Characterization of bacteriocin-like substance produced by *Lactobacillus songhuajiangensis*isolated from raw pap and antimicrobial Activity against Food borne *Campylobacter* spp.

ABSTRACT

*Lactobacillussonghuajiangensis*strain 7-19 isolated from raw pap, produced bacteriocins-like substance whichwas able to inhibit *Campylobacter jejuni* and *Campylobacter coli* a leading cause of bacterial food borne diarrhoea disease worldwide. Crude bacteriocin of *L. songhuajiangensis* inhibited the growth of the *Campylobacter* spp. significantly ($p < 0.05$) from Day 3 to Day 15, thereafter, slightly stimulated growth. This can be seen in the decrease in the absorbance from 0.710 on Day 0 to 0.081 on Day 15 for *C. Jejuni*and from 0.710 on Day 0 to 0.053 on Day 15 for *C. coli*. This represented 88% and 93% growth inhibition for the respective organisms. The bacteriocin produced by *Lactobacillus songhuangiensis strain* 7-19 was heat stable at temperature of 80^{o}C for 15mins maintained full stability for 15days at 37^{o}C. Bacteriocins produced by these organisms was stable at pH range of 2-8. It was also observed that there was stability of the activity of the bacteriocins' inhibition in the presence of amylase and lipase enzyme while unstable in the presence of proteinase k and pepsin enzyme.The molecular weight of the bacteriocin was at 2.6kDa for *Lactobacillussonghuajiangensis*. It is classified as class I bacteriocins. This study revealed the possibility of using Bacteriocin as food bio-preservatives to control food spoilage and pathogenic bacteria. The paper concluded that the ability of bacteriocins produced by the test isolates in inhibiting a leading food-borne pathogen, is of potential interest for food safety and have future applications as food preservatives.

Keywords: Bacteriocin, food-borne pathogens, indicator organisms, inhibition, antimicrobial activity

INTRODUCTION

Bacteriocins are proteins or complexed proteins biologically active with antimicrobial action against other bacteria, principally closely related species. They are produced by bacteria and are normally not termed antibiotics in order to avoid confusion and concern with therapeutic antibiotics, which can potentially illicit allergic reactions in humans and other medical problems [1].

For the past years, bacteriocins have attracted considerable interest for their use as safe food preservatives, as they are easily digested by the human gastrointestinal tract [2]. The use of bacteriocins as natural food preservatives fulfills consumer demands for high-quality and safe foods without the use of chemical preservatives. However, the application of bacteriocins as food additives can be limited for various reasons, such as the effectiveness of pathogen elimination or its high price [3]. Nevertheless, research interest in bacteriocins has continued over the past years, as investigators continue to search for new and more effective bacteriocins to address both

biological and economic concerns.One of the concerns in the food industry is contamination by pathogens, which are frequent causes of food-borne diseases. Over the past decade, recurrent outbreaks of diarrhoea, combined with the natural resistance of the causative agents, contributed to its status as a hazard. The problem of selection of resistant bacteria to antibiotics [4-6] and the increasing demand for safe foods, with fewer chemical additives, has increased the interest in replacing these compounds with natural products, which do not injure the host or the environment. Biotechnology in the food-processing sector targets the selection, production and improvement of useful microorganisms and their products, as well as their technical application in food quality. The use of non-pathogenic microorganisms and/or their metabolites to improve microbiological safety and extend the shelf life of foods is defined as bio preservation [7]. Antagonistic properties of lactic acid bacteria (LAB) allied to their safe history of use in traditional food fermented products make them very attractive to be used as bio preservatives [8]. Antibiotics are at present restricted for use in foods and feeds, and bacteriocins are an interesting group of bio molecules with antimicrobial properties that may represent a good alternative [9]. The increasing interest in these compounds has stimulated the isolation of LAB producers and the characterization of many novel peptides [9]. The successful development of nisin from an initial biological observation through regulatory approval for commercial applications is a model that has stimulated new contributions in the field of bacteriocin research [10].The application of bacteriocins for biopreservation of foods usually includes the following approaches: inoculation of food with the bacteriocin-producer strain; addition of purified or semi-purified bacteriocin as a food additive; and use of a product previously fermented with a bacteriocin-producing strain as an ingredient in food processing [11]. An increasing number of bacteriocins have been isolated and identified from Gram-positive and Gram-negative microorganisms. As a result, databases have been created to compile the information that can be used for the automated screening of bacteriocin gene clusters [12,13].Most of the bacteriocins produced from LAB, in particular those inhibiting Gram-positive bacteria, exert their antibacterial effect by targeting the cell envelope-associated mechanisms [14]. Several antibiotics and some class II bacteriocins target Lipid II, an intermediate in the peptidoglycan biosynthesis machinery within the bacterial cell envelope and, by this way they inhibit peptidoglycan synthesis [15].LAB have long been used in a variety of food fermentation by converting lactose to lactic acid, as well as producing additional antimicrobial molecules such as other organic acids, diacetyl, acetoin, hydrogen peroxide, antifungal peptides, and bacteriocins [16] As a result of their extensive use in traditional fermented products, most of the LAB are Generally Regarded as Safe (GRAS), granted by the American Food and Drug Agency (FDA). In recent years bacterial antibiotic resistance has been considered a problem due to the extensive use of classical antibiotics in the treatment of human and animal diseases [17]. As a consequence, multiple resistant strains appeared and spread causing difficulties and the restricted use of antibiotics as growth promoters. So, the continue development of new classes of antimicrobial agents has become of increasing importance for medicine [17-19]. Bacteriocin production could be considered as an advantage for food and feed producers since, in sufficient amounts, these peptides can kill or inhibit pathogenic bacteria that compete for the same ecological niche or nutrient pool. This role is supported by the fact that many bacteriocins have a narrow host range, and is likely to be most effective against related bacteria with nutritive demands for the same scarce resources.Intensive research into the bacteriocins produced by LAB received considerable attention during recent years for their possible use as preservatives in food, with a resultant reduction in the use of chemical preservatives. In this study, bacteriocins produced by LAB isolates obtained from

