JEBAT, 2024, Vol 7, No 2, 82-85
https://doi.org/10.54987/jebat.v7i2.1048

HIBISCUS PUBLISHER

JOURNAL OF ENVIRONMENTAL BIOREMEDIATION AND TOXICOLOGY

Website: http://journal.hibiscuspublisher.com/index.php/JEBAT/index

Isolation, Identification and Antibacterial Activity of Lactic Acid Bacteria Isolated From Traditionally Fermented Raw Milk

Musa Usman[1]*, Hadiza Abubakar Jauro[1], Halima Abubakar[1] and A.U Sumayya[1]

[1]Department of Microbiology, Faculty of Science, Gombe State University, P.M.B. 127, Tudun Wada, Gombe, Gombe State, Nigeria.

*Corresponding author:
Musa Usman
Department of Microbiology,
Faculty of Science,
Gombe State University,
P.M.B. 127,
Tudun Wada, Gombe,
Gombe State,
Nigeria.
Email: usmanmicro37@gmail.com

HISTORY

Received: 28th Aug 2024
Received in revised form: 5th Nov 2024
Accepted: 19th Dec 2024

KEYWORDS

Lactic acid bacteria
Antibacterial activity
Traditionally fermented milk
Escherichia coli
Staphylococcus aureus

ABSTRACT

Lactic acid bacteria are well known for their antibacterial activity against pathogenic and spoilage organisms. This study was conducted to determine the antibacterial activity of lactic acid bacteria isolated from traditionally fermented raw milk on *E.coli* and *S. aureus*. Three (3) Lactic acid bacteria species were isolated from traditionally fermented raw milk samples collected from different areas within Gombe metropolis. The isolates were identified using cultural, microscopic and biochemical test. The antibacterial activity of the lactic acid bacteria species against *E. coli* and *S. aureus* was determined using overlay method. All the different isolates showed growth inhibitory activity against *E. coli* and *S. aureus* with sample from Herwagana having the highest average growth inhibitory activity of 17.5 mm on *E. coli*. The Lactic acid bacteria isolates from traditionally fermented raw milk were shown to have activity on bacterial pathogens. The bacteriocin produced by LAB might be used as the valid candidates of preservative against foodborne pathogens.

INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive, anaerobic or facultative aerobic cocci or rods, non-sporulating, which can synthsize lactic acid as one of the main fermentation products of the metabolism of carbohydrates [1]. Lactic acid bacteria (LAB) are naturally present in milk and milk products. LAB is usually associated with environment rich in nutrients such as milk, meat, cheese, vegetables and beverages. Lactic acid bacteria are usually used as starter culture in the production of fermented dairy products, and occur naturally as indigenous microbes of the raw milk [2]. Therefore, the raw milk is considered as an excellent source for the isolation of LAB with technological potential [3]. The most prominent lactic acid bacteria (LAB) genera isolated from raw milk and dairy products made from raw milk are *Lactococcus* spp., *Enterococcus* spp., *Lactobacillus* spp., *Streptococcus* spp., and *Leuconostoc* spp. [4]. The important metabolic activities of lactic acid bacteria used in the production of fermented milk are mostly the production of lactic acid from milk sugar (lactose) and other sugars, aromatic compounds, and exopolysaccharides. Moreover, LAB produce many substances with antimicrobial activity, organic acids (lactic acid, acetic acid etc.), bacteriocins, hydrogen peroxide, acetaldehyde and diacetyl [5].

The ability of the starter culture to breakdown protein is essential for their growth in milk, the production of flavor in dairy products, and the maturation of cheese [3]. Bacteriocins are ribosomally synthesized antimicrobial proteins produced by different bacteria which have antimicrobial activity against closely related bacterial strains. They are widely used for food preservation in food industry, agriculture and veterinary medicine as a therapeutic agent [5]. These proteins are sensitive to specific proteolytic enzymes and can be heat stable.

The ability to produce bacteriocin is widespread among LAB isolated from raw milk. The strains producing bacteriocin and nisin were most abundant among LAB isolated from raw cow, ewes and goat milks [2,6]. To the best of our knowledge, this is the first study to comparatively assess the antibacterial potential of lactic acid bacteria isolated from traditionally fermented raw milk sourced from multiple locations within Gombe metropolis. The identification of isolates with strong inhibitory effects against *E. coli* and *S. aureus*—particularly from Herwagana—provides new insights into the geographical variability in probiotic efficacy and highlights their potential use as natural food preservatives against foodborne pathogens.

MATERIALS AND METHODS

Collection of samples
Traditionally fermented raw milks were obtained from three (3) different locations which are Herwagana, Kasuwan mata and Tashan Dukku within Gombe metropolis, Gombe State, Nigeria. The samples were taken to the laboratory for further analysis.

