International Journal of Pathogen Research

Volume 13, Issue 6, Page 9-20, 2024; Article no.IJPR.124457
ISSN: 2582-3876

Antagonistic Effect of Crude Bacteriocin Produced by *Chungangia koreensis* Strain CAU 9163 against *Campylobacter* spp.

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpr/2024/v13i6317

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124457

Original Research Article

Received: 01/08/2024
Accepted: 04/10/2024
Published: 09/10/2024

ABSTRACT

This study aims to detect the antagonistic effect of crude bacteriocin produced by *Chungangia koreensis* strain CAU 9163, isolated from fermented cereal slurry (pap) and determine its inhibitory spectra against two species of Campylobacter, a leading cause of bacterial foodborne diarrheal disease worldwide. The *Chungangia koreensis* CAU 9163, a Gram-staining-positive, strictly aerobic, non-spore-forming, rod-shaped bacterial strain isolated from pap, could produce crude bacteriocin that inhibited *Campylobacter jejuni* and *coli*. The crude bacteriocin was stable at 60^0C for 15 minutes and at acidic to slight alkali pH of 4 to 8. It was stable in the presence of amylase and lipase enzymes and deactivated in the presence of pepsin and proteinase k enzyme. The

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Cite as: *Uzor, B. C., and S.O. Umeh. 2024. "Antagonistic Effect of Crude Bacteriocin Produced by Chungangia Koreensis Strain CAU 9163 Against Campylobacter Spp". International Journal of Pathogen Research 13 (6):9-20. https://doi.org/10.9734/ijpr/2024/v13i6317.*

bacteriocin had a molecular weight of 5.6KDa. This study shows that bacteriocin producing *Chungangia koreensis* CAU 9163 could potentially be used as a bio-preservative for food products.

Keywords: Bio-preservative; Chungangia koreensis; crude bacteriocin; Campylobacter sp.; diarrhoea.

1. INTRODUCTION

"Gastrointestinal microflora helps to maintain a microbial barrier against the colonization and proliferation of pathogens in the digestive tract" [1]. "Pathogens are responsible for inciting intestinal infections that negatively affect the normal functions of the gastrointestinal tract, leading to diseases such as cholera, typhoid, salmonellosis, acute gastroenteritis, traveller's diarrhoea and Shigellosis. These diseases are characterised by a common symptom known as diarrhoea. Diarrhoea is an illness characterized by stools in increased frequency and fluidity and it is one of the most common illnesses causing infant death in developing countries. Consumption of contaminated food, poor hygiene, and proximity to animals are the reasons why pathogens (such as *Campylobacter jejuni, Escherichia coli, Salmonella* species, *Shigella* species, *Staphylococcus aureus, Clostridium difficile* etc.) find their way to the gastrointestinal tract" [2,3]. Diarrhoea, although self-limiting, may sometimes require antibiotic therapy.

"Pap, an acid-fermented cereal gruel, is a major staple sour porridge food widely taken in West Africa. It also serves as the traditional infant weaning food. It is generally obtained by fermenting maize grains (*Zea mays*). sorghum (*Sorghum vulgare,*), or millet (*Pennisetum americanum*)" [4]. "It is the most popular traditional health-sustaining fermented food in Western and Eastern, Nigeria, and serves as weaning food for infants in these regions. Raw cereal pap when dissolved, is normally administered to people suffering from gastroenteritis to reduce discomforts" [5]. "It is assumed to contain some lactic acid bacteria that are known for their beneficial importance. These lactic acid bacteria are widely used for the production of fermented food products, they play an important role by improving the texture, flavour, boost immunity and shelf life of fermented food products. The production of diverse antibacterial compounds, such as organic acids, diacetyl, hydrogen peroxide and proteinaceous molecules, known as bacteriocins leads to the inhibition of food spoilage and pathogenic bacteria. Bacteriocins are antimicrobial peptides produced by ribosomes of bacteria, which have the characteristics of inhibiting other bacteria. A wide range of pathogens especially bacteria, are already developing resistance to most of the conventional antibiotics" [6]. "Therefore it is necessary to look into the scientific basis of some traditional remedies for diarrhea through the use of fermented foods that naturally contain beneficial microorganisms that will help to successfully compete with, and inhibit the growth of gastrointestinal pathogens. *Chungangia koreensis,* a Lactic acid bacteria producing a bacteriocin have been isolated from marine sediments in Southern Asia" [7].

This research isolated the same Lactic acid bacteria *Chungangia koreensis* CAU 9163 able to produce crude bacteriocin which could inhibit *Campylobacter jejuni* and *Campylobacter coli* a food borne pathogens isolated from fermented pap. The detection and characterization of the bacteriocin as well as their inhibitory spectra against *Campylobacter* sp. are present in this work.

