World Journal of Advanced Research and Reviews, 2020, 06(03), 050–058

World Journal of Advanced Research and Reviews

e-ISSN: 2581-9615, Cross Ref DOI: 10.30574/wjarr

Journal homepage: https://www.wjarr.com

(RESEARCH ARTICLE)

Bio-control of Vibrio species in cultured milk by in situ bacteriocin production from lactic acid bacteria

Ogunbanwo Samuel Temitope [1], Odubanjo Oluwadamilola Rashidat [1], Adegoke Caleb Oladele [2,*] and Oramadike Chigozie [3]

[1] *Department of Microbiology, University of Ibadan, Oyo State, Nigeria.*
[2] *Ogun State College of Health Technology, Ilese Ijebu, Department of Medical Laboratory Science P.M.B. 2081.*
[3] *Fish Technology Department, Nigerian Institute for Oceanography and marine Research, P. M. B.12729, Victoria Island Lagos, Nigeria.*

Publication history**:** Received on 22 March 2020; revised on 12 June 2020; accepted on 14 June 2020

Article DOI: https://doi.org/10.30574/wjarr.2020.6.3.0073

Abstract

The growth of resistance to antibiotic by *Vibrio* signifies a possible risk to human health; hence, there is a need to deploy another technique for controlling species of *Vibrio*. This study was undertaken to demonstrate the antagonistic activity of bacteriocin-producing Lactic acid bacteria against *Vibrio alginolyticus, Vibrio vulnificus, Vibrio harveyi, Vibrio parahaemolyticus, Vibrio fluvialis,* and *Vibrio cholera in vitro* and *in situ.* Lactic acid bacteria (LAB) were isolated from milk products and identified phenotypically. They were initially screened for antagonistic activity against the *Vibrio* species by the agar well diffusion assay, bacteriocins produced by the LAB were characterized with respect to pH, enzymes and temperatures. The effect of *in situ* bacteriocin production by LAB on the survival of *Vibrio* species was determined in *Nono,* after fermentation of milk during the storage period of 72 h (12 h interval). Of the 112 strains of LAB tested for antagonistic activity against *Vibrio* species, only twelve were selected based on the bacteriocin production and large zone of inhibition against *Vibrio* species. They were characterised phenotypically and identified to be *Pediococcus damnosus, Pediococcus acidilactici, Lactobacillus brevis* and *Lactobacillus plantarum*. The bacteriocins produced by the LAB were heat stable at 90°C for 20 min, active over a wide pH range (2 to 6), stable in the present of catalase but lost their activity in the present of proteolytic enzymes. Bacteriocins produced by the LAB showed antagonistic activity against *Vibrio* species with zones of inhibition ranges from 12 to 20mm. *Vibrio* species counts were reduced significantly to different extents in all samples of *Nono* and undetectable within 48 to 60 hours of *Nono* storage. On the contrary, *Vibrio* species survived for 72 h of storage in the control experiment that lack bacteriocin producing LAB. This work demonstrates that the use of selected bacteriocin-producing starter in milk fermentation might contribute to safety of dairy products.

Keywords: Lactic acid bacteria; Vibro species; Biocontrol; Fermentation; in situ Bacteriocins; production in Milk.

1. Introduction

Lactic Acid Bacteria are gram-positive, catalase negative, usually non-motile, non-spore-forming rods and cocci which produce lactic acid as the major end product of carbohydrate fermentation [1]. They cannot generate ATP because they lack the ability to synthesize cytochromes and porphyrins which are components of respiratory chains. They can only obtain ATP by fermentation, usually from sugars. They are facultative anaerobes and are protected from oxygen byproducts (e.g. H_2O_2) because they possess peroxidases [2]. Lactic Acid Bacteria are usually found in the environment related with rich nutrients, such as different food products (milk, meat, vegetables), although some of their members are of the flora of the intestine, mouth and vagina of mammals [3; 4]. One of the most important and significant values of lactic acid bacteria to humans is their beneficial role in health and inhibition of pathogenic bacteria. Some LAB are reputed to act directly as probiotics when ingested or incorporated into food products [5], or indirectly by action of the

* Corresponding author: Adegoke Caleb Oladele

antimicrobial substances they produce in foods. Several antimicrobial substances are produced by various starter cultures of LAB, which include organic acids, hydrogen peroxide, CO_2, diacetyl and bacteriocins [6] which have received increasing attention during the past two decades.

