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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Crude Bacteriocins Produced by Lactobacillus Species Isolated from Nono (Fermented Milk)

Ruqayyah, Abbas Usman

Department of Microbiology, Bayero University, Kano, Nigeria.

ABSTRACT
Bacteriocins are newly discovered exometabolites of lactic acid bacteria (LAB) with antimicrobial activity and biopreservative potential; however, studies investigating the potential of LAB from locally fermented milk (Nono) in Northern Nigeria for bacteriocin production and their antimicrobial effects on foodborne pathogenic bacteria are still insufficient. The present study aims to evaluate the antibacterial activity of bacteriocins produced by *Lactobacillus* spp isolated from *Nono* against foodborne pathogens. A total of 30 samples of *Nono* were purchased from Bayero University Kano old campus and K/Wambai market Kano State. *Nono*'s physicochemical parameters (pH and Titratable acidity) and Lactic acid Bacteria (LAB) counts were determined according to standard methods. The samples were further screened for *Lactobacillus* spp based on routine cultural characteristics, general morphological, biochemical tests and API 50CHL techniques. Crude bacteriocins were extracted and evaluated for antibacterial activity by Agar well diffusion method. The analyses revealed that *Nono* had pH values ranging from 2.84- 4.08, titratable acidity (1.16- 1.34%) and LAB counts ranged from $8.60x10^6$ – $3.00x10^8$. Thirty – eight (38) *Lactobacillus* spp based on distinct morphology were isolated from *Nono*. The different species of the genus *Lactobacillus* were identified phenotypically based on their carbohydrates fermentation profiles (API 50 CHL) as *Lactobacillus plantarum* 1, *Lactobacillus fermentum* 1 , *Lactobacillus pentosus, Lactobacillus fermentum* 2 and *Lactobacillus delbrueckii* ssp *lactis* 1. Ten (10) out of the 38 isolates were potential bacteriocin producers. Extracted crude bacteriocins exhibited broad-spectrum activity against *E. coli* (9.5±0.3 – 20 ±0.8mm) and *S. aureus* (9.47±0.6 -18.3 ± 0.6mm). Bacteriocins produced by *Lactobacillus spp* isolated from *Nono* could be used to solve the problem of contaminating microorganisms in food industries.

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Antibacterial Activity, Bacteriocins, *Lactobacillus* species, *Nono*

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INTRODUCTION

One of the food industry's top concerns must be the manufacture of safe food that is free of microorganisms, especially bacteria. Due to the accomplishment of this crucial goal, a number of approaches have been looked at, and attention has been directed to using naturally occurring antimicrobial agents including bacteriocins and essential oils (Carocho *et al*., 2015; Mokoena, 2017; Yousefi *et al*., 2020). Bacteriocins are ribosomally generated peptides or proteins that are produced by G+ and G- bacteria like lactic acid bacteria (LAB), *Staphylococcus* strains, *Bacillus* strains, and *E. coli* strains as natural antibacterial agents. Bacteriocins have inhibitory effects on numerous groups of undesirable microorganisms without having a negative impact on the bacteria that produce them, and they primarily target the cell wall of microorganisms in their activities (Espitia *et al*., 2016; Gutiérrez-Cortés *et al*., 2018; Lopetuso *et al*., 2019, Ng *et al*., 2020; Benítez-Chao *et al*., 2021).). They are tiny cationic molecules that form amphiphilic helices and contain 30 to 60 amino acids, and they exhibit high stability at 100 °C for 10 min. According to Parada *et al.* (2007), Mokoena (2017), Sidooski *et al.* (2019), they differ in terms of genetic make-up, biochemical characteristics, molecular weight (MW), and activity spectrum. In contrast to broad-spectrum bacteriocins, which inhibit a wide range of bacteria, narrow-spectrum bacteriocins may inhibit bacteria that are taxonomically near to them (Cotter *et al*., 2005; O'Connor *et al*., 2020). The bacteriocins are divided into four categories (Class I, II, III, and IV) based on the producer organism, MW, chemical structure, presence of changed amino acids, and temperature stability (López-Cuellar *et al*., 2016; Johnson *et al*., 2018; Kumariya *et al*., 2019; Cui *et al*., 2021). The most researched bacteriocins that could be used commercially as natural preservatives are nisin and pediocin (Acuna *et al*., 2011). The only bacteriocin that is currently permitted for usage as an additive is nisin. However, pediocin can be used as a food additive created by *P. acidilactici*, a strain that produces pediocin, and its