2. MATERIALS AND METHODS

Four hundred and fifty samples of three different types of pap (ogi) were collected from different markets in Elele, Rivers State. The samples were made from either corn (*Zea mays*), sorghum (*Sorghum vulgare*,), or millet (*Pennisetum americanum*).

2.1 Isolation and Phenotypic Identification of Lactic Acid Bacteria

LAB was isolated from pap samples by pour plate method using de Man Rogosa and Sharpe (MRS) agar (Himedia, Laboratory, India) according to Fossi et al. [8]. For this purpose, ten-fold serial dilution was realised with saline solution (NaCl, 0.85% w/v). One ml aliquot of the 10^{-4} and 10^{-5} dilutions were aseptically introduced in sterile plates. MRS agar was poured onto it, swirled and allowed to set. All plates were incubated at 30°C for 48 hr under anaerobic conditions. After the incubation, a

preliminary catalase test was carried out. Catalase negative discrete colonies on the plates with distinct morphological differences such as colour, shape and size were picked and subcultured 2-3 times by re-streaking on fresh MRS agar plate. The pure cultures were characterised using Gram staining test and cell morphology examinations. Catalase-negative and Gram-positive isolates were preserved in 15% (v/v) glycerol agar at -20°C until identification. The bacteriocin activity was expressed as the diameter of the zone of inhibition caused by microbial test strain and expressed in mm. Carbohydrate fermentation patterns of LAB were determined using API 50 CHL kit (bioMerieux, France). The APILAB PLUS database software was used to interpret the results.

2.2 Isolation of *Campylobacter Species*

The test organism was isolated from green vegetables, using a selective agar Campylobacter growth supplement media (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. Four *Campylobacter*-like colonies were picked up from each plate and subjected to Gram staining and oxidase, catalase, indoxyl acetate, hippurate hydrolysis test, H_2S production and nitrate reduction test and then ready for standardization.

2.3 Preparation and Standardization of Inoculum

A loopful of each of the test organisms *Campylobacter jejuni* and *Campylobacter coli* were separately inoculated into 10 ml of nutrient broth contained in 20 ml test tube and incubated overnight at 35^{o}C for 18 hours. Thereafter, the cultures were standardized by transferring 0.1ml into test tubes containing 9.9ml sterile distilled water to obtain culture concentration of 10^{-2}. The inoculum was standardized in conformation with the McFarland No 2 using spectrophotometric adjustments of optical density at 0.6 at 600n m.

2.4 Screening of Lactic Acid Bacteria for their Antimicrobial Activity

The ability of LAB isolate to exert an antibacterial effect against a food-borne pathogen *Campylobacter* species was examined by the well-diffusion method according to Kos et al. [9]. The isolated LAB strains were inoculated in 5ml MRS broth and incubated under anaerobic conditions at 30°C for 48 hours. Cell-free supernatant (CFS) was obtained by centrifugation of this culture using Biofuge fresco centrifuge at 10.000xg for 10min at 4°C. To clarify whether the antimicrobial activity detected was derived from an organic acid or hydrogen peroxide (H_2O_2), the CFS was adjusted to pH 7.0 by adding 1N NaOH to eliminate the inhibitory effect of organic acids and 3000U/ml of catalase was added to eliminate the potential inhibitory effect of hydrogen peroxide produced by the isolates. The so-treated CFS was filtered through 0.45µm filter and used as a crude bacteriocin solution. Using the standardized inoculum, 0.1ml was inoculated and spread evenly on the Muller Hilton Agar plate surface with a glass spreader. Agar wells were bored in the plates by the use of a sterile cork borer. The 0.1µl crude bacteriocin fluid was transferred into the wells with micro Pasture pipette. Ampicillin (10µg/100 µl) was used as a positive control and sterile (uninoculated) media as a negative control. Antibacterial activity was determined by the measurement of zone of inhibition (ZOI)) around the wells after 24 hours of incubation at 37°C.The diameter of the zone was measured (mm) and compared with that of positive and negative control. The experiment was performed in triplicates and the average was calculated.

2.5 Molecular Characterization of the Bacteriocin-Producing Isolates

2.5.1 Extraction of template DNA

A Single colony from each pure culture was picked by a transfer loop and suspended in 100 µl of sterile distilled water in Eppendorf tubes and each tube was vortex for 30 seconds. Each suspension was boiled using thermomixer comfort at 100°C for 10 minutes to lyse the cells and inactivate nucleases. The suspensions were centrifuged using Biofuge fresco centrifuge at 9500 g for 5 mins. Each supernatant was carefully collected and put in clean PCR tubes and used as templates for PCR.

