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Isolation, Identification and Mode of action of Partially Purified Bacteriocins from Lactic Acid Bacteria in Fermented Cassava Grits

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study on the isolation, identification and mode of action of partially purified bacteriocin from lactic acid bacteria found in fermented cassava grits was carried out. Fermented cassava grits were collected from different garri processing plants and transported with cold box to the laboratory for analysis. The viable microbial count after the partially purified bacteriocin from the various lactic acid bacteria isolates were grown against the food borne bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) ranged from 0.98 x 10^3 CFU/ml for partially purified bacteriocin from isolate 6 at 8 hrs to 9.2 x10^3 CFU/ml for isolate 3 at 24 hrs. Similar results were obtained against *Bacillus subtilis* with microbial counts that ranged from 1.02 x10^2 CFU/ml for isolate 3 at 8 hrs to 9.2 x 10^2 CFU/ml at 24 hrs. Isolates 6, 7, 10 and 11 were bactericidal to both *Staphylococcus aureus* and *Bacillus subtilis* while isolate 3 was bacteriostatic. The viable microbial count after the partially purified bacteriocin was grown against the foodborne bacteria (*Escherichia coli* and *Salmonella typhi*) ranged from 1.0 x10^2 CFU/ml for partially purified bacteriocin from isolate 3 at 8 hrs to 7.1 x 10^2 CFU/ml for partially purified bacteriocin from isolate 6 at 24 hours. The microbial count against *Salmonella typhi* ranged from 6.50 x 10^2 CFU/ml for isolate 6 at 8 hrs to 8.5 x 10^2 CFU/ml for

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isolate 7 at 24 hrs. Partially purified bacteriocins from isolates 3 and 7 were bacteriostatic while isolates 6, 10 and 11 were bactericidal to *Escherichia coli* and *Salmonella typhi.* This result showed that the partially purified bacteriocins were very efficacious in killing or inhibiting the growth of some foodborne pathogens which can be applied in biopreservation.

Keywords: Bactericidal; biopreservation; mode of action; cassava grits; microbiological.

1. INTRODUCTION

Lactic acid bacteria (LAB) are a large group of beneficial bacteria belonging to different taxonomic groups, but unified on the basis of their shared metabolic and physiological characteristics. Morphologically, they are gram positive, generally non-sporulating, non-respiring, either rod-shaped (bacilli) or spherical (cocci) bacteria which produce lactic acid as the major metabolic end product of carbohydrate fermentation. Their importance is associated mainly with their safe metabolic activity while growing in foods utilizing available sugar for the production of organic acid and other metabolites. Their common occurence in foods along with their long lived uses contributes to their natural acceptance as GRAS (Generally Recognised as Safe for Human Consumption [1]. Bacteriocins are ribosomally synthesized peptides that when secreted act selectively on other bacteria, permeabilizing its membrane and potentially leading to cell death [2]. In recent times, bacteriocins eg nisin, a bacteriocin produced by *Lactococcus lactis,* is generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and is currently being used as a preservative agent in the food industry to prevent the growth of *Listeria monocytogenes* and other food pathogens [3]. The commercialization of nisin since the 1950s, triggered the research interest to isolate new bacteriocins from different sources so that by the 1990s, there was a variety of bacteriocins with different activity spectra, some of which are still in the process of seeking approval for use as food preservative. The aim of this research was to isolate, identify and determine the mode of action of partially purified bacteriocins from the lactic acid bacteria isolated from fermented cassava grits which could be applied in biopreservation.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fifteen samples of fermented cassava grits were randomly collected from different garri processing plants in Abakaliki metropolis. The samples were collected in cold box and transported to the Applied Microbiology Laboratory of Ebonyi State University, Abakaliki for analysis.

2.2 Isolation and Identification of Lactic Acid Bacteria

Ten grams of the fermented cassava grits was added to 90 ml of distilled water and homogenized in a stomacher (Seward Somacher Lab Blenders, UK) for 5 minutes. After ten- fold serial dilution, 0.1ml of the sample homogenate was plated out on De Man Rogosa sharp agar [4] which was prepared and sterilized according to the manufacturer's instructions. The streaked plates were incubated anaerobically using an anaerobic jar (Gas pak) with CO_2 generating kit at 30^{o}C for 48 hours. The pure colonies obtained were identified by morphological and biochemical characterization according to Cheesbrough [5].