Correspondence: Ruqayyah, A.U. Department of Microbiology, Bayero University, Kano, Nigeria.
✉ Muhsina1907@gmail.com; Phone: 07066354976
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use is protected by a number of US and European patents (El-Ghaish *et al*., 2011; Espitia *et al*., 2016). These peptides are GRAS (generally acknowledged as safe), and many scientists are interested in using them in the food and pharmaceutical industries in the future (Pato *et al*., 2022). For a very long time, the food industry has employed bacteriocin to stop food spoiling and food-borne illnesses (Perez *et al*. 2014). High stability, low toxicity, a narrow to broad spectrum of activity, and other characteristics of these bacteriocins make them ideal for use as natural food preservatives (Bharti *et al*., 2015; Pato *et al*., 2022). Pathogen-driven foodborne outbreaks pose a serious risk to public health and have a substantial financial impact on global health care. The US federal government believes that there are still roughly 48 million incidents of foodborne disease each year, despite the American food supply being among the safest in the world (Dumen *et al*., 2020). Food-borne pathogens have become serious social issues that have drawn a lot of attention from consumers and food safety regulatory authorities globally as a result of these frequent outbreaks of microbial infections (Pato *et al*., 2022). Bacteriocin has demonstrated significant antibacterial action despite being obtained from different strains of *Lactobacillus bulgaricus, Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus lactis*. Nonetheless, the study of bacteriocins as an antibacterial agent from indigenous foods is crucial given the pressing need in the modern world to identify efficient means of reducing pathogenic and food spoilage bacteria.

METHODOLOGY

Samples Collection

A total of thirty (30) samples of *Nono* were aseptically collected in sterile bottles from commercial producers in the *Kofar Wambai* market and BUK old campus. All the samples were transported to the laboratory in ice packs for further analyses. Foodborne pathogens isolated from vegetables used for the bioassay were collected from the department of microbiology.

Determination of Physicochemical Parameters of *Nono*

These parameters were determined according to the method of the Association of Official Analytic Chemists (AOAC, 2005).

pH

The glass electrode of the pH meter (Jenway- U.K) was inserted into the *Nono* sample to a depth of 3/4 of the sample, then swirled for 5 seconds and allowed to become steady before taking reading on the pH meter (AOAC, 2005).

Titratable acidity

Ten milliliters (10mL) of *Nono* samples were titrated with 3 drops 0.1N of sodium hydroxide using phenolphthalein as an indicator, till a faint pink color which persisted appeared. The acidity of the sample was calculated using the following equation:

$$\text{Titratable acidity}\ (\%) = 0.009\ x\ Vol.of\ NaOH\ used\ x \frac{100}{Weight\ of\ the\ sample} \quad (AOAC, 2005)$$

Enumeration of Lactic acid Bacteria (LAB)

In 225ml of buffered peptone water, 25mL of *Nono* samples were aseptically added before being shaken to homogenise. Additional serial dilutions up to 10^6 followed these. Using the pour plate technique, 1mLof each dilution was added to duplicate petri dishes of de Man Rogosa and Sharpe agar (OXOID), then set at a pH of 5.5. The plates were placed in the anaerobic jar and immediately sealed before being incubated under anaerobic conditions for 48 hours at 37°C using anaeroGen (Pyar and Kok, 2019).

Isolation and Identification of *Lactobacillus* species.