2.5.2 Amplification of 16S rRNA region of the selected strains by PCR reaction

The 16S rRNA genes were amplified using a set of 0.3 µl each of 16s forward (5'-AGAGTTTGATCCTGGCTCAG-3') and16s reverse (5'GCTGATCCGCGATTACTAGC-3') primers. The reaction mixture contained Taq polymerase 5X Master Mix (2.0 µl), 2.0 µl of the

DNA template and 5.4 µl of nuclease-free water in a total volume of 10 µl. the mixture was heated at an initial denaturation of 95^{o}C for 5 minutes and subjected to 30 rounds of thermal cycling at 95^{o}C for 5 minutes. 54^{o}C for 30 seconds for annealing, 72^{o}C for 2 minutes for Elongation and 72^{o}C for 2 minutes for final extension then it was held at 10^{o}C. The amplicons were further purified before gel electrophoresis (2.0% [w/v]).

2.5.3 Separation of amplified fragments

After the completion of the PCR reaction, amplified products were separated in a 1.5% (w/v) agarose gel. For this purpose, 1.5g agarose was dissolved in 100ml 1xTAE buffer and the agarose solution was boiled. Agarose solution was cooled to nearly 40°C. After cooling, 1.5µl ethidium bromide solution (10mg/ml) was added. The agarose gel was poured into the gel casting stand and the combs were placed. When the gel was solidified, the combs were removed. For loading, 10µl of amplification was loaded into wells. After the loading of samples, 5µl of DNA molecular weight marker (Gene Ruler, Fermentas) were loaded into the first well. Finally, electrophoresis was performed using instrument H5 horizontal gel electrophoretic system, at 100mA. Amplification products were visualized in a Bluelight transilluminator. The presence of DNA fragments sized between 1500-2000bp indicated that targeted amplification was achieved.

2.6 Characterization of the Antimicrobial Compounds

The tests were carried out with cell-free supernatant extracts from *Chungangia koreensis* CAU 9163 grown at 35^{o}C for 48 hours.

2.6.1 Effect of temperature on bacteriocin activity

The culture supernatant of inhibitory substance-producing strains, which were grown in MRS broth for 24 hours, was exposed to various heat treatments. The culture supernatants were incubated at 30^{o}C, 60^{o}C and 80^{o}C, as well as at 121^{o}C for 15 minutes and the antimicrobial activity was then determined, as described above.

2.6.2 Effect of pH on bacteriocin activity

To determine the pH sensitivity of the culture supernatant, recovered during the stationary growth phase of the isolates, pH values were adjusted ranging from 2 – 10 by using 1 M NaOH for alkaline values and 1 M HCl for acidic values [10]. After incubation for 4 h, the pH was readjusted to 6.5 and the antimicrobial the activity was determined, as described above.

2.6.3 Effect of enzyme inhibitors on bacteriocin activity

The effect of the enzymes; amylase, and proteinase k, at a concentration of 1.0 mg/ml was added to the culture supernatant and incubated for 1 hour at 37°C. After incubation, the bacteriocin activity was determined as described above.

2.7 Broth Assay

Experimental setups were made according to the method of Cadmus and Adesokan [11]. Broth assays were performed as follows: 20µl volumes of crude cell-free supernatant, 20µl of diluted cell-free supernatant of *Chungangia koreensis* CAU 9163 i.e. (10ml of crude cell-free supernatant in 20ml of sterile distilled water, 1:2 dilution), were pipetted into a different screwed caped test tube containing 20ml of raw pap diluted with sterile water, followed by inoculation of 20µl volume of indicator bacteria (*Campylobacter jejun*i and *Campylobacter coli*) containing a 10^{5}CFU/ml. Incubation of the tubes was carried out at 35^{o}C for 18 days. Growth of *Campylobacter species* was monitored at 72-hour intervals for 18 days. This was done spectrophotometrically by measuring the absorbance (optical density) in a spectrophotometer. *Campylobacter* growth was monitored at 600nm

2.8 Determination of Molecular Size of Bacteriocin

The molecular size of the purified bacteriocin was determined using SDS PAGE gel as described by Sambrook et al. [12]. Briefly, sterile glass plates were assembled, 20 ml of 15% resolving gel was dispensed, 2 ml of butanol was overlaid onto the gels, allowed to polymerize, after which the overlay was poured off and then the gel surface was rinsed with deionized water. To the gel, 8 ml of 5% stacking gel was overlaid and fixed in an electrophoresis apparatus. To the electrophoresis wells, equal volumes 20 ml of 1 x SDS and test sample preheated at 100°C in a test tube for 30 min and marker (2,500 - 40,000 KDa) respectively was

loaded in the gel. The gel was run 100 V for 5 hrs at 4°C, after which was stained with Coomassie brilliant blue.