fermented corn slurry (pap), were first characterized. In addition, bacteriocins were partially purified and estimated their molecular weight.

MATERIALS AND METHODS

Four hundred and fifty Samples of three different species of ogi were collected from different markets in Elele, Rivers State. The species include maize or corn (*Zea mays*), sorghum (*Sorghum vulgare,*), and millet (*Pennisetum americanum*).

Isolation and Phenotypic Identification of Lactic Acid Bacteria

LAB was isolated from raw 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 realized with saline solution (NaCl, 0.85% w/v). One mL aliquot of the 10^{-4} and 10^{-5} dilutions was aseptically disposed on sterile plates. MRS agar was poured onto it and allowed to set. All plates were incubated at 30°C for 48 hours under anaerobic conditions. After the incubation, a preliminary catalase test was carried out. Catalase-negative discrete colonies which appeared on the plates with distinct morphological differences such as colour, shape and size were picked and purified 2-3 times by re-streaking on fresh MRS agar. The pure cultures were further characterized using a 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.

Isolation of inoculum

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 from each plate and subjected to gram staining, oxidase, catalase, indoxyl acetate, hippurate hydrolysis test, H_2S production and nitrate reduction test

Preparation and Standardization of Inoculum

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

Molecular characterization of bacteriocin-producing lactic acid bacteria Extraction template DNA

A Single colony from each pure culture was picked by a transfer loop and suspended in 100µl 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 10mins to lyse the cells and inactivate nucleases. The suspensions were centrifuged using Biofuge fresco centrifuge at 9500g

for 5mins. Each supernatant was carefully collected and put in clean PCR tubes and used as templates for PCR.

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 95^0C for 5 min. and subjected to 30 rounds of thermal cycling at 95^0C for 5mins. 54^0C for 30 sec. for Annealing, 72^0C for 2 min. for Elongation and 72^0C for 2min. for final Extension then it was held at 10^0C. The amplicons were further purified prior to gel electrophoresis (2.0% [w/v])

Separation of amplified Fragments After the completion of 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 were 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.

Screening of lactic acid bacteria for their bacteriocigenic activity

The ability of each LAB isolate to exert an antibacterial effect against a food-borne pathogen *Campylobacter sp*was examined by well diffusion method according to [20]. The isolated LAB strains were inoculated in 5ml MRS broth and incubated under anaerobic condition 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 derived from an organic acid or hydrogen peroxide (H_2O_2), the CSF 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 treated CFS was filtered through 0.45µm filter and used as crude bacteriocin solution. 0.1ml from each cell-free supernatant or crude bacteriocin. Using the standardized inoculum(Campylobacter) sp, 0.1ml was inoculated and spread evenly on the Muller Hilton Agar plate surface with a glass spreader. Agar wells was bored in the plates by the use of a sterile cork borer. The 0.1ml 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 negative control. Antibacterial activity was determined by the measurement of zone of inhibition (ZOI)) around the wells after 24 h 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.

Characterization of the antimicrobial compounds

The tests were carried out with cell-free supernatant extracts from *Lactobacillus songhuajiangensis*strain 7-19 hours grown at 35^0C for 48hours.