Isolation of *Lactobacillus* species

The *Lactobacillus* species were isolated from the collected *Nono* samples. For the purpose of serial dilutions process, ten (10) mL of each of the obtained samples was added to 90ml of sterilised distilled water. The isolation was done using homogenised *Nono* samples that had been serially diluted on sterile plates of MRS (De Man Rogosa Sharpe) agar supplemented with 50 mg/L Nystatin and pH – adjusted to 5.5, aliquots of 10^{-6} and 10^{-7} (0.1mL) dilutions were aseptically dispensed. The plates were placed in the anaerobic jar immediately and then anaerobically incubated for 48 hours at 37°C using anaeroGen. Discrete colonies were streaked onto new agar to create pure cultures of each isolate (Adebayo *et al*., 2014). The pure cultures were then stored for later use in MRS broth that contained 40% glycerol (v/v) at -80°C. The cultures were propagated twice in MRS broth during a 48-hour period at 37°C before being used in experiments.

Identification of *Lactobacillus* spp Using Phenotypic Characterisation

The pure colonies were characterised using colonial, morphological characteristics and biochemical tests which included Gram staining, catalase test, oxidase test, citrate utilisation test, Endospore test, growth at different temperatures 10 ºC and 42ºC), growth under anaerobic conditions, growth at 6.5% NaCl concentration gas production from glucose and motility test. Non-spore forming bacilli, non-capsule, catalase-negative and Gram-positive isolates were maintained on MRS agar slants and stored at 4°C for further tests. The identification of the cultures to species level were based on the phenotypic characteristics of the Lactobacilli as described in Bergey's manual of determinative bacteriology (Ludwig *et al*., 2009). Phenotypic characterisation was conducted twice for each strain. Pure isolates of *Lactobacillus* species were inoculated

into API 50 CHL medium (Biomerieux, Marcy L' Etoile, France) to study carbohydrate metabolism profiles.

Identification of *Lactobacillus* spp using (API 50 CHL) kit.

The API 50CHL system (BIOMÉRIEUX, France, V 5.1) specific for Lactobacilli identification, was used to further establish the synthesis of acids from carbohydrates and associated compounds by the five (5) selected *Lactobacillus* species. The manufacturer's instructions were followed during every step of the *Lactobacillus* identification process. A swab was used to aseptically transfer pure cultures of each isolate from a freshly inoculated stock culture to an ampule of API 50 CHL (10.0ml) basal medium. The ampule was then emulsified to produce a final turbidity equivalent to McFarland standard ♯2. Using a sterile pipette, the standardised bacterial suspension was injected into each API 50 CHL strip tube. According to the directions, the strip was placed in the incubation tray with honeycombed wells each filled with distilled water and covered with mineral oil. Each well's color was checked 48hours after incubation to see if it had changed. Except for well ♯26 (for esculin hydrolysis test) where the bromocresol purple indicator's color changed from purple to black, the change from purple to yellow indicated a positive result. While a lack of color change indicated a negative result. The control was the first well on the strip. To identify *Lactobacillus* species the outcome was examined using api-web™ identification software database (BIOMÉRIEUX, France V5.1). Identification listed in the index as excellent, very good or acceptable based on percentages were accepted as correct (Pyar and Kok, 2019).

Extraction of Crude Bacteriocin

To determine bacteriocin production, overnight grown culture of 10 different isolates of *Lactobacillus* spp (NL1-M39) *were* propagated separately in 500ml of de Man Rogosa and Sharpe broth at 10% inoculum and incubated at 37°C for 48 hours anaerobically. After incubation the broth was centrifuged at 20,000 rpm for 20 minutes at 4°C and then sterilized by filtration through 0.22µm Syringe filter. To eliminate growth inhibition caused by organic acids, the resulting cell free supernatant fluids was adjusted to pH 7.0 with 1moL^{-1} NaOH. Inhibitory activity of hydrogen peroxide was eliminated by adding 1mg mL^{-1} catalase (Sigma-Aldrich Corporation) to the cell free supernatant. The crude extract of bacteriocins were tested for Antibacterial activity (Sure *et al*., 2016).