3. RESULTS

3.1 Lactic Acid Bacteria Count Isolated from Pap

The lactic acid bacteria obtained from the three different species of grains used for pap, was identified as closely related to *Chungangia koreensis* strain CAU 9163 (Table 1).

3.2 Effect of Enzymes, pH and Temperature Treatments on Antimicrobial Activity of the Isolated Bacteriocin-like Substance

The action of enzymes on the antimicrobial activity of crude bacteriocin against the two different species of Campylobacter. The enzymes used were α-amylase, proteinase K, pepsin and lipase. The cell-free supernatant of *Chungangia koreensis* CAU 9163 treated with α-amylase and lipase enzyme revealed strong antimicrobial activity against *Campylobacter jejuni* and *Campylobacter coli.* Meanwhile, proteinase K and pepsin enzymes showed no antimicrobial activity against the two species of Campylobacter.

The effect of pH on the antimicrobial activity of crude bacteriocin against *Campylobacter jejuni* and *Campylobacter coli* was determined. It was observed that bacteriocin produced by *Chungangia koreensis*, was stable at pH of 4 to 8. Strain CAU 9163 bacteriocin, was able to exhibit antimicrobial activity against *Campylobacter jejuni* and *Campylobacter coli* at a pH of 4.0. At a pH of 6.0, it showed inhibitory action against *Campylobacter jejun*i than in *Campylobacter coli*. As the pH increased, the antimicrobial activity was still active against *Campylobacter jejuni* and *Campylobacter coli* at a pH of 8.0. The bacteriocin activity was destroyed as it approached pH of 10.

The thermal resistance of the bacteriocin produced by strain CAU 9123 was determined. The treatment of the extracellular extract of bacteriocin-producing Lab strain at 60^{O}C for 15 minutes led to antimicrobial activity against the *Campylobacter jejuni* and *Campylobacter coli*. At 80^{O}C, 100^{o}C and 121^{o}C for 15 minutes there was no inhibition against *Campylobacter jejuni* and *Campylobacter coli.* Hence, the inhibitory activity was destroyed by heat at 80^{o}C, 100^{o}C and 121^{o}C for 15 minutes.

3.3 Bioassay

The effect of cell-free supernatant (bacteriocin) from *Chungangia koreensis* on the growth of *Campylobacter* spp showed that Crude bacteriocin of *Chungangia koreensis* inhibited the growth of the *Campylobacter* spp. significantly ($p \leq 0.05$) from day 6 to day 18. This can be seen in the decrease in the absorbance from 0.670 on Day 0 to 0.070 on day 18 for *C. jejuni* and from 0.692 on day 0 to 0.389 on day 18 for *C. coli*. This represented 84% and 44% growth inhibition for the respective organisms. However, the inhibition was still effective on the test organisms.

The 1:2 bacteriocin dilution reduced the absorbance from 0.680 from day 0 to 0.534 on day 18 in *C. jejuni* and from 0.680 from day 0 to 0.523 on day 15 for *C. coli*. This represented 22% and 23% growth inhibition of the organisms respectively, with a slight growth stimulation from 0.523 to 0.621 on day 18 for *C. coli*, representing 18% growth stimulation in Figs. 1 and 2.

In the control, there was a constant growth increase of both *Campylobacter jejuni* and *Campylobacter coli.*

3.4 Molecular Size of Bacteriocin

Results presented in Plate 1 showed the molecular size of the bacteriocins produced from *Chungangia koreensis.* It was observed that the molecular weight of the bacteriocin produced by the *Chungangia koreensis* falls at the 5.6KDa band in a SDA-PAGE analysis. Hence, grouping it as a class I bacteriocin group.