2.3 Isolation of Bacteria Pathogens from Spoilt Cucumber Samples

The cucumber samples were processed by first removing the outer leaves and 20 g of each of the samples were weighed, washed with distilled water and placed on an electric blender. The blended samples were put in a clean beaker containing 20ml of sterile water and sieved. The resulting filtrate were plated on Cysteine Lactose Electrolyte Deficient (CLED) agar, Mannitol salt agar, *Salmonella-shigella* (SS) agar and Nutrient agar which were used to isolate different foodborne bacteria present and incubated at 37^{o}C for 24 hrs. The discrete colonies of each of the bacterial isolates were identified by standard morphological and biochemical tests [5].

2.4 Production and Assay of Crude Bacteriocin

The preliminary isolation and characterization of lactic acid bacteria was carried out using standard microbiology technique after which the bacteriocin was extracted by growing the bacteriocin producing bacteria in 1000 ml of De

Man Rogosa sharp (MRS) broth and incubated for 72 hours at 30^{o}C under anaerobic conditions. Extract was obtained by centrifuging the culture at 12, 000 rpm for 15 minutes to pellet down the cells [6].

2.5 Partial Purification of Bacteriocins

The cell free supernatant (CFS) from each of the bacterial culture centrifuged at 10,000 x g, 4^{o}C, 10 minutes and the pH was adjusted to 6.5. The bacteriocin was precipitated by the addition of 40% ammonium sulphate in conical flask and mixtures were stirred overnight at 4^{o}C. Then the mixtures were centrifuged at 10, 000 x g, 4^{o}C, 10 min. The precipitated bacteriocins that adhered on the wall of the tube were resuspended in 1ml of 0.2M phosphate – buffered saline (PBS). The precipitate was subjected to dialysis through a membrane (Dialysis bag). The partially purified bacteriocins obtained were stored at – 20^{o}C [7].

2.6 Mode of Action of the Partially Purified Bacteriocin

The procedure followed was described by Nilsen et al. [8] in which 5 ml aliquots of the PPB from each of the LAB isolates was added to 20 ml of a suspension of 24 hour old 0.5 MacFarland standard of *Staphylococcus aureus, B. subtilis, E. coli and S. typhi* in Nutrient Broth in a McCartney bottle. *S. aureus, B. subtilis, E.coli and S. typhi* cells were allowed to grow. The optical densities (OD at 640 nm) were measured and recorded at 8 hours intervals. The investigation was terminated by plating aliquots on nutrient agar plates and incubated at 37^{o}C for 24 hours to determine presence or absence of growth and the microbial count.

3. RESULTS

3.1 Colony and Biochemical Characteristics of Isolated LAB

Table 1 revealed that a total of five isolates were identified out of the 15 samples collected from locally fermented food. Out of the five isolated, all were Gram positive and catalase negative.

The isolated foodborne bacteria isolated from cucumber samples were shown in Table 3. It revealed that the probable bacteria isolated were *E. coli*, *S. aureus*, *Bacillus subtilis* and *Salmonella* species.

3.2 Production and Assay of Crude Bacteriocin

Fig. 1 below showed the crude bacteriocin produced by the various LAB isolates. This showed that isolate 10 produced the highest crude bacteriocin.

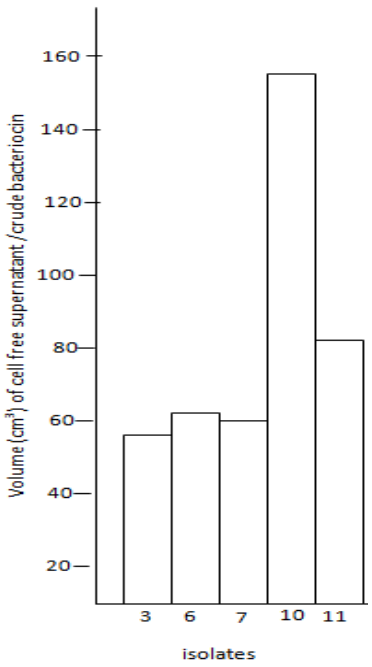

Fig. 1. The volume of crude bacteriocin produced by each lactic acid bacteria isolates

Table 1. Morphological and biochemical characteristics of lactic acid bacteria from fermented cassava grits