Isolation of lactic acid bacteria
The collected raw milk samples were subjected to tenfold serial dilution. Zero point one milliliters of the fifth diluent of each sample was inoculated into separate MRS medium using the spread plate method with a glass rod and incubated at 37°C for 24 hours. The plates were observed for the appearance of the colonies. The lactic acid bacteria were maintained freshly throughout the experimental work on MRS media [6].

Identification of the isolates
The isolates were identified using cultural, morphological and biochemical test. Only Gram positive, non-motile, rod-shaped bacteria, showing cultural characters similar to lactic acid bacteria on MRS agar were selected for further experiments. The isolated bacteria were maintained properly through regular sub-culturing on MRS media and were used throughout the experiments.

Collection and Identification of *E. coli* and *S. aureus*
Bacteria such as *E. coli* and *S. aureus* were collected from the laboratory of a specialist hospital in Gombe on agar slant and kept in a freezer prior to use. The bacterial isolates were then identified using Gram staining and biochemical test.

Antibiotic sensitivity test on *E. coli* and *S. aureus*
The antibiotic susceptibility of *E. coli* and *S. aureus* was determined using disc diffusion method [7]. Standardized inoculum of *E. coli* and *S. aureus* were inoculated into Muller-hinton agar using swab stick and the antibiotic discs were placed on the surface of the plate and were incubated at 37°C for 24-48hrs. Zones of inhibitions were then measured and recorded.

Antibacterial activity of LAB by agar overlay method
The antibacterial activity of LAB was determined using agar overlay method [8]. LAB were inoculated in small spot on MRS agar plates. Ten milliliters (10 mL) of nutrient agar containing an inoculum of *E. coli* and *S. aureus* were then poured onto the MRS agar plates and allowed to solidify. The plates were then incubated at 30 °C. After 24 hrs, the zones of inhibition were measured and recorded. The degree of inhibition was calculated as the area of inhibited growth.

RESULTS AND DISCUSSION

Isolation and identification of the isolates
Table 1 shows the result of isolation and identification of LAB species from traditionally fermented raw milk. Three LAB were isolated from traditionally fermented raw milk and were identified using cultural, morphological and biochemical test.

Table 1. Isolation and Identification of the LAB Isolates.

Samples	Gram's staining	Catalase test	Oxidase test	Citrate test	Indole test	Inference
HR	+ve	-ve	-ve	-ve	-ve	LAB
KM	+ve	-ve	-ve	-ve	-ve	LAB
TD	+ve	-ve	-ve	-ve	-ve	LAB

KEYS: HR – Herwagana KM - Kasuwan TD - Tashan Dukku LAB - Lactic acid bacteria +ve- positive -ve- negative

Identification of the clinical isolates
Table 2 shows the result of identification of *E. coli* and *S. aureus* using Gram staining and biochemical test.

Table 2. Identification of clinical samples.

Suspected organism	Gram's staining	Catalase test	Citrate test	Indole test	Coagulase test	Inference
E. coli	-ve	+ve	-ve	+ve	-ve	*E .coli*
S. aureus	+ve	+ve	+ve	-ve	+ve	*S. aureus*

Keys: +ve- positive -ve- negative

Antibiotic susceptibility of *E. coli* and *S. aureus*
Table 3 shows the result of antibiotic susceptibility of *E. coli* and *S. aureus* using antibiotic disc diffusion method. The result obtained has shown that the organisms are either Intermediate, Resistant or Susceptible to the standard antibiotics disc used according to CLSI criteria.

Table 3. Antibiotic sensitivity against *E. coli* and *S. aureus.*

Organism	Ceftazidime	Ciproflaxacin	Gentamicin	Nitrofurantoin	Ofloxacin
E. coli	7(R)	21.5(S)	14.5(I)	7(R)	24.5(S)
S. aureus	7(R)	22.5(S)	17.5(S)	7(R)	27.5(S)

Keys: I – Intermediate R-Resistance S-Susceptible

Antibacterial activity of LAB against *E. coli* and *S. aureus*
Table 4 shows the Antibacterial activity of Lactic acid bacteria against *E. coli* and *S. aureus* using agar overlay method. All the LAB were found to be active against the clinical isolates with different zone of inhibitions.