4. DISCUSSION

Bacteriocin-like substance produced by *Chungangia koreensis* strains were isolated from pap and were identified using a 16S rRNA-targeted PCR method [12] and by morphological, cultural and biochemical characteristics using the API-50CH system. The bacteriocin-like substances from *Chungangia koreensis* CAU 9163 are interesting antimicrobial compounds that were tested against two species of Campylobacter which

Table 1. Blast from molecular characterization of *Chungangia koreensis*

S/N	Description	Max score	Total score	Query cover	E value	Identity	Accession
1	*Chungangia koreensis* strain CAU 9163 16S ribosomal RNA gene, partial sequence	191	233	40%	4e-49	75%	NR_117554.1
2	*Lactobacillus equicursoris* strain DI70 16S ribosomal RNA gene, partial sequence	189	231	40%	1e-48	75%	NR_112652.1
3	*Lactobacillus crispatus* strain DSM 20584 16S ribosomal RNA, partial sequence	183	225	40%	6e-47	75%	NR_119274.1
4	*Lactobacillus crispatus* strain DSM 20584 16S ribosomal RNA gene, partial sequence	183	225	40%	6e-47	75%	NR_117063.1
5	*Lactobacillus crispatus* strain ATCC 33820 16S ribosomal RNA gene, partial sequence	183	225	40%	6e-47	75%	NR_041800.1
6	*Streptococcus dentiloxodontae* strain NUM2404 16S ribosomal RNA, partial sequence	176	218	39%	1e-44	75%	NR_152054.1
7	*Abiotrophia defectiva* strain GIFU 12707 16S ribosomal RNA gene, partial sequence	176	218	40%	1e-44	75%	NR_025863.1
8	*Streptococcus ratti* strain ATCC 19645 16S ribosomal RNA gene, partial sequence	176	218	39%	1e-44	75%	NR_025516.1
9	*Eremococcus coleocola* strain M1832/95/2 16S ribosomal RNA gene, partial sequence	176	218	40%	1e-44	75%	NR_026481.1
10	*Lactobacillus kefiranofaciens* subsp. *kefirgranum* strain JCM 8572 16S ribosomal RNA gene, partial sequence	172	214	40%	1e-43	75%	NR_117067.1

Table 2. The effect of enzymes, pH and temperature on the antimicrobial activity of crude bacteriocin against *Campylobacter jejuni*

Variables		Bacteriocin activity
Enzyme	α -Amylase	+
	Proteinse K	-
	Pepsin	-
	Lipase	+
pH	2	-
	4	+
	6	+
	8	+
	10	-
Temperature	60	+
	80	-
	100	-
	121	-

Key: positive= + Negative= -

Table 3. The effect of enzymes, pH and temperature on the antimicrobial activity of crude bacteriocin against *Campylobacter coli*

Factor		Bacteriocin activity
Enzyme	α -Amylase	+
	Proteinse K	-
	Pepsin	-
	Lipase	+
pH	2	-
	4	+
	6	-
	8	+
	10	-
Temperature (ºC)	60	+
	80	-
	100	-
	121	-

Key: positive= + Negative= -

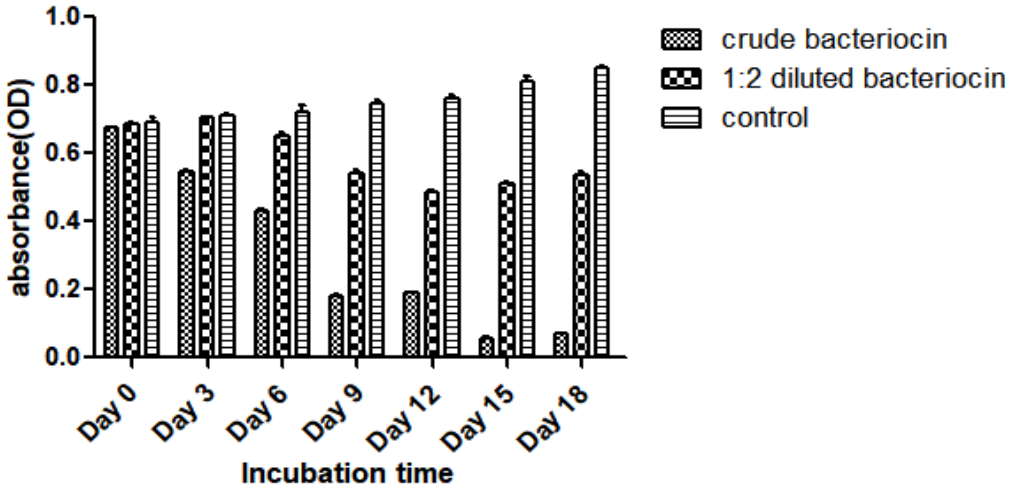

Fig. 1. Growth of *Campylobacter jejuni* in the presence of bacteriocin from *Chungangia koreensis* in raw pap