Effect of heat treatment 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 at 30^oC, 60^0C, 80^0Cand 100^0C as well as at 121^oC for 15 min and the antimicrobial activity was then determined, as described above.

Effect of pH on bacteriocin activity

To determine the pH effects of bacteriocin activity, an aliquot of bacteriocin extract (0.5 ml) was added into MRS broth (4.5 ml) at different pH values (2 to 10) using 1M NaOH or 1M HCl and incubated for 30 minutes at 37°C[21]. Bacteriocin samples were exposed to different pH values and were assayed against indicator organisms(*Campylobacter* spp.) by the agar well diffusion method, and activities were compared to non-exposed bacteriocins as a control.

Effect of enzyme inhibitors on bacteriocin activity

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

Broth Bioassay

Experimental setups were made according to the method of Cadmus and Adesokan [22]. Brothassays were performed as follows: 20µl volumes of crude cell-free supernatant of *L.songhuajiangensis* , 20µl of diluted cell-free supernatant of *L.songhuajiangensis*i.e (10ml of crude cell free supernatant in 20ml of sterile distilled water, 1:2 dilution), were pipetted into 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 10^5Cfu/ml . Incubation of the tubes was at 35^0C for 18 days. Growth of *Campylobacter* species was monitored at 72-hourintervals for 18 days. This was done spectrophotometrically by measuring the absorbance (optical density) in a spectrophotometer. *Campylobacter* growth was monitored at 600nm.

Determination of molecular size of bacteriocin

The molecular size of the purified bacteriocin was determined using SDS PAGE gel as described by [23]. 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 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.

Results

Lactic Acid bacteria count isolated from pap.

The total mean count of Lactic acid bacteria count obtained from the three different species of grains used for pap, showed that zea maize had higher lactic acid bacteria count of about 3.92 ± 0.2 log $_{10}$cfu/g, followed by guniea corn, 3.54 ± 0.1 $\log_{10}$ cfu/g and sorghum, 3.09 ±0.1 $\log_{10}$cfcfu/g respectively. There was no significant difference at ($p \geq 0.05$).

Table 1: Total mean count of Lactic acid bacteria isolated from different species of grains used for pap production

Species of grains	**LAB Count (log $_{10}$ cfu/g)**
Guinea corn	3.544 ± 0.1 (35×10^2)
Zea maize	3.929 ± 0.2 (85×10^2)
Sorghum	3.079 ± 0.1 (12×10^2)

Table 2: Biochemical identification of Campylobacter sppusing a selective media

Test	*C. jejuni*	*C. coli*
Colour of Colony	slimy white	moist white
Cell shape	rod	rod
Gram reaction	-	-
Hippurate hydrolysis	+	-
Oxidase test	+	+
Catalase test	+	+
Reduced nitate	+	+
Indoxyl acetate	+	-
Glucose utilization	-	-

Table 3: Morphological and Biochemical characteristics of Bacteriocin producing Lactic Acid Bacteria

Isolates	*Lactobacillussonghuajiangensis*
Colour	transparent white
Microscopic appearance	short rods
Cellular arrangement	short chains
Motility	-
Spore	-
Catalase	-
Gram reaction	+
Sugar fermentation(acid production)	
Glucose	-
Xylose	-
Fructose	+
Sucrose	+
Galactose	+
Lactose	+
Maltose	+
Manitol	+
Turanose-	
Ribose	+
Aesculin	+
Glycerol	-
Gentiobiose	-
Trehalose	+

The strains were identified using 16SrRNA- targeted PCR, and demonstrated homology levels and phenotypic characteristics using the API- 50CH system.

Table 4:Blast from molecular characterization of *Lactobacillus songhuajiangensis.*