Collection and Standardisation of the bioassay Isolates

The foodborne bioassay isolates collected from the department of Microbiology were further confirmed using standard biochemical tests including: The Triple Sugar Iron (TSI) Agar test was used to determine sugar formation, CO_2 and H_2S production (Harrigan 1998). The IMViC (Indole, Methyl Red, Voges- Proskauer, and Citrate). These tests were done to determine the various end products (*Escherichia coli*). Catalase and Coagulase tests were used for *Staphylococcus aureus*. The bioassay isolates were adjusted to 0.5 McFarland standard (1.5 X 10^8 CFU/mL) using sterile normal saline. The 0.5 McFarland standard was made by continuously swirling 9.95mL of 0.18M H_2SO_4 (1.0% w/v) with 0.05mL of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$). The standard was compared with a black line standing out against a white background to facilitate comparison (Kalpana *et al.,* 2013).

Antibacterial Assay

The antibacterial activity of the crude bacteriocin was determined using the agar well diffusion technique as described by Dhewa, (2012). Nutrient agar used for the sensitivity was prepared according to manufacturer's instructions. Twenty (20mL) millilitres of nutrient agar was poured into sterile Petri dishes and allowed to solidify and then labelled. Each plate was uniformly inoculated with standardised test bacteria (1.5x10^8 cfu/mL). A sterile cork borer of 6mm diameter was used to bore 4 wells on the nutrient agar into which 100 µL of crude bacteriocins were filled. The inoculated plates were kept on the bench for 30mins to allow the bacteriocins to diffuse into the agar medium. The plates were then incubated for 24 hours at 37˚C, and the diameter of the inhibition zone was measured using calipers in millimeter. The antibacterial activity were assayed in triplicates.

RESULTS

Physicochemical Characteristics and Enumeration of Lactic Acid Bacteria in *Nono*

The results of the physico chemical characteristics and LAB count of the *Nono* samples showed that the mean pH ranges from 2.84 –4.30. While the mean titratable acidity ranges from 1.16 – 1.34%. Out of the 30 *Nono* samples collected, the result shows that twenty- five (83.33%) samples analysed contained lactic Acid Bacteria (LAB). The result showed that *Nono* had LAB counts ranging from 8.60 x 10^6 – 3.00x10^8 cfu/ml (Table)

Table 1: Chemical Properties and LAB counts of Nono Samples

Sample	pH	Titratable acidity (%)	LAB COUNT (cfu/mL)
1.	3.65	1.20	1.30x10^{8}
2.	3.76	1.24	7.90x10^{7}
3.	3.68	1.30	8.60x10^{6}
4.	3.99	1.25	1.08x10^{8}
5	3.73	1.23	1.99x10^{8}
6	3.85	1.21	2.85x10^{8}
7.	3.72	1.23	2.45x10^{8}
8	3.74	1.24	1.35x10^{7}
9	4.30	1.20	1.14x10^{7}
10	2.84	1.24	1.22x10^{7}
11	3.84	1.24	1.22x10^{7}
12	3.99	1.20	8.25x10^{7}
13	4.08	1.32	1.30x10^{8}
14	3.77	1.32	2.92x10^{8}
15	3.76	1.20	2.72x10^{7}
16	3.81	1.22	1.99x10^{8}
17	3.76	1.25	3.00x10^{8}
18	3.73	1.25	1.68x10^{8}
19	3.70	1.23	1.21x10^{8}
20	3.69	1.24	1.21x10^{8}
21	3.70	1.34	1.23x10^{7}
22	3.70	1.23	8.20x10^{8}
23	3.86	1.22	1.48x10^{8}
24	3.84	1.20	9.10x10^{8}
25	3.68	1.16	9.70x10^{7}

Isolation of *Lactobacillus* Species

Lactobacillus spp selectively isolated on MRS agar appeared as creamy, smooth and round colonies. Their microscopic appearance revealed them as gram positive rods (plates I).