Isolate code	Source	Cell shape	Oxidase	Gram reaction	Catalase activity	Spore formation	Anaerobic growth	Citrate
3	FCG	C	-	+	-	-	+	-
6	FCG	R	-	+	-	-	+	-
7	FCG	C	-	+	-	-	+	-
10	FCG	R	-	+	-	-	+	-
11	FCG	C	-	+	-	-	+	-

C = Cocci, R = Rod, FCG = Fermented Cassava Grits

Table 2. Carbohydrate utilization patterns of five isolates

Carbohydrates	Isolates				
	3	6	7	10	11
Lactose	+	+	+	+	+
Xylose	+	+	+	+	+
D-fructose	+	+	+	+	+
Dextrose	+	+	+	+	+
Galactose	+	+	+	+	+
Maltose	+	+	+	+	+
Raffinose	+	+	+	+	+
Trehalose	+	+	+	+	+
Melibiose	+	+	+	+	+
Sucrose	+	+	+	-ve	+
L-Arabinose	+	+	+	-ve	-ve
Mannose	+	+	+	+	+
Sorbitol	+	+	+	+	+
Ribose	+	+	+	+	+
Adonitol	+	+	+	-	+
	Lactobacillus pentosus	*Lactobacillus spp*	*Lactobacillus spp*	*Lactobacillus plantarum*	*Lactobacillus fermentum*

Table 3. Morphological, microscopic and biochemical characteristics of bacteria isolated from cucumber sample

Morphological Characteristics		Biochemical Tests						Sugar Fermentation Test							
Shape	Colour	Gram reaction	Motility Test	Citrate Test	Oxidase Test	Coagulase Test	Indole Test	Lactose	Glucose	Sucrose	Catalase Test	Urease Test	Voges Proskauer	Methyl Red	Probable isolates
Cocci	Yellow	+	-	-	-	+	-	+	+	-	+	+	+	+	*E. coli*
Rods	opaque yellow colonies with slightly yellow center on CLED	-	+	-	-	-	+	+	+	+	+	-	-	+	*S. aureus*
															Bacillus subtilis
Gray-white round, opaque, flat on NA		+	+	+	+	-	-	-	+	+	+	-	+	-	
Rods and	black	-	+	-	-	+	-	-	+	-	+	-	-	+	*Salmonella specie*

Key: + = Positive, - Negative, NA- Nutrient Agar

Table 4. Microbial counts against Gram positive foodborne bacteria showing mode of action of PPB

Isolates	Microbial counts (*S. aureus*)		Microbial counts (*B. subtilis*) CFU/ml	
	8 hours	24 hours	8 hours	24 hours
3	1.22×10^3	9.2×10^3	1.02×10^2	8.8×10^2
6	0.98×10^3	8.5×10^2	7.1×10^3	6.2×10^2
7	1.12×10^3	8.2×10^2	7.0×10^3	9.2×10^2
10	1.42×10^3	1.3×10^2	4.2×10^3	3.0×10^2
11	1.65×10^3	1.2×10^2	1.42×10^3	1.05×10^2

Table 5. Microbial counts against Gram negative foodborne bacteria showing mode of action of PPB

Isolates	Microbial counts (*E. coli*)		Microbial counts (*S. typhi*) CFU/ml	
	8 hours	24 hours	8 hours	24 hours
3	1.0×10^2	3.6×10^2	8.50×10^2	7.2×10^2
6	8.5×10^3	7.1×10^2	6.50×10^2	5.8×10^1
7	8.2×10^2	7.0×10^2	9.10×10^2	8.5×10^2
10	1.28×10^3	1.1×10^2	1.10×10^3	2.3×10^2
11	1.16×10^3	1.7×10^2	1.25×10^3	2.50×10^2

Table 4 showed the viable microbial count of *S. aureus* and *B. subtilis* after incubation with PPB from the various isolates at 8 hours and 24 hours interval. The result revealed that PPB from isolates 6,7,10 and 11 were bactericidal while isolate 3 was bacteriostatic.

Table 5 shows the viable microbial count of *E. coli* and *S. typhi* after incubation with PPB from the various isolates at 8 hours and 24 hours interval. The result revealed that PPB from isolates 6, 10 and 11 were bacteriocidal while isolates 3 and 7 were bacteriostatic.