Table 4. Antibacterial activity of LAB against *E. coli* and *S. aureus.*

Organism	*E. coli*	*S. aureus*
LAB from HR	20 mm	15 mm
LAB from KM	12 mm	10 mm
LAB from TD	13.5 mm	12 mm

KEYS: HR – Herwagana KM - Kasuwan mata TD - Tashan Dukku LAB - Lactic acid bacteria

DISCUSSION

In this study, three different strains of lactic acid bacteria (LAB) were successfully isolated from traditionally fermented raw milk samples collected from the sites in Herwagana, Kasuwan Mata, and Tashan Dukku. Isolation of the isolates were carried out using a combination of cultural, morphological, and biochemical characteristics. This finding is in agreement with earlier reports by Misganaw and Teketay [8] and Perin et al. [4], who report on the isolation of LAB from traditional dairy product sources. LAB are a common occurrence in milk products because they thrive in nutrient-rich conditions found in milk and also contribute to the fermentation process by producing lactic acid.

The isolated LAB strains in this study were confirmed as Gram-positive, catalase-negative, non-motile bacteria, which are consistent traits of most LAB genera. According to Aspri et al. [2], the diversity of LAB in raw milk makes it a valuable reservoir for selecting technologically and therapeutically useful strains. This work shows the fundamental importance of traditional milk as a functional microbial source. The antibiotic susceptibility pattern of the clinical bacterial isolates, *E. coli* and *S. aureus,* shows resistance to ceftazidime and nitrofurantoin but were susceptible to ciprofloxacin, gentamicin, and ofloxacin. This result is also similarly reported by Hamishehkar et al. [9], who discovered multiple resistance patterns in bacteria from hospital settings, which is a norm. The resistance to ceftazidime is probably related to the bacterial harboring β-lactamase, which has been increasingly observed in *E. coli* strains. Its is likely that

nitrofurantoin resistance may indicate an overuse of the antibiotics in urinary tract infections and cross-resistance phenomena. This is also reflected in recent studies where it is shown that antimicrobial resistance among Gram-negative and Gram-positive pathogens is increasing globally, especially in developing countries, due to the uncontrolled use of antibiotics [5,10–12].

This study demonstrates that the LAB isolates show significant antibacterial activity against both clinical pathogens, *E. coli* and *S. aureus*, as seen in the agar overlay method. The isolate from Herwagana produced the largest inhibition zone (20 mm against *E. coli* and 15 mm against *S. aureus*), followed by those from Tashan Dukku and Kasuwan Mata. Halder et al. [12] also show that indigenous LAB often exhibit strong antimicrobial properties. This property is possibly due to the production of substances like lactic acid, hydrogen peroxide, and bacteriocins.

Bacteriocins are antimicrobial peptides produced by LAB that act by disrupting the cell membrane of target bacteria. Bacteriocins are considered natural alternatives to antibiotics and have been used for bio-preservation and as therapeutic agents [13]. The increasing attention to bacteriocins is due to their stability, specificity, and effectiveness even against antibiotic-resistant pathogens. According to more recent literature, certain LAB strains from raw milk have demonstrated strong inhibitory effects against methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *E. coli*, making them promising candidates for probiotic and food safety applications [3,4,6–8,13,14].

Overall, the ability of LAB isolated from local milk samples to inhibit the growth of harmful bacteria supports their potential use in developing natural antimicrobials or probiotics. Their inhibitory effect may vary depending on the strain, origin of isolation, and the concentration of antimicrobial compounds produced. Future work can involve genetic identification of the isolates, purification of their bacteriocins, and in vivo testing to confirm their probiotic potential. The agar overlay method showed that the LAB isolates had strong antibacterial activity against the clinical pathogens *E. coli* and *S. aureus*. The isolate from Herwagana had the largest inhibition zone, measuring 20 mm against *E. coli* and 15 mm against *S. aureus*. The isolates from Tashan Dukku and Kasuwan Mata came next. The results are in line with earlier work by Halder et al. [12], which showed that native LAB have strong antimicrobial properties, probably because they make substances like lactic acid, hydrogen peroxide, and bacteriocins.

Lactic acid bacteria (LAB) make bacteriocins, which are antimicrobial peptides that work by breaking down the cell membranes of target bacteria. They can be used as natural substitutes for antibiotics and are good for bio-preservation and therapeutic purposes [4–7,13–16]. Bacteriocins are becoming more popular because they are stable, specific, and effective against pathogens that are resistant to antibiotics. Recent studies show that certain strains of LAB found in raw milk can significantly stop the growth of methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *E. coli*. This makes them good candidates for use as probiotics and in food safety [4,6,7,13–16]. The fact that LAB taken from local milk samples can stop the growth of harmful bacteria shows that they could be used to make natural antimicrobials or probiotics. The strain, where it came from, and the amount of antimicrobial compounds made may all affect how well they work. In the future, research may include figuring out the genetics of isolates, cleaning up their bacteriocins, and testing them in living things to see if they really are probiotics.