Bacteriocins are definitely regarded as antimicrobial peptides that are produced from their ribosomes (ribosomally) which are active against other bacteria, either of the same species or across genera [7 ; 8] . They may be produced by both Gram negative and Gram positive bacteria [9]. In recent years, bacteriocin producing LAB have attracted significant attention due to their Generally Recognized as Safe (GRAS) status and potential use as safe additives for preservation of food [10] . Bacteriocins have potent antagonistic effect against important clinical pathogens as observed by [11]. Different types of bacteriocins from food-associated lactic acid bacteria have been characterised and identified, of which the important ones are nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins, and plantaricins [12]. Quite a lot of studies showed that LAB starter cultures or co-cultures are able to produce bacteriocins in food media, and as a result exhibited inhibitory potentials towards labile foods capable of spoilage or pathogenic bacteria. The genus *Vibrio* is oxidase-positive, Gram-negative, rod or curved shaped and facultative anaerobes that are motile, which produces cholera enterotoxin. They are responsible for food-borne disease, morbidity, life threatening and mortality [13; 14]. Several foods include vegetables, fruits, dairy products, sea foods, poultry, milk, meat products and others can become contaminated with *Vibrio* species as a result of improper handling, undercooking, washing with unhygienic water and by the use of untreated soil [15;16]. These organisms are capable of producing a thermostable toxin known as hemolysin.

The principal cause of mortality in the developed, developing and the rest of the world is the *Vibro vulnificus* because of its association with sea food consumption [17]. However, the use of antibiotics in regulating *Vibrio* species is not encouraging as a result of the growth of antibiotic-resistance and the negative impact of antibiotic consumption on the host. An efficient microbiological protection and safety of milk is vital to ensure its purity and to avoid adverse human heath implication. However, research in this area has not been fully explored, therefore, the focus of this work centered on the testing of Lactic Acid Bacteria against multiple-drug resistance *Vibrio* species in milk and its products.

2. Material and methods

2.1. Sample collection

Samples of milk products ("Nono", "Wara" and Yoghurt) used in this study were obtained from local markets in Ibadan, Oyo State. Two replicate samples were collected and transported to the laboratory in sterile bottles. The samples were refrigerated at 4°C and analyzed within 24 hours of collection [18]. The multidrug resistance Vibrio species used as indicator organisms in this work had been previously phenotypic and molecular characterised as reported by [19].

2.2. Sterilization

All media were sterilized in an autoclave at 121˚C for 15 minutes, except otherwise stated. Used glass wares were soaked in solutions containing antiseptic (Jik) overnight. They were then washed with liquid soap and rinsed in several changes of tap water. The glass wares were arranged on the drain board to drip off.

2.3. Isolation procedure

One mL/1g of the samples was homogenized with 9ml of sterile distilled water to make an initial dilution 10^{-1}. The suspensions were used for making suitable serial dilutions up to10^{-6} by incorporating 1ml into 9ml of sterile distilled water in sterile tubes. Using different sterile 1.0ml pipette, 0.1ml of 10^{-4} and 10^{-6} dilutions of the various samples were plated out.

2.4. Culture preservation

The isolates of Lactic acid bacteria were sub-cultured onto maintenance medium consisting of MRS broth with 12% (v/v) glycerol and incubated at 30°C until growth becomes visible. The stock cultures were stored at 4°C for subsequent use for a period of 2 to 4 weeks before sub-culturing into fresh medium. *Vibrio* species was stored in Tryptone Soy broth (TSB) and maintained at room temperature for further studies.

2.5. Characterization of isolates

This was carried out by employing macroscopic, microscopic and biochemical tests (all Gram positive, catalase negative and non-spores forming isolates were selected for sugar fermentation test).

2.6. Identification of Isolates

The isolates were identified based on the results of the various biochemical tests using Bergey's Manual of Systematic Bacteriology [20].

2.7. Antimicrobial activity of bacteriocin producing LAB

The agar diffusion assay described by [21]. Freshly prepared *Vibrio* species were used for this assay. One (1) ml of the indicator organism (*Vibrio* species) was inoculated into 15 ml of semisolid Mueller Hinton agar (MHA plus 0.75% bacteriological agar) and then poured into a petri dish. After solidification, three wells (7 mm diameter) were cut and 50 µl of cell-free supernatant (CFS) from each LAB isolate were added to each well. Cell-free supernatant was prepared as follows; one ml of frozen LAB isolate was cultured overnight in 20 ml MRS broth, then 1 ml culture was sub-cultured for 72 hours in 20 ml MRS broth. Cells were removed by centrifuging at 14,000g for 5mins and 50 µl of the unadjusted aliquot of cell-free supernatant was added to the wells. . The plates were incubated at 37˚C aerobically for 24 h. Inhibition zones were measured and recorded appropriately.