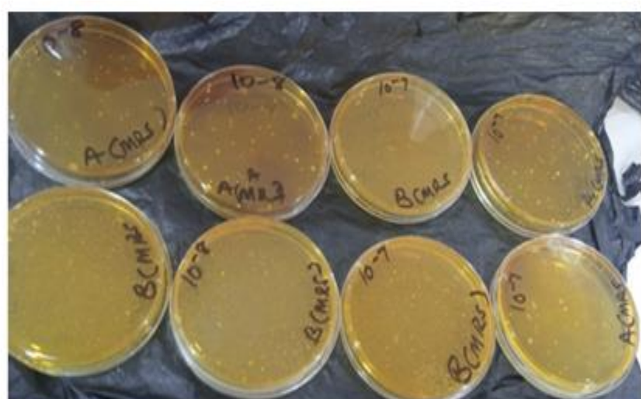


Plate I: Morphology of *Latobacillus* species on MRS Agar

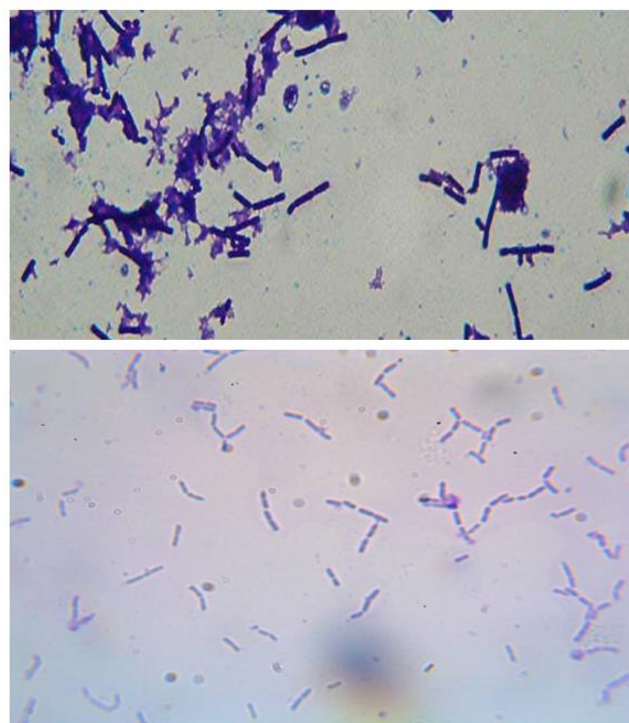


Plate II: Microscopic Appearance of *Lactobacillus* spp

Identification of *Lactobacillus* spp using (API 50 CHL) kit.

The results of carbohydrates fermentation test of API 50 CHL identification kit are shown in Table 2. The result obtained coincided with characteristics of *Lactobacillus* species as described by Jones and Versalovic, (2009). API 50 CHL database indicates that NK2, NL1, N64, N33 and N38 were *Lactobacillus plantarum 1, Lactobacillus fermentum 1, Lactobacillus pentosus, Lactobacillus fermentun 2* and *Lactobacillus delbrueckii* ssp *lactis* 1 with percentage 1.Ds of 99.9%, 99.7%, 98.6%, 97.6% and 99.5% respectively.

