bases, which are often found at the beginning and end of a sequence, were deleted, after which an optimized BLAST was run for each 16S rRNA sequence. Strains with sequences that were 97% identical to the database match were presumed to belong to the same species as the matching organism in the database and a 95% cut-off was used to define genera [15].

4.5.6. Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignments and phylogenetic tree construction were performed using the Bioedit and MEGA software (http://www.megasoftware.net/), following directions and recommendations by MEGA [16]. The alignment process utilized the default settings for DNA. These settings included gap penalties with values of -400 for gap opening and 0 for gap extension. The memory and iteration parameters were set to a maximum memory of 1685 MB and a maximum of 8 iterations. More advanced options involved using the UPGMB clustering method for the first two iterations and subsequent iterations. The minimum diagonal length (lambda) was set to 24. Before the analysis, all sequences were trimmed to the same length. The alignment was then performed, and the session was saved. The obtained alignment data was exported and saved as a MEGA file, which was used to construct a phylogenetic tree using the MEGA platform. The tree construction process involved selecting the "Construct/Test Maximum Likelihood Tree" option from the phylogeny menu [17].

4.6. Data analysis

All the assays were done in triplicates. The statistical factors were bacteriocin from *kunu* (k) and bacteriocin from *nono* (n) while the variable of response was the zone of inhibition. Analysis of variance and Descriptive statistics were employed to examine the data gotten from the study using Statistical Package for the Social Sciences (SPSS) version 21, PAST version 2.17c and Microsoft Excel version 2010 [3].

Limitations

Not applicable.

Ethics Statement

The authors have read and follow the ethical requirements for publication in Data in Brief and confirming that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Data Availability

Limosilactobacillus pontis strain EOINONO (Original data) (NCBI)
Limosilactobacillus pontis strain OGENONO (Original data) (NCBI)
Limosilactobacillus pontis strain OGENONO (Original data) (NCBI)
Limosilactobacillus pontis strain EEIKUNU (Original data) (NCBI)
Lactiplantibacillus plantarum strain MJIKUNU (Original data) (NCBI)

CRediT Author Statement

Emmanuel Edoghogho Imade: Conceptualization, Writing – original draft, Writing – review & editing; **Solomon Esharegoma Omonigho:** Methodology, Validation; **Olubukola Oluranti Babalola:** Funding acquisition, Resources, Supervision, Validation, Visualization; **Ben Jesuorsemwen Enagbonma:** Formal analysis; **Ozede Nicholas Igiehon:** Investigation; **Abraham Goodness Ogofure:** Investigation.

Acknowledgements

The authors would like to thank the staff of FIIRO for their technical support in this research work. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

References

[1] A.M.E. Sulieman, E.M. Abdallah, N.A. Alanazi, A. Ed-Dra, A. Jamal, H. Idriss, A.S. Alshammari, S.A. Shommo, Spices as sustainable food preservatives: a comprehensive review of their antimicrobial potential, Pharmaceuticals 16 (2023) 1451.
[2] H. Cissé, B. Kagambèga, A. Sawadogo, A. Tankoano, G. Sangaré, Y. Traoré, I.I.L. Ouoba, A. Savadogo, Molecular characterization of Bacillus, lactic acid bacteria and yeast as potential probiotic isolated from fermented food, Scientific African 6 (2019) e00175.
[3] E.E. Imade, S.E. Omonigho, O.O. Babalola, B.J. Enagbonma, Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria, Ann. Microbiol. 71 (2021) 1–14.
[4] Y. Wang, Y. Qin, Q. Xie, Y. Zhang, J. Hu, P. Li, Purification and characterization of plantaricin LPL-1, a novel class IIa bacteriocin produced by Lactobacillus plantarum LPL-1 isolated from fermented fish, Front. Microbiol. 9 (2018) 2276.
[5] Ş. Suyabatmaz, Ş.A. Karaoğlu, A. Bozdeveci, R. Akpınar, Honeybee-associated lactic acid bacteria and their probiotic potential for human use, World J. Microbiol. Biotechnol. 39 (2023) 2.
[6] Y. Ismail, C. Yulvizar, B. Mazhitov, Characterization of lactic acid bacteria from local cow s milk kefir, IOP Conference Series: Earth and Environmental Science, IOP Publishing, 2018.
[7] M.S. Abdel-Hamid, A. Anis, R.H. Elbawab, A.A. Mohammed, S.H. Orabi, S.I. Fathalla, Distinctive antagonistic role of new Enterococcus faecium ER-3M strain and its bacteriocin effect against Staphylococcus aureus Pneumonia, Rendiconti Lincei, Scienze Fisiche e Naturali 29 (2018) 675–690.
[8] M.P. Rivarez, E. Parac, Rapid molecular detection and transmission of bacterial leaf streak pathogen, Xanthomonas Oryzae Pv. Oryzicola, in rice seeds, Biosci. Biotechnol. Res. Asia 16 (2019) 509–520.
[9] S.A. Rangwala, D.M. Desai, Antibacterial activity of some common species used in indian kitchen and effect of it in combination, World J. Adv. Res. Rev. 16 (2022) 084–087.
[10] E.E. Imade, T.O. Ajiboye, A.E. Fadiji, D.C. Onwudiwe, O.O. Babalola, Green synthesis of zinc oxide nanoparticles using plantain peel extracts and the evaluation of their antibacterial activity, Scientific African 16 (2022) e01152.
[11] D. Borah, P. Mipun, J. Sarma, C. Mili, D. Narah, Quantitative documentation of traditionally used medicinal plants and their significance to healthcare among the Mishing community of Northeast India, Ecol. Questions 32 (2021) 1–62.
[12] C. Amarantini, D. Satwika, T. Budiarso, E. Yunita, E. Laheba, Screening of antimicrobial-producing lactic acid bacteria isolated from traditional fish fermentation against pathogenic bacteria, J. Phys. Conf. Ser. 1397 (2019) 1–8.
[13] G.O. Onipede, B.R Aremu, A.I. Sanni, O.O. Babalola, Molecular study of the phytase gene in lactic acid bacteria isolated from Ogi and Kunun-Zaki, African fermented cereal gruel and beverage, Appl. Food Biotechnol. 7 (1) (2020) 49–60.
[14] A. Moshood, H. Tengku, Lactic acid bacteria: bacteriocin producer, IOSR J. Pharmacy 3 (2013) 44–50.
[15] O.O. Bello, O.O. Babalola, M.F. Adegboye, O.M. Fashola, K.T. Bello, Partial purification characterization and application of bacteriocin from bacteria isolated Parkia biglobosa seeds, Nat. Eng. Sci. 3 (2018) 72–94.
[16] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (1) (2018) 1547–1549.
[17] S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: molecular evolutionary genetics analysis across computing platforms, Mol. Biol. Evol. 35 (2018) 1547.