2.8. Characterization of bacteriocins produced by LAB

2.8.1. Treatment of antimicrobial compounds with NaOH and catalase enzyme

The isolates that exhibited antagonistic activity against the pathogenic organism were investigated for their antimicrobial compounds using a modified method of [22]. The cell-free supernatant was adjusted to pH 6.0 with 1 mol l^{-1} NaOH in order to rule out possible inhibition effects due to organic acids. 50 µl of the pH adjusted cell-free supernatant were filtered (0.2 µm pore-size cellulose acetate filter) and added to the second well. The neutralized cell-free supernatant was then treated with 1 mg ml-1 of catalase at 25 °C for 30 min to eliminate the possible inhibitory action of H_2O_2 and then was placed in the third well. The Mueller Hinton plates were incubated at 37°C aerobically for 24 h. Inhibition zones were measured and recorded appropriately. If inhibition zones are found in the third well, the isolates were considered to be able to produce bacteriocin-like substance.

2.8.2. Sensitivity of bacteriocin produced by LAB to proteinase

To confirm production of a proteinaceous compound, cell-free supernatant displaying antimicrobial potential after acid neutralization and H_2O_2 elimination were treated with 1 mg ml-1 of proteolytic enzymes, including Pepsin and Trypsin at 37 °C for 2 h [23; 24]. Each enzyme is dissolved in plug phosphate buffer and sterilized by filtration (0.2 µm). Antimicrobial activity of treated culture broth was determined by the agar diffusion bioassay as described above.

2.8.3. Thermal stability of bacteriocins produced by LAB isolates:

Sensitivity of the bacteriocins to heat was investigated using a modified method of [25]. The pH-adjusted and H_2O_2 eliminated cell-free supernatant described above were treated at 60 °C, 90 °C for 20 mins and at 121 °C for 15 mins. pH -adjusted and H_2O_2- eliminated cell-free supernatant without any heat treatments served as control. Residual antimicrobial activity of heat-treated culture broth was determined by the agar diffusion bioassay.

2.8.4. Effects of pH on the bacteriocins:

In order to determine the sensitivity of the bacteriocins to pH, a modified method of [25] was employed. The cell-free supernatant of each strain was adjusted to pH levels ranging from 2 to 10 (intervals of 2) with HCl and NaOH, incubated at 37 °C for 5h and then tested for bacteriocin activity using the agar well diffusion assay. The supernatant of unadjusted pH served as controls.

2.9. Production of a model cultured milk (Nono)

The effect of in situ bacteriocin production on the behavior of *Vibrio* spp in Nono was determined during storage using a modified method of [26]. Fresh milk was aseptically collected from the teat of a cow and transported to the laboratory. The milk was pasteurised at 72°C for 20 minutes. After pasteurisation the milk was cooled to 40 - 45°C. The milk was then inoculated with the bacteriocin producing LAB (1.5 X 10^8cfu/ml) and stirred well for 3-5 minutes to ensure uniform distribution of starter culture. The milk was fermented at 30°C for a period of 24 hours. None inoculated milk served as control. The fermented milk was then artificially contaminated with *Vibrio* species (1.5 X 10^6 cfu/ml). The food product was tested at selected intervals (12 h) for 72 hours for the presence or absence of *Vibrio* species. Counts of *Vibrio* species were performed on TCBS (Thiosulphate citrate bile salt sucrose) agar after incubation at 37°C for 48 hours.

3. Results

A total of 112 LAB strains were isolated from fermented milk products and were initially screened for antagonistic activity against six *Vibrio* species (*V. alginolyticus, V. parahaemolyticus, V. cholera, V. fluvialis, V. harveyi, V. vulnificus*) by the agar well diffusion assay using cell free supernatant of broth culture (Table 1). Of the 112 strains tested, 13 produced bacteriocin that inhibited *Vibrio* spp. Subsequently, only twelve were selected based on their zone of inhibition for identification to species level.