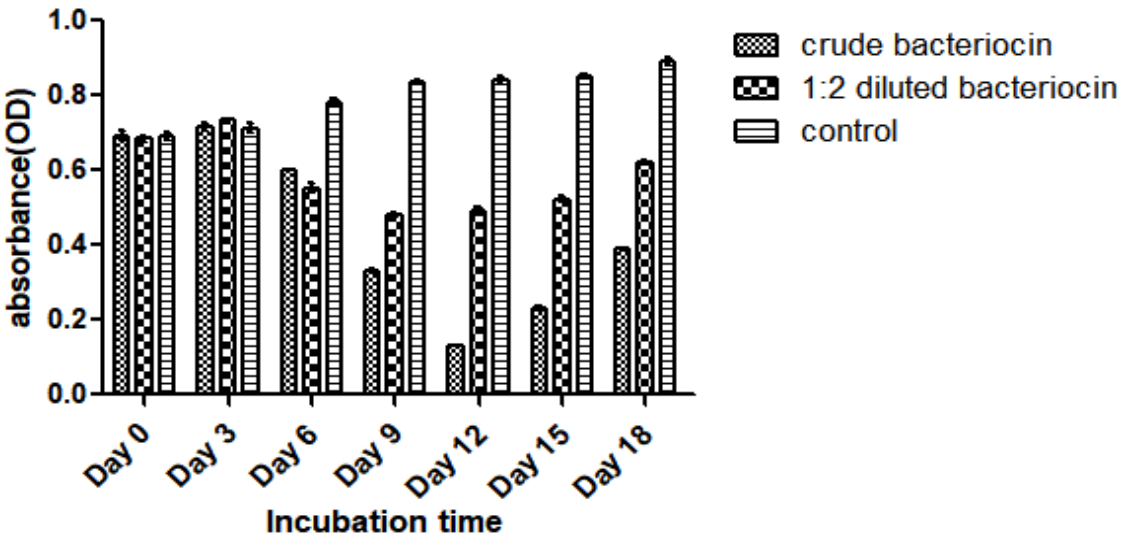

Fig. 2. Growth of *Campylobacter coli* in the presence of bacteriocin from *Chungangia koreensis* in raw pap

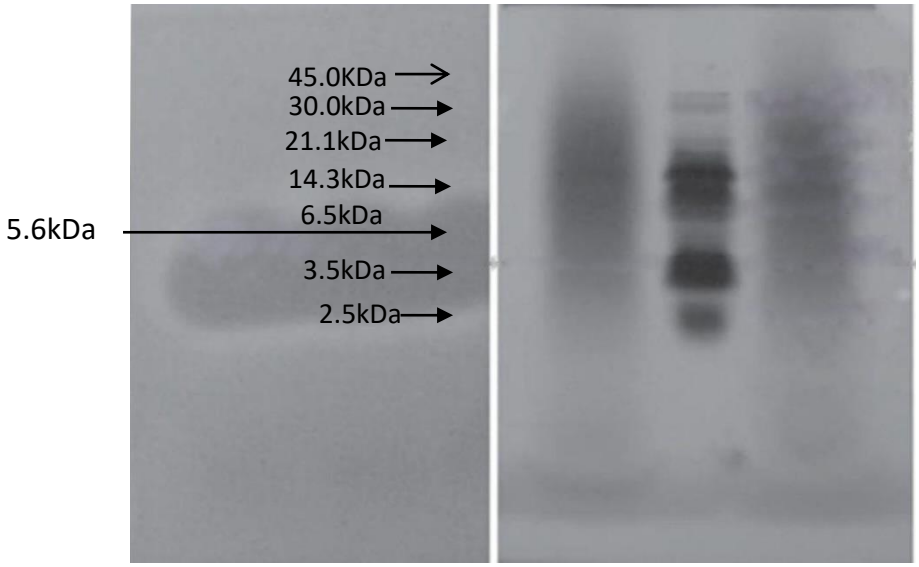

Plate 1. 5.6 KDA SDS-PAGE gel of partial purified bacteriocin of *Chungangia koerensis* strain

include the *Campylobacter jejuni* and *Campylobater coli*. The antimicrobial substances researched in this work were referred to as BLIS as they have not been isolated and their amino acid sequence have not been characterized. The result of the present study shows the presence of bacteriocin in *Chungangia koreensis* and can be used as a natural substitutes for chemical food preservatives and in eradication of diarrhoea causing food infection.