Table 2: Sugar Fermentation by Isolates Using API 50CHL

S/N	Carbohydrates	NK2	NL1	N64	N33	N38
1	Control	-	-	-	-	-
2	Glycerol	-	-	-	-	-
3	Erythirol	-	-	-	-	-
4	D – arabinose	-	-	-	-	-
5	L – arabinose	+	-	+	+	-
6	Ribose	+	+	+	+	-
7	D – xylose	-	-	+	-	-
8	L – xylose	-	-	-	-	-
9	Adonitol	-	-	-	-	-
10	β-metil –D – xyloside	-	-	-	-	-
11	Galactose	+	+	+	+	-
13	D – glucose	+	+	+	+	-
13	D – fructose	+	+	+	+	+
14	D – mannose	+	-	+	+	+
15	L – sorbose	-	-	-	-	+
16	Rhamnose	+	-	-	-	-
17	Dulcitol	-	-	-	-	-
18	Inositol	-	-	-	-	-
19	Manitol	+	-	+	+	-
20	Sorbitol	+	-	+	+	-
21	α-methyl-D-mannosidse	+	-	-	-	-
22	α-methyl-D-glucoside	-	-	+	-	-
23	N-acetyl-glucosamine	+	-	+	+	+
24	Amigdalin	+	-	+	+	-
25	Arbutin	+	-	+	+	-
26	Esculin	+	-	+	+	-
27	Salicin	+	-	+	+	-
28	Cellobiose	+	-	+	+	-
29	Maltose	+	+	+	+	-
30	Lactose	+	+	+	+	+
31	Melibiose	+	+	+	+	-
32	Saccharose	+	+	+	-	+
33	Trehalose	-	-	+	-	+
34	Inulin	-	-	-	+	-
35	Melezitose	+	-	+	+	-
36	D-raffinose	+	+	+	-	-
37	Amidon	-	-	-	-	-
38	Glycogen	-	-	-	-	-
39	Xylitol	-	-	-	+	-
40	β-gentiobiose	+	-	+	-	-
41	D –turanose	+	-	+	-	-
42	D – lyxose	+	-	-	+	-
43	D – tagarose	-	-	-	-	-
44	D – fuccose	-	-	-	-	-
45	L – fuccose	-	-	-	-	-
46	D – arabitol	-	-	-	-	-
47	L – arabitol	-	-	-	-	-
48	Gluconate	+	-	-	-	-
49	2 – keto – gluconate	-	-	-	-	-

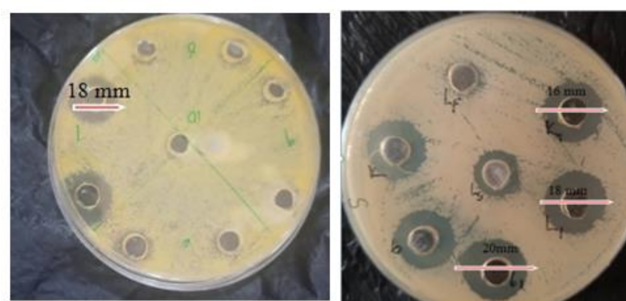
Key: NK2 = *Lactobacillus plantarum, NL1 = Lactobacillus fermentum1, N64 = Latocatobacillus pentosus, N33 = Lactobacillus fermentum2 and N38 = Lactobacillus debrueckii ssp lactis1*

Antibacterial Activity of Crude Bacteriocin

The result of the antibacterial activity of bacteriocins extracted from *Lactobacillus* spp against indicator foodborne pathogens are presented in Table 3. The inhibitory effects were higher on *E. coli* (9.5+0.3– 20+0.8) than *S. aureus* (9.47+0.6–18.3+0.6).

Table 3: Antibacterial Activity of Crude Bacteriocins (100 µL)

Crude Bacteriocins:	Zone of inhibition (mm)	
	S. aureus	***E.coli***
NL1	17±00	17±00
NK2	16±0.8	16±00
N33	13±0.5	12±0.6
N64	18.3±0.6	20±0.8
NP5	12.7±0.2	11±0.3
NM6	12±1.0	11±0.3
N71	10.8±0	18±0.3
NJ9	11.4±0.3	10±0.3
B33	9.47±0.6	9.5±0.5
M39	10.67±0.3	10.83±0.3
	(P<0.05)	



Antibacterial activity of crude bacteriocin against *S. aureus*

Antibacterial activity of crude bacteriocin against *E. coli*

Plate III: Antibacterial activity of crude bacteriocin against *S. aureus* and *E. coli*