Table 1 Antagonistic activity of LAB metabolites isolated from milk products against Vibrio species

	Bacteriocin producing Lactic Acid Bacteria/Zone of Inhibition(mm)												
Indicator organisms	**W18**	**W23**	**N28**	**N29**	**N31**	**N39**	**N42**	**N43**	**W45**	**N46**	**N54**	**N56**	**N58**
V. fluvialis	17	17	14	15	15	18	15	16	16	14	15	17	18
V. cholera	16	16	16	16	16	15	18	14	14	15	13	13	15
V. parahaemolyticus	20	17	19	17	16	16	15	18	19	16	16	17	17
V. vulnificus	16	14	14	14	13	13	15	14	14	14	13	13	13
V. alginolyticus	18	14	14	14	13	12	13	14	14	13	14	14	14
V. harveyi	16	17	15	14	18	18	15	15	15	14	17	15	15

All the LAB isolates were Gram positive, catalase negative, non-spores forming, non-motile, cocci and rods. Carbohydrate utilization pattern of the isolates was used to differentiate and identified the isolates to species level. The isolates were identified as *Pediococcus damnosus, Pediococcus acidilactici, Lactobacillus brevis* and *Lactobacillus plantarum.* The cell free supernatant of the 12 strains of LAB were treated with catalase, NaOH, proteolytic enzymes (trypsin and pepsin) and tested by the agar well diffusion assay against the *Vibrio* species as shown in Table 2. Catalase and NaOH had no effect on the inhibition, that is, the antimicrobial activity was still maintained, indicating that hydrogen peroxide and organic acids respectively did not account for the observed inhibition. However, the antimicrobial activity from all the strains was completely inactivated by treatment with trypsin and pepsin (Table 2).

Table 2 Effects of NaOH, Catalase enzyme, Trypsin and Pepsin on the activity of bacteriocin produced by LAB

Isolates	Untreated	NaOH	Catalase	Trypsin	Pepsin
P. damnosus	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. brevis	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. brevis	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
P. damnosus	+	+	-	+	+

The bacteriocins produced by these isolates were heat-treated at 60°C, 90°C for 20 mins and at 121°C for 15 mins and were observed to be stable at 60°C - 90°C conditions as indicated by their inhibitory effects against *Vibrio* species (Table 3).

Table 3 Thermal stability of the bacteriocin produced by LAB isolates

Isolates	Temperature (°C)			
	Untreated	60	90	121
P. damnosus	+	+	+	-
P. acidilactici	+	+	+	-
L. brevis	+	+	+	-
P. acidilactic	+	+	+	-
L. brevis	+	+	+	-
P. acidilactic	+	+	+	-
L. plantarum	+	+	+	-
L. plantarum	+	+	+	-
L. plantarum	+	+	+	-
L. plantarum	+	+	+	-
L. plantarum	+	+	+	-
L. plantarum	+	+	+	-

Stability of the bacteriocin was evaluated at different pH values ranging from 2 to 10 at 37 °C for 5 h, it was observed that the antimicrobial activity against *Vibrio* species was retained in pH ranges form 2-6, however the inhibitory activity was lost at alkaline pH (Table 4).

Table 4 Stability of bacteriocin produced by LAB isolates at different pH

Isolates	pH 2	pH 4	pH 6	pH 8	pH 10
P. damnosus	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. brevis	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. brevis	+	+	+	-	-
P. acidilactici	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-
L. plantarum	+	+	+	-	-

The effect of *in situ* bacteriocin production on the survival of *Vibrio* spp in *Nono* was determined after fermentation during the storage period of 72 hours (12 hours interval). Figures 1a-d shows the survival of *Vibrio* species in *Nono* produced with bacteriocin-producing *Pediococcus damnosus*, *Pediococcus acidilactici*, *Lactobacillus brevis* and *Lactobacillus plantarum* respectively. It was observed that *Vibrio* species counts were reduced to different extents in all samples of Nono and undetetable within 48 to 60 hours of *Nono* storage. On the contrary, *Vibrio* species survived for 72 hours of storage in the control experiment that contained no bacteriocin-producing LAB.