4. DISCUSSION

A total of 15 fermented cassava grits were collected from different garri processing plants. The colony morphology, Gram's reaction, catalase test and other biochemical tests were used to identify the five (5) lactic acid bacteria in these fermented foods. This result was similar to the work of Ohenhen et al. [9] who isolated 5 different species of *Lactobacillus* species from fermented ogi samples. The result of the morphological and biochemical characteristics of bacteria isolated from spoilt cucumber showed that *E. coli, Staphylococcus aureus, Bacillus subtilis* and *Salmonella* sp were present. These bacteria are mostly enteric microbes which suggests possible fecal contamination from faecal materials. This is in agreement with the work of Sujeet and Vipin [10] who reported the presence of the same bacterial species in cabbage and other salad vegetables. The viable microbial count after the partially purified bacteriocin from the various lactic acid bacteria isolates were grown against the food borne bacteria (*S. aureus* and *B. subtilis*) ranged from 0.98×10^3 CFU/ml for PPB from isolate 6 at 8 hrs to 9.2×10^3 CFU/ml for PPB from isolate 3 and 1.02×10^2 CFU/ml for PPB from isolate 3 at 8 hours to 9.2×10^2 CFU/ml for PPB from isolate 7 at 24 hrs. The microbial count for PPB from isolate 3 at 8 hours and 24 hour intervals were 1.22×10^3 CFU/ml and 9.2×10^3 CFU/ml for *S. aureus*. There was no remarkable reduction in the microbial count over a long period of time, hence they are bacteriostatic. For PPB from isolate 6, isolate 7, isolate 10 and isolate 11, microbial counts at 8 hrs and 24 hrs intervals were from 0.98×10^3 CFU/ml to 8.5×10^2 CFU/ml. This showed that the partially purified bacteriocin (PPB) was bactericidal in its mode of action. This result corroborates the work of Wayah and Philip [11] who reported that a bacteriocin, Pentocin MQ1 was bactericidal to *L. monocytogenes* and *B. cereus*. Similarly, the results for PPB against *B. subtilis* followed the same trend as PPB from isolates 6, 7, 10 and 11 were bacteriocidal to the foodborne bacteria while isolate 3 was bacteriostatic. The viable microbial count after the PPB was grown against the foodborne bacteria (*E. coli* and *S. typhi*) ranged from 1.0×10^2 CFU/ml for PPB from isolate 3 at 8hrs to 8.2×10^2 CFU/ml for PPB from isolate 7 at 24 hours. The microbial count for partially purified bacteriocin from isolate 3 at 8 hours and 24 hours intervals were 8.5×10^2 CFU/ml and 7.2×10^2 for *S. typhi*. There was no appreciable reduction in the microbial count over a long period of time, hence they are bacteriostatic. It was observed that they exhibited bacteriostatic mode of action. Partially purified

bacteriocin from isolates 6, 10 and isolate 11 had microbial counts of 8.5 x 10^3 to 7.1 x 10^2 CFU/ml, 1.28 x 10^3 to 1.1 x 10^2 CFU/ml, 1.16 x 10^3 to 1.7 x 10^2 CFU/ml at 8 hrs and 24 hrs respectively. It was observed that the PPB were bactericidal. This was consistent with the work of Zhao et al. [12] who reported the bactericidal mode of action of plantaricin 827 on *S. aureus* thereby extending the shelf life of skin milk. Similarly, Yi et al. [13] equally stated that a novel bacteriocin produced by *L*. *crustorum* MNO47 from China, had bactericidal action on indicator organisms. Similarly, the microbial count for PPB from isolate 3 against *S. typhi* were 8.50 x 10^2 CFU/ml at 8 hrs and 7.2 x 10^2 CFU/ml after 24 hours. Same trend of results were recorded for PPB from isolate 7 which were from 9.10 x 10^2 to 8.5 x 10^2 CFU/ml. This result showed that the partially purified bacteriocins had bacteriostatic activity on *S. typhi*. There was observed decrease in microbial counts for PPB from isolate 6 against *S. typhi* from 8 hrs to 24 hrs with a value of 6.50 x 10^2 to 5.1 x 10^1 CFU/ml and isolate 10 with a value of 1.10 x 10^3 to 2.3 x 10^2 CFU/ml. These indicated bactericidal mode of action of the PPB on *S. typhi*. This corroborates the report of Jiang et al. [14] who reported the bactericidal mode of action of Pentocil JL-1 from *L. pentosus* against *S. aureus*. The result in this research corroborates the work of Xinran et al. [15] who reported that plantaricin JY22, a novel bacteriocin isolated from *L. plantarum* JY22 had bactericidal activity on *B. cereus*.