DISCUSSION

The results of the physico chemical characteristics of the *nono* samples showed that the pH values of *Nono* in this study (2.84 – 4.30) were slightly lower than that obtained by Omola *et al.* (2019) (4.22 – 4.70) in Kano, and that of Sanusi and Salamatu (2019) (4.45 – 4.85) also in Kano, but differed from that obtained by Okonkwo (2011) (3.59 - 5.36) in Maiduguri, and also that reported by Adesokan *et al.* (2011) (5.51- 6.29) in Ibadan. The variations in pH could be explained by the fact that the samples were collected in the hotter months and the ambient temperatures at which the natural fermentation took place probably were high. The low pH of *Nono* prevents the growth of most spoilage and pathogenic microorganisms (Varga, 2007). The mean titratable acidity values (1.16 – 1.34%) relatively corresponded to that obtained by Omola *et al* (2019) (1.34 – 1.50%). The high acidity explains why

nono has a sour taste and it may be due to the variations in fermentation duration and method of production (Omola *et al*., 2019). Presence of Lactic acid bacteria in *nono* has been associated to increased titratable acidity and corresponding decrease in pH (Achi and Akubor, 2000). The traditionally fermented milk (*Nono*) contained Lactic acid bacteria (LAB) in high numbers. The high counts of the lactic acid bacteria shows that this group of bacteria constitutes a prominent part of the microflora of *Nono*. This corroborated with previous studies that have reported lactic acid bacteria to be associated with food and feed fermentations (Syukur *et al.,* 2014; Iranmenesh *et al.,* 2012; Akabanda *et al*., 2010). The high LAB counts (8.60 x 10^6 – 3.00 x 10^8) recorded in this work is in conformity with the reports of Oyeleke *et al.* (2006), Akabanda *et al.* (2010), Adesokan *et al.* (2011), Mohammed and Ijah, (2013), where they all reported that *Nono* and other fermented foods analyzed had high LAB counts. This high numbers of LAB, coupled with the low values of pH and high acidity may be responsible for the sour taste, flavor and unique aroma of *nono* (Akabanda *et al*., 2010). The synthesis of lactic acid results in the formation of a smooth gel as well as a sour flavor in the fermented product. Additionally, many flavor compounds are created, and these are what give varied products their unique taste. It has been reported that traditional fermented milks in regions with a cold climate favor the growth of mesophilic bacteria such as *Lactococcus* and *Leuconostoc spp.* whereas, in warm regions, thermophilic bacteria like *Lactobacillus* and *Streptococcus* prevailed (Savadogo *et al*., 2004*)*. The dominance of thermophilic bacteria in our samples could be explained by the fact that the samples were collected in the hotter months and the ambient temperatures at which the natural fermentation took place probably were high. This result supports the theory that the microorganisms that are found in traditionally fermented milks depend on the particular climatic region and the distribution of lactic acid bacteria depends on the nature of fermented milk or fermented food (Savadogo *et al*., 2004*)*. The present study revealed that most of the strains isolated from *Nono* where Gram positive bacilli, catalase negative, non – spore formers and non – motile. These are typical characteristics of *Lactobacillus* spp as reported by Axelson (1993), Arokiyamar and Sivakumar (2011); Jothi *et al.* (2012) and Usman *et al.,* (2022). The different species of the genus *Lactobacillus* were identified phenotipically based on their carbohydrates fermentation profiles (API50 CHL). They were identified as *L. plantarum 1, L. fermentum 1, L. pentosus, L. fermentum 2 and L. delbrueckii* ssp *lactis*. API 50 CHL identification kit has been reported as an important tool for Lactobacilli identification. It can be used for taxonomic identification which is based on phenotypic characteristics to identify different species of *Lactobacillus* (Pyar and Kok, 2019). This result is in agreement with the findings of other researchers (Pancheniak and Soccol, 2005; Mattia and Merker, 2008; Jones and Versalovic, 2009). Many studies had reported the diversity of LAB in milk and milk products. Syukur *et al.* (2014) isolated different *Lactobacillus* spp from 'Dadih' (fermented buffalo milk) in Indonesia, the presence of *Lactobacillus* strains in sheep and cow milk was also reported by Mobarez *et al.* (2008). Adesokan *et al.* (2011) also isolated *Lactobacillus brevis L. casei, L. fermentum* and *L. plantarum*, from traditionally fermented *Nono* in Ibadan. Okagbue and Bankole (1992) also reported isolation of LAB from *nono*, which they used singly and in combination with yeast to develop starter cultures for *nono* production. Furthermore, Gran *et al.* (2003) isolated LAB from Amasi, a Zimbabwean naturally fermented milk product. It is therefore a well – established fact that the composition of LAB in *Nono* is varied. Crude Bacteriocins i.e. (cell free supernatant) from 10 *Lactobacillus* spp showed various degree of activity against the gram positive and Gram negative foodborne isolates used. Statistical analysis revealed there was no significant difference ($P < 0.05$) in the antibacterial activity. Higher inhibitory activity was observed against *E. coli* than *S. aureus*. The higher activity of bacteriocin against *E. coli* may be due to the variation in the structure of these bacteriocins and/or differences in the structure of the putative target molecules (receptors). Bacteriocins with high structural similarity may even differ remarkably in their antimicrobial spectrum of activity (Drider *et al.,* 2006; Pal *et al.,* 2009). Stevens *et al.* (1991) reported that bacteriocins are not frequently active against Gram negative bacteria because the outer membrane of this class of bacteria acts as a permeability barrier for the cell. It is also responsible for preventing molecules such as antibiotics, detergents and dyes from reaching the cytoplasmic membrane. Cleveland *et al.* (2001) also reported that bacteriocins are supposed to act only on closely related species which limits their application as a natural preservative, but in contrast, our study revealed that bacteriocins produced by *Lactobacillus* spp were active against both Gram positive and gram negative isolates suggesting their broad spectrum of activity. Our result was in agreement with the work of Sure *et al.* (2016) where they reported that bacteriocins of *Lb. viridenscence* (NICM 2167) showed better inhibition against *E. coli*. Jothi *et al.* (2012) also reported that inhibitory effect of antibacterial substance produced by *Lactobacillus* was more significant against Gram negative bacteria*, Salmonella* sp (12mm) in their study. Similar results were also obtained by Sharma *et al.* (2011) in their research, purification and characterization of bacteriocin produced by *Lactobacillus* sp. A75 isolated from fermented chunks of '*Phaseolus radiata*'. Thus, this proves that bacteriocins from different lactic acid bacteria have specific inhibition spectra.