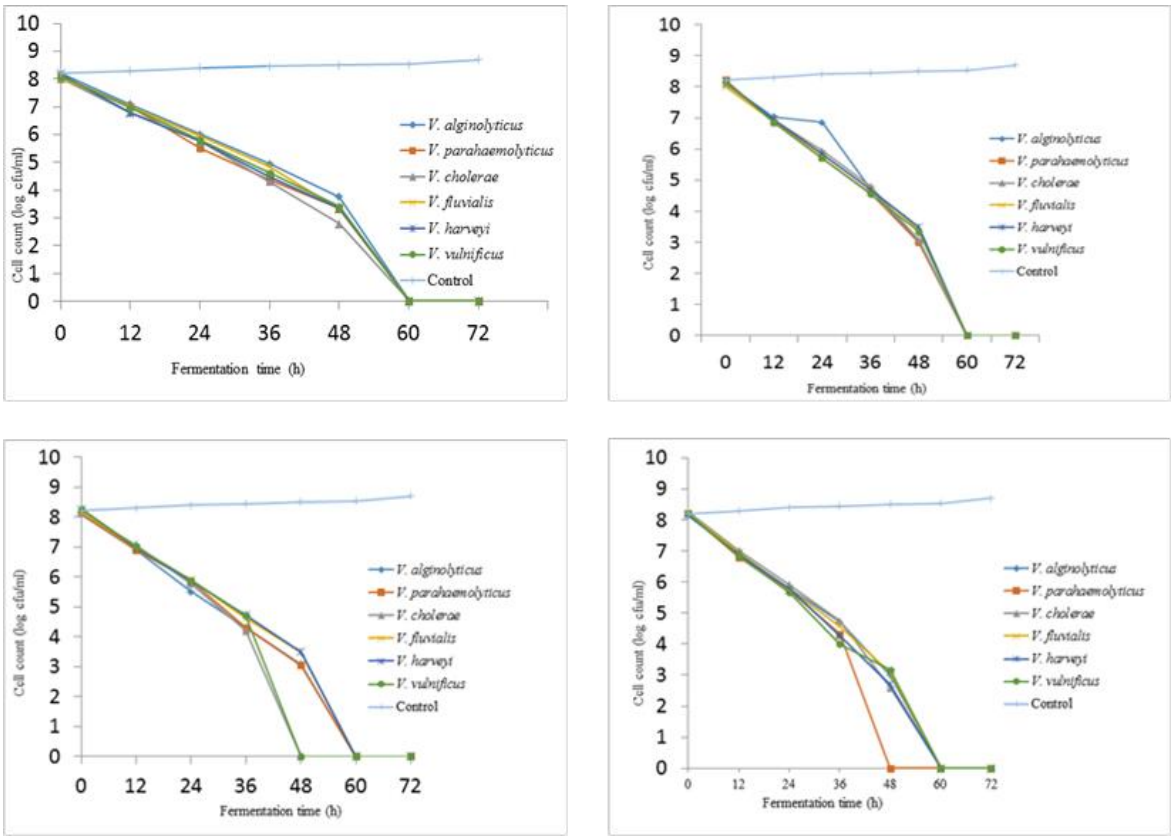

Figure 1 Survival of Vibrio spp. in Nono produced with bacteriocin-producing a- *Pediococcus damnosus*; b-*Pediococcus acidilactici*; c- *Lactobacillus brevis*; d- *Lactobacillus plantarum*

4. Discussion

This study was undertaken to demonstrate the antagonistic property of bacteriocin-producing Lactic acid bacteria on Vibrio cholera, Vibrio alginolyticus, Vibrio vulnificus, Vibrio parahaemolyticus, Vibrio harveyi, and Vibrio fluvialis in vitro and in situ

The fermented milk products (yoghurt, nono, wara) analyzed was found contained lactic acid bacteria (LAB) in different numbers. Nono had higher lactic acid bacteria counts than wara and yoghurt. This is in conformity with the report of [27] and [28] that in the locally fermented foods analyzed, nono had higher LAB count than wara, fufu and akamu. Phenotypic and biochemical identification of the twelve selected isolates were carried out.