The results from this study showed the cell-free supernatant of *Chungangia koreensis* inhibiting *Campylobacter jejuni* more than the *Campylobacter coli*. However, it supports the finding that the *l. utilitarians* could inhibit strains of *Campylobacter jejuni* more than *coli* [13]. The inhibitory activity is as a result of pore formation, dispersion of membrane potential and leakage of internal low molecular-weight substances [14]. According to Todorov and Dicks [15], bacteriocin production was strongly dependent on temperature, pH and nutrient source. Different physicochemical factors seems to affect bacteriocin as well as its activity [16]. In the present study, the temperature effect of these BLIS on Campylobacter strains revealed stability and non-stability in Table 4. The BLIS under the

temperature of 60^{o}C for 15mins was stable and showed inhibition against both *Campylobacter jejuni* and *Campylobacter coli*. While at temperatures of 80^{o}C, 100^{o}C and 121^{o}C, their stability was low or non-active. This could be a result of the proteinaceous nature of the BLIS, which is easily affected by high temperature. Their growth rate occurs optimally at temperature of 30^{o}C and can survive as high as 60^{o}C with stability of its BLIS [6]. According to De Vuyst and Vandamme [17], non-thermal stability may be as a result of non-formation of small globular structures and the occurrence of low hydophobic regions, low stable cross linkages and low glycine content. Hence, the BLIS produced by the *Chungangia koreensis* isolated described here may suggest possible use as biopreservatives in combination with heat processing in order to preserve food products, in procedures like pasteurization, drying, refrigeration and freezing.

The pH can enhance the antimicrobial activity of LAB. The pH of the LAB isolated was active at a pH range of between 4 and 8 against both Campylobacter species. According to Kim et al. [7], it was stated that *Chungangia koreensis* sp grows best at a pH of 8.0. Hence, its ability to maintain a stable activity at a pH range of 4-8. These results state that the BLIS described can be used in bio-preservation of food products with low, medium and high pH.

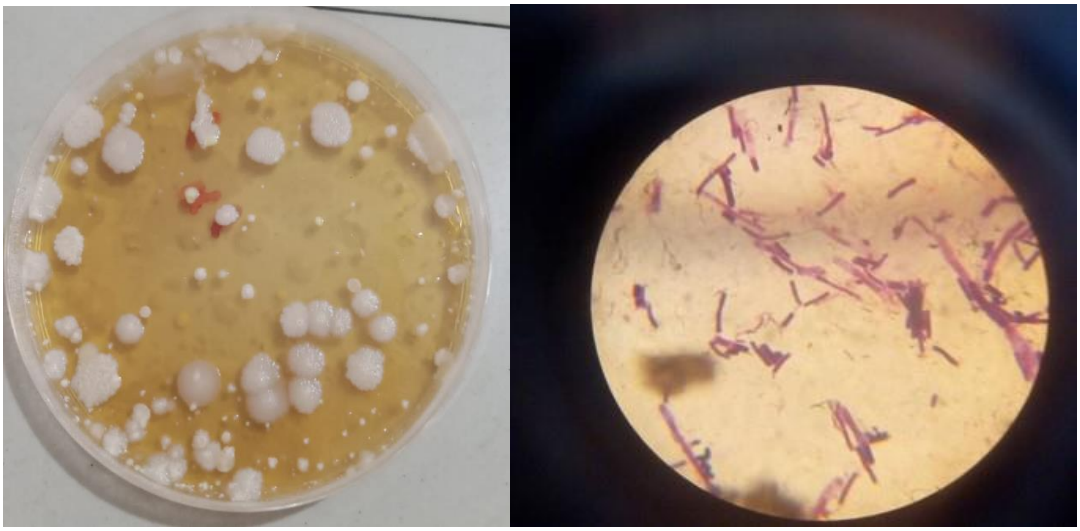

Plate 2. (a) *Chunguangia koreensis* on cultured media plate (b) Gram positive rods