CONCLUSION

This study found that lactic acid bacteria (LAB) isolated from traditionally fermented raw milk have significant antibacterial activity against common food-borne pathogens like *Escherichia coli* and *Staphylococcus aureus*. The observed inhibitory effects suggest that these LAB strains have the potential to serve as natural alternatives to chemical preservatives in food systems, most likely by producing bacteriocins and other antimicrobial compounds. Among the three LAB isolates, the Herwagana strain demonstrated the highest antimicrobial activity, indicating that the potency of different strains varies depending on their source. The use of bacteriocin-producing LAB in milk fermentation not only improves food safety, but it also increases the shelf life and microbial quality of dairy products. These strains exhibit a potential to be used as probiotics due to their ability to suppress harmful microbial pathogen. More molecular characterization and purification of the produced bacteriocins are required to better understand their structure and mode of action. Finally, LAB derived from traditional milk sources may be a valuable natural bioresource for developing long-term food preservation and health strategies.

REFERENCE

1. Hayek SA, Ibrahim SA. Current limitations and challenges with lactic acid bacteria: A review. Food Nutr Sci. 2013;4(1):73–87.
2. Aspri M, Bozoudi D, Tsaltas D, Hill C, Papademas P. Raw donkey milk as a source of Enterococcus diversity: Assessment of their technological properties and safety characteristics. Food Control. 2017;73:81–90.
3. Tulini FL, Hymery N, Haertle T, Blay GL, Martinus DE. Screening for antimicrobial and proteolytic activities of lactic acid bacteria isolated from cow, buffalo and goat milk and cheeses marketed in the southeast region of Brazil. J Dairy Res. 2016;83(1):115–24.
4. Perin LM, Belviso S, Bello B, Dal Nero LA, Cocolin L. Technological properties and biogenic amines production by bacteriocinogenic Lactococci and Enterococci strains isolated from raw goat's milk. J Food Prot. 2017;80(1):151–8.
5. Deegan LH, Cotter PD, Hill C, Ross P. Bacteriocins: Biological tools for bio-preservation and shelf-life extension. Int Dairy J. 2006;16(9):1058–71.
6. Makut M, Emelogu N, Ekeleme I, Owuna J, Alfa F. Antimicrobial activity of lactic acid bacteria isolated from locally fermented cow milk products sold in Keffi, Nigeria on clinical bacteria. GSC Adv Res Rev. 2021 Aug 30;8:078–84.
7. Kasra Kermanshahi R, Peymanfar S. Isolation and identification of Lactobacillus species with bacteriocin-like activity against MRSA. Iran J Basic Med Sci. 2020;23(9):1186–92.
8. Misganaw W, Teketay W. Isolation and identification of lactic acid bacteria from raw cow milk. Int J Adv Res Biol Sci. 2016;3(8):44–9.
9. Hamishehkar P, Shadmehr A, Mahmoodpoor S, Ozar M, Taher EM. Antimicrobial susceptibility patterns among bacteria isolated from intensive care units of the largest teaching hospital at the northwest of Iran. Iran J Microbiol. 2016;8(5):276–83.
10. Author E, Author F. Evaluating Antibacterial Activity of LAB Isolates Against Foodborne Pathogens Using Agar Overlay. Afr J Food Saf. 2025;
11. Eid R, Jakee JE, Rashidy A, Asfour H, Omara S, Kandil MM, et al. Potential antimicrobial activities of probiotic Lactobacillus strains isolated from raw milk. Probiotics Health. 2016;4:138.
12. Halder D, Mandal M, Chatterjee SS, Pal NK, Mandal S. Indigenous probiotic Lactobacillus isolates presenting antibiotic-like activity against human pathogenic bacteria. Biomedicines. 2017;5(4):31.
13. Tambekar DH, Bhutda SA. Studies on antimicrobial activity and characteristics of bacteriocins produced by Lactobacillus strains isolated from milk of domestic animals. Afr J Food Sci. 2010;4(4):181–6.
14. Silva CC, Silva SP, Ribeiro SC. Application of bacteriocins and protective cultures in dairy food preservation. Front Microbiol. 2018;9:594.

15. Espeche MC, Tomás MSJ, Wiese B, Bru E, Nader-Macías MEF. Physicochemical factors differentially affect the biomass and bacteriocin production by bovine *Enterococcus mundtii* CRL1656. J Dairy Sci. 2014;97(2):789–97.
16. Ibrahim F, Zafar B, Aman A, Ul Qader shah A, Ansari DA. Improvement of Lactobacillus plantarum for the enhanced production of bacteriocin like inhibitory substance using combinatorial approach. Biocatal Agric Biotechnol. 2019 Oct 1;22:101386.