A total of 112 LAB strains were isolated from fermented milk products and were initially screened for antagonistic activity against the Vibrio species by the agar well diffusion assay. Of the 112 strains tested, 13 produced an inhibition zone against the Vibrio spp. In this step, the possible inhibitory effect of the organic acids and hydrogen peroxide was not excluded. Subsequently, only twelve were selected by the large zone of inhibition for their identification at species level. The cell free supernatant of the 12 strains were treated with catalase, NaOH, proteolytic enzymes (trypsin and pepsin) and tested by the agar well diffusion assay against the Vibrio spp. Catalase and Sodium hydroxide (NaOH) treatment of cell free supernatant had no effect on the inhibitory activity, that is, the antimicrobial activity was still maintained, indicating that hydrogen peroxide and organic acids did not account for the observed inhibition. However, the antimicrobial activity from all the strains was completely inactivated by treatment of the cell free supernatant with trypsin and pepsin. This confirms that the inhibition is as a result of a proteinacious compound and provides evidence

that growth inhibition of the Vibrio species was caused by a bacteriocin. Bacteriocins have been reported to be inhibitory against several other bacteria [11; 29; 30; 31; 25; 22].

Phenotypic, morphological and biochemical characterization of the LAB isolates identified the organisms as *Pediococcus damnosus*, *Pediococcus acidilactici*, *Lactobacillus brevis* and *Lactobacillus plantarum*. The characterization of these isolates agreed with Bergey's Manual of Systematic Bacteriology [20].

The bacteriocins produced by LAB isolates were observed to be stable at 60°C, 90°C for 20 mins but not at 121°C for 15 mins. This is in agreement with the work of Andersson [32] who reported the loss of bacteriocin activity after heating at 121°C for 10 mins. Also, Ogunbanwo et al. [11] recorded the loss of inhibitory activity of bacteriocin produced by L. plantarum at 121°C for 10 mins. The thermal stability of the bacteriocins produced by these LAB isolates may constitute an advantage for potential use as biopreservatives in combination with thermal processing in order to preserve food products [22]. This resistance is also known for other bacteriocin produced by lactic acid bacteria: lactacin B [33], lactacin F [34], nisin [35] and bacteriocin ST15 [36].

Bacteriocins were stable at different pH values ranging from 2 to 6 (intervals of 2) at 37°C for 5h, it was observed that the antimicrobial activity against Vibrio species was retained in pH range 2-6. However the inhibitory activity was lost at pH 8 and 10. This is in agreement with the work of Ogunbanwo et al. [11] who reported that the activity of bacteriocin elaborated by the test isolates was pH dependent and recorded that the highest antibacterial activity was exhibited in an acidic pH range of 2 to 6, while inactivation occurred at pH 8 to 12. The work carried out by Lade et al. [37] also stated that bacteriocin were stable in acidic to neutral range i.e. from pH 4.0 to 7.0, but, inactive in the alkaline range. These data suggest that the bacteriocin described in this study could be applied in both low and medium-acidic food products with final pH values in such range; this includes a number of fermented and ripened dairy and meat products [25].

The effect of in situ bacteriocin production on the survival of Vibrio species in Nono was determined after fermentation during the storage period of 72 hours. It was observed that Vibrio species counts were reduced to different extents in all samples of Nono and undetetable within 48 to 60 hours in Nono produced with bacteriocin producing LAB during storage periods. On the contrary, Vibrio species survived for 72 hours of storage in the control experiment (unfermented milk) that contained no bacteriocin-producing LAB. This is in consonance with the work of Benkerroum et al. [38] that reported a significant decrease in the amount of L. monocytogenes in a 1-mL sample within 24 h of storage at 7°C in lben fermented with the bacteriocin-producing starter culture. The work demonstrates that the use of selected bacteriocin-producing starter in milk fermentation might contribute to ensuring the safety of dairy products, especially when they are obtained from raw or minimally processed milk.

5. Conclusion

From the present study, bacteriocin-producing LAB were obtained from fermented milk products and were able to inhibit the growth of *Vibrio* species with multiple antibiotic drug resistance. The selected bacteriocin-producing LAB isolated from milk fermentation could be used to inhibit *Vibrio* species and act as biopreservation of milk and its products. *Vibrio* species have shown to be sensitive to the bacteriocin produced by LAB *in vitro* and *in situ*.

Compliance with ethical standards

Acknowledgments

The authors are grateful to the department of Microbiology University of Ibadan and the Department of medical Laboratory Science, Ogun State College of Health Technology, Ilese Ijebu for making available their facilities for usage.

Disclosure of conflict of interest

Authors declared that there is no conflict of interest.

References

[1] Michaela S, Reinhard W, Gerhard K and Christine ME. (2009). Cultivation of anaerobic and facultatively anaerobic bacteria from spacecraft-associated clean rooms. Applied and Environmental Microbiology, 11(75), 3484-3491.