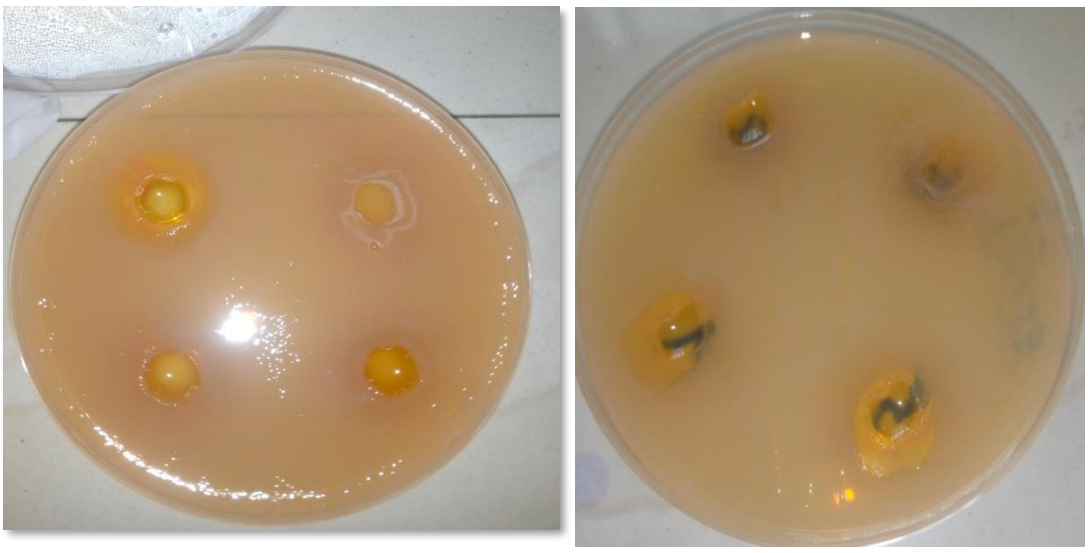

Plate 3. Zone of inhibition of LAB on *Campylobacter* sp

Bacteriocins may be degraded by proeolytic enzymes resulting in loss in their antimicrobial activities. In the study, the BLIS maintained their antimicrobial stability in the presence of amylase and lipase enzymes and lost their stability in the presence of proteinase k and pepsin. According to Yang et al. [18], it stated that the low activity of BLIS against proteinase k and pepsin may be as a result of its proteinaceous compounds and also due to the presence of unusual amino acids in the bacteriocin structure, or cyclic N- and C-terminal blocked peptides [19]. The resistance of cylic peptides to hydrolysis by protease may be mainly due to their cyclic structure rendering them relatively inflexible, which may make cleavage sites inaccessible due to steric hindrances [20].

As stated by Felis and Dellaglio [21], “phenotypic methods alone are inadequate for the identification of lactic acid bacteria and should be confirmed by molecular methods to achieve a reliable identification. Theoretically, the amplicon size of 16S rDNA region on the genomic DNA of lactic acid bacteria is 1500bp. In the present study, bacteriocin-producing strains were identified by PCR technique to confirm if they were actually LAB. The 16S rDNA region of each bacteriocin-producing strain was amplified. The length of amplification products varied between 1000 to 2000bp”.

“These results show that the bacteriocin-producing strains isolated in this study were lactic acid bacteria because the amplicon sizes of their 16S rDNA region amplified was approximately 1500bp. Many research works have focused on the application of molecular biology techniques for the rapid detection and differentiation of LAB. The use of primers and probes that target genes encoding ribosomal ribonucleic acid (rRNA) is promising. Due to the high inter species variability of this region, rDNA sequences coding for the 16S and 23S rRNA has been validated as a means of identification. However, the data in the literature concern the identification of strains known to be lactobacilli to the species level” [22]. “The approaches in preliminary identification of isolated bacterial strains of various bacterial species were morphological characterization like positive growth on MRS agar medium, colony colour and outcomes of microscopic analysis and all were to suggest the presence of lactic acid bacteria in the pap samples under consideration. These identification approaches were in agreement with almost every study on lactic acid bacteria in that all the isolated strains could grow on MRS agar, bacteria colonies were whitish, circular and convex when observed with the eye and the bacteria cells were Gram-positive when observed under the microscope after gram staining and catalase negative partially purified bacteriocins obtained during antimicrobial test were analyzed on SDS-PAGE (15%w/v) and stained Coomassie brilliant blue. These partially purified bacteriocins appeared as single bands in SDS-PAGE with molecular weight approximately ~ 5.6kDa for bacteriocin. The bacteriocins of lactic acid bacteria belonging to class-I and II have molecular weight (<5kDa) and (<10kDa) respectively e.g., *Pediococcus acidolactici* (3.5kDa), L. cinC-TA33a (4.6kDa) and *L. curvatus* SB13 (10kDa)” [23]. “The lower molecular mass of CAU 9163 bacteriocin (5.6KDa) suggest that it belongs to class I bacteriocin group” [24].

5. CONCLUSION

The raw pap commonly consumed in traditional environments in western and eastern parts of Nigeria is a potential source of lactic acid bacteria. *Chungangia koreensis* a novel Lactobacillus strain appeared as one of the strains found in the fermented maize slurry (pap). These lactic strains produce bacteriocins having a significant inhibitory activity against foodborne pathogens (*Campylobacter* sp.). The remarkable fact in this work is the ability of these bacteriocin to also inhibit Gram-negative strains suggesting their potential application in the biomedical field and their uses as probiotic bacteria. The ability to significantly inhibit *Campylobacter jejuni* and *Campylobacter coli* which is an emerging food-borne pathogen of concern gives it an enabling status to be used as a bio preservatives in fermented food products and as probiotics for health benefits of individual.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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https://www.sdiarticle5.com/review-history/124457