5. CONCLUSION

The different partially purified bacteriocins from the various isolates exhibited different modes of action against the different foodborne bacteria. This showed that some partially purified bacteriocins or bacteriocins can be used for biopreservation and this study will help researchers and industries to choose the most appropriate bacteriocins to be used specifically to prevent and preserve certain foods from foodborne pathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bourdichon F, Casaregola S, Farrokh C, Frisvad JC, Gerds ML, Hammes WP, Harnett J, Huys G, Laulund S, Ouwehand A, Powell IB, Prajapati JB, Seto Y, Schure ET, Boven AV, Vankerckhoven V, Zgoda A, Tuijtelaars S, Hansen EB. Food fermentations: Microorganisms with technological beneficial use. International J Food Microbiol. 2012;154(3):87- 97.
2. Jordan K, Dalmasso M, Zentek J, Mader A, Bruggeman G, Wallace J. Microbes versus microbes: control of pathogens in the food chain. J. Sci. Food Agric. 2014;94:3079–3089.
3. Singh VP. Recent approaches in food bio-preservation - a review. Open Vet J. 2018; 8(1):104-111.
4. Uzoh CV, Orji JO, Okeh CO, Nworie CO, Igwe PC. Preliminary probiotic properties of lactic acid bacteria isolated from locally fermented food condiment - Ogiri. International J Advanced Res Biol Sci. 2022;9(4):21-30.
5. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edition Cambridge University Press, Cambridge Edition. 2006;72-75.
6. Hashim GM, Almasaudi SB, Azhar E, Al Jaouni SK, Harakeh S. Biological activity of Cymbopogon schoenanthus essential oil Saudi J Biol Sci. 2017;24(7):1458-1464.
7. Zhang J, Yang Y, Yang H, Bu Y, Yi H, Zhang L, Han X, Ai L. Purification and Partial Characterization of Bacteriocin Lac-B23, a Novel Bacteriocin Production by Lactobacillus plantarum J23, Isolated From Chinese Traditional Fermented Milk. Front. Microbiol. 2018;9:2165-2172.
8. Nilsen T, Nes IF, Holo H. Enterolysin A, a Cell Wall-Degrading Bacteriocin from Enterococcus faecalis LMG 2333. Appl. Environ. Microbiol. 2003;69:2975–298.
9. Ohenhen RE, Isibor JO, Emonfonmwan G, Enabulele SA. Effect of pH and Storage Temperature on Antimicrobial Activity of Bacteriocin Produced by Lactic Acid Bacteria Isolated from Ogi. British Microbiol. Research J. 2015;9(3):1-9.
10. Sujeet KM, Vipin K. A Study on Prevalence of Microbial Contamination on the Surface of Raw Salad Vegetable. International J Food Microbiol. 2017;6:411–418.
11. Wayah SB, Philip K. Pentocin MQ1: A novel, broad spectrum, pore forming bacteriocin from Lactobacillus pentosus CS2 with Quorum sensing regulatory mechanism and biopreservative potential. Front Microbiol. 2018;9:564-569.
12. Zhao D, Wang Q, Lu F, Bie X, Zhao H, Lu Z, Lu Y. A novel plantaricin 827 effectively

inhibits Staphylococcus aureus and extends shelf life of skim milk LWT - Food Science and Technol. 2022;154:1-11.

13. Yi L, Dang Y, Wu J, Zhang L, Liu X, Liu B, Zhou Y, Lu X. Purification and characterization of a novel bacteriocin produced by Lactobacillus crustorum MN047 isolated from koumiss from Xinjiang, China J. Dairy Sci. 2016;99: 7002–7015.
14. Jiang H, Zou J, Cheng H, Fang J, Huang G. Purification, Characterization, and Mode of Action of Pentocin JL-1, a Novel Bacteriocin isolated from Lactobacillus pentosus, against Drug-Resistant *Staphylococcus aureus*. BioMed Res International. 2017;20:1-12.
15. Xinran L, Luhuan M, Huanhuan M, Fengling B, Yang L, Mengtong S, Jianrong L. Purification, characterization and action mechanism of plantaricin JY22, a novel bacteriocin against Bacillus cereus produced by Lactobacillus plantarum JY22 from golden carp intestine Food Sci Biotechnol. 2018;27(3):695–703.

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