[2] Axelsson L. (2004). Lactic Acid Bacteria: Classification and physiology. In: Lactic Acid Bacteria, Microbiological and Functional Aspects. Salminen, A.V. and A.O. Wright (Eds) ouwehand. Marcel Dekker, New York, 1-66.

[3] Whittenbury R. (1964). Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. Journal of General Microbiology, 35, 13-26.

[4] Oladele AC, Deji-Agboola AM and Ogunbanwo ST. (2010). Antibacterial Activity of Lactic Acid Bacteria Isolated from Healthy Human Vagina Against Sexually Transmitted Disease Organisms. Ethiopian Pharmaceutical Journal, 28, 1-11.

[5] Salminen S, Von WA, Morelli L, Marteau P, Brassart D, de Vos WM, Fonden R, Saxelin M, Collins K., Mogensen G, Birkeland SE and Mattila-Sandholm T. (1998). Demonstration of safety of probiotics—a review. International Journal of Food Microbiology, 44, 93–106.

[6] Vandenbergh RA. (1993). Lactic acid bacteria, their metabolic products and interference with microbial growth. FEMS Microbiology Reviews, 12, 221–238.

[7] Bowdish DM, Davidson DJ and Hancock RE. (2005). A re-evaluation of the role of host defence peptides in mammalian immunity. Current Protein and Peptide Science, 6, 35–51.

[8] Diop MB, Dibois-Dauphin R, Tine E, Jacqueline ANand Thonart P. (2007). Bacteriocin producers from traditional food products. Biotechnology, Agronomy, Society and Environment, 11, 275–281.

[9] Ogunbanwo ST, Sanni AI and Onilude AA. (2003). Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. African Journal of Biotechnology, 2, 219-227.

[10] Nettles CG and Barefoot SF. (1993). Biochemical and genetic characteristics of bacteriocins of food associated lactic acid bacteria. Journal of Food Protection, 56, 338-356.

[11] Drake SL, Depaola A and Jaykus L. (2007). An Overview of Vibrio vulnificus and Vibrio parahaemolyticus. Comprehensive Reviews in Food Science, 6, 120-144.

[12] Sasaki S, Suzuki H, Igarashi K, Tambatamba B and Mulenga P. (2008). Spatial analysis of risk factor of cholera outbreak for 2003–2004 in a periurban area of Lusaka, Zambia. American Journal of Tropical Medicine and Hygiene, 79, 414-421.

[13] Huq A, Small EB, West PA, Huq MI, Rahman R and Colwell RR. (1983). Ecological relationships between Vibrio cholerae and planktonic crustacean copepods. Applied and Environmental Microbiology, 45, 275-283.

[14] Rabbani GH and Greenough WB. (1999). Food as a Vehicle of Transmission of Cholera. Journal of Diarrhoeal Diseases Research, 17(1), 1-9.

[15] Shapiro RL, Altekruse S, Hutwagner L, Bishop R, Hammond R, Wilson S, Ray B, Thompson S, Tauxe RV, Griffin PM and the Vibrio Working Group. (1998).The role of Gulf Coast oysters harvested in warmer months in Vibrio vulnificus infections in the United States, 1988-1996. Journal of Infectious Diseases, 178, 752-759.

[16] Abdullahi IO, Umoh VJ and Galadima M. (2004). Hazards associated with Kilishi preparation in Zaria, Nigeria. Nigeria Journal of Microbiology, 18(1-2), 338-345.

[17] Chigozie Oramadike and Samuel Temitope Ogunbanwo. (2014). Incidence of Vibrio species in seafood samples collected from Lagos Lagoon, Nigeria. American Journal of Food Science and Nutrition, 1(5), 76-82.

[18] Sneath PA, Mair NS, Sharpe ME and Holts JG. (1986). Bergey's Manual Systematic Bacteriology, Vol. 2, Baltimore MD: Willam and Wilkins.

[19] Ogunbanwo ST, Oloketuyi SF and Adegoke CO. (2015). Potency of Bacteriocin Porduced by Enterococcus species isolated from Wara a Nigerian White Soft Unripened Cheese against Pathogenic Organisms Journal of Antimicrobials. Photon, 130, 382-390.

[20] Yang E, Fan Lihua F, Jiang Y, Doucette C and Fillmore S. (2012). Antimicrobial activity of bacteriocin-producing Lactic acid bacteria isolated from cheeses and yogurts. AMB express, 2, 48.