CONCLUSSION

The Present study revealed a high density of *Lactobacillus spp* (8.60x10^6 – 3.00x10^8) in Nigerian traditionally fermented milk 'Nono'. The *Lactobacillus spp* based on API50 CHL profiles were identified as *L. plantarum 1, L. fermentum 1, L. pentosus, L. fermentum 2 and L. delbrueckii ssp lactis*. The isolated *Lactobacillus* spp has ability of bacteriocin production with antibacterial activity against *S. aureus* and *E. coli*. Higher inhibitory activity was observed

against *E. coli* (9.5+0.3– 20 + 0.8) than *S. aureus* (9.47+0.6–18.3+0.6). Different bacteriocin exhibits different inhibition profile on food spoilage and pathogenic bacteria, therefore they could serve as natural replacements for synthetic food preservatives. More research related to the mode of action, absorption, metabolism and pharmacodynamics of bacteriocins are needed to explore their utility.

RECOMMENDATION

treatments and block sizes for the two factors is found in Bailey and Cameron (2019).

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Incomplete Block Design was developed by adopting the method in Mills (2014). An additive model with both treatments and block fixed was proposed; the unknown parameters in the model were estimated using the least square method. The sum of squares of the sources of variation in the design was also estimated, and finally, an ANOVA Table was presented for the given design.

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