[21] Bonadè A, Murelli F, Vescovo M and Scolari G. (2001). Partial characterization of a bacteriocin produced by Lactobacillus helveticus. Letters in Applied Microbiology, 33, 153.

[22] Herreros MA, Sandoval H, González L, Castro JM, Fresno JM and Tornadijo ME. (2005). Antimicrobial activity and antibiotic resistance of lactic acid bacteria isolated from Armada cheese (a Spanish goats‟ milk cheese). Food Microbiology, 22, 455-459.

[23] Khay E, Idaomar M, Castro L, Miguel P, Bernardez P F, Senhaji SN and Abrini J. (2011). Antimicrobial activities of bacteriocin-like substatnces produced by Lactic acid bacteria isolated from Moroccan dromedary milk. African Journal of Biotechnology, 10(51), 10447-10455.

[24] Adesokan IA, Odetoyinbo BB, Ekanola YA, Avanrenren RE and Fakorede S. (2011). Production of Nigerian Nono using Lactic Starter Cultures. Pakistan Journal of Nutrition, 10(3), 203-207.

[25] Odunfa SA. (1985). African fermented foods. In. B.J.B Wood, ed. Microbiology of Foods. London: EL Sevier, 155-191.

[26] Oyeleke SB, Faruk AK, Oyewole OA and Nabara HY. (2006). Occurrence of lactic acid bacteria in some locally fermented food products sold in Minna markets. Nigerian Journal of Microbiology, 20(2), 927- 930.

[27] Flythe MD and Russsell JB. (2004). The effect of pH and a bacteriocin (bovicinHC5) on Clostridium sporogenes MD1, a bacterium that has the ability to degrade amino acids in ensiled plant materials. FEMS Microbiology Ecology, 47, 215-222.

[28] Moghaddam MZ, Sattari M, Mobarez AM and Doctorzadeh F. (2006). Inhibitory effect of yogurt Lactobacilli bacteriocins on growth and verotoxins production of enterohemorrhgic Escherichia coli O157:H7. Pakistan Journal of Biological Science, 9(11), 2112-2116.

[29] Karthikeyan V and Santosh SW. (2009). Isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum*. African Journal of Microbiology Research, 3(5), 233-239.

[30] Andersson R. (1986). Inhibition of Staphylococcus aureus and spheroplasts of gram-negative bacteria by antagonistic compound produced by a strain of *Lactobacillus plantarum*. International Journal of Food Microbiology, 3, 149-160.

[31] Barefoot SF and Klaenhammer TK. (1983). Detections and activity of lactacin B on bacteriocin produced by Lactobacillus acidophilus. Applied and Environmental Microbiology, 45, 1808-1815.

[32] Muriana PM and Klaenhammer TR. (1987). Conjugal transfer of pasmid-encoded determinants for bacteriocin production and immunity in Lactobacillus acidophilus 88. Applied and Environmental Microbiology, 53, 553-560.

[33] Bailey FJ and Hurst A. (1971). Preparation of a highly active form of nisin from Streptococcus lactis. Canadian Journal of Microbiology, 17, 61-67.

[34] De Kwaadsteniet M, Todorov SD, Knoetze H and Dicks LMT. (2005). Characterization of a 3944 Da bacteriocin, produced by Enterococcus mundtii ST15, with activity against Gram-positive and Gram-negative bacteria. International Journal of Food Microbiology, 105, 433–444.

[35] Lade HS, Chitanand MP, Gyananth G and Kadam TA. (2006). Studies on some properties of Bacteriocins produced by Lactobacillus species Isolated from Agro-Based waste. International Journal of Microbiology, 2(1), 1937-8289.

[36] Benkerroum N, Ghouati Y, Ghalfi H, Elmejdoub T, Roblain D, Jacques P and Thonart P. (2002). Biocontrol of Listeria monocytogenes in a model cultured milk (lben) by in situ bacteriocin production from Lactococcus lactis ssp. Lactis. International Journal of Dairy Technology, 55, 145–151.

How to cite this article

Ogunbanwo ST, Odubanjo OR, Adegoke CO and Oramadike C. (2020). Bio-control of Vibrio species in cultured milk by in situ bacteriocin production from lactic acid bacteria. World Journal of Advanced Research and Reviews, 6(3), 50-58.