S/	Description	Max	Total	Query	E	Ide	Accessi

N		score	score	cover	value	nt	on
1	*Lactobacillus songhuajiangensis* strain 7-19 16S ribosomal RNA gene, partial sequence	529	529	91%	7e-151	76%	NR_125562.1
2	*Agitococcuslubricus*strain DSM 5822 16S ribosomal RNA gene, partial sequence	381	381	64%	2e-106	75%	NR_104868.1
3	*Lactobacillus ginsenosidimutans* strain EMML 3041 16S ribosomal RNA, partial sequence	370	370	64%	4e-103	76%	NR_132607.1
4	*Jeotgalibacadankookensis* strain EX-07 16S ribosomal RNA gene, partial sequence	370	417	71%	4e-103	76%	NR_125553.1
5	*Lactobacillus iners* strain DSM 13335 16S ribosomal RNA gene, partial sequence	370	370	64%	4e-103	76%	NR_036982.1
6	*Lactobacillus fructivorans* strain KCTC 3543 16S ribosomal RNA, partial sequence	370	370	64%	4e-103	76%	NR_036789.1
7	*Jeotgalibacaarthritidis* strain 1805-02 16S ribosomal RNA, partial sequence	359	359	64%	9e-100	75%	NR_156899.1
8	*Lactobacillus delbrueckii* subsp. *bulgaricus* strain ATCC 11842 16S ribosomal RNA, partial sequence	359	425	72%	9e-100	75%	NR_075019.1
9	*Lactobacillus delbrueckii* subsp. *bulgaricus* strain NBRC 13953 16S ribosomal RNA gene, partial sequence	359	425	72%	9e-100	75%	NR_113639.1
10	*Lactobacillus delbrueckii* subsp. *bulgaricus* strain ATCC 11842 16S ribosomal RNA gene, partial sequence	359	425	72%	9e-100	75%	NR_117075.1

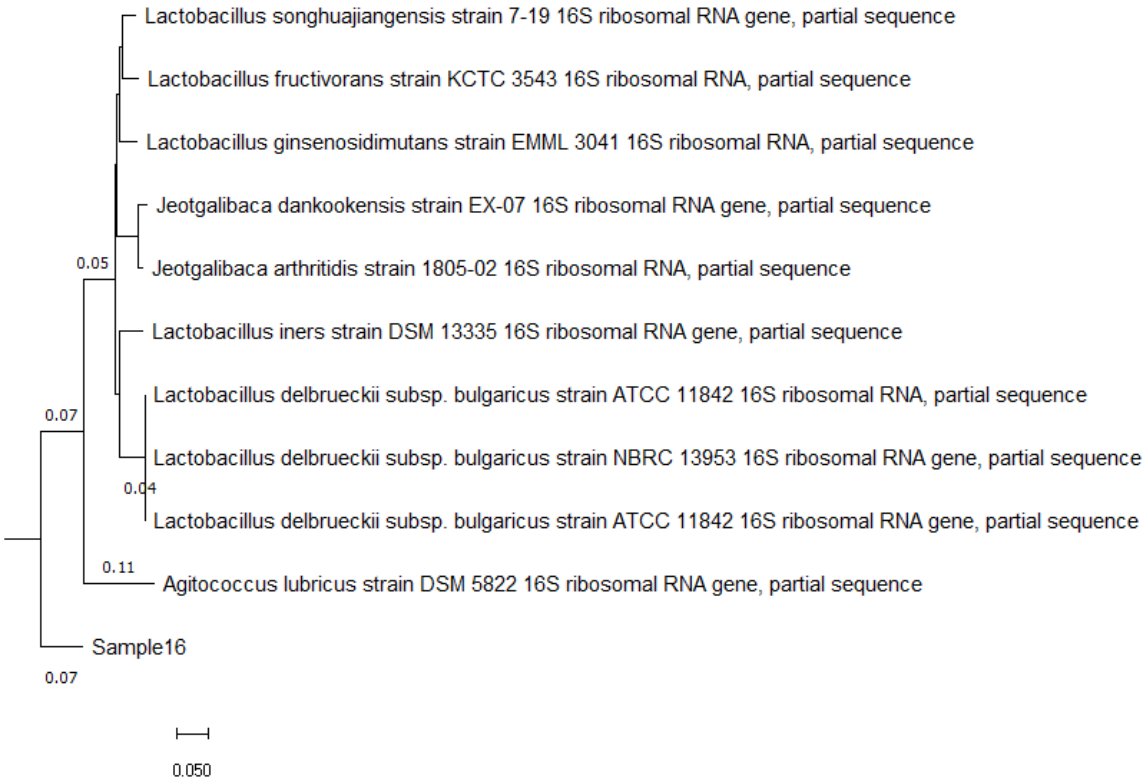

Figure 1: Phylogenetic tree showing evolutionary relatedness of isolates

Effect of temperature treatments on antimicrobial activity of the isolated bacteriocin-like substance.

The thermal resistance of the bacteriocin like substance(BLIS) produced by *Lactobacillus* s*onghuajiangensis*was determined. The treatment of the extracellular extract of bacteriocin producing Lab strain at 60^{O}C and 80^{o}C for 15mins led to an antimicrobial activity against the *Campylobacter jejuni*and *Campylobacter coli* while at 100^{O}C and 121^{O}C for 15mins there was no inhibition against *Campylobacter jejuni* and *Campylobacter coli*.Hence, the inhibitory activity was completely destroyed by heat at 100^{0}C and 121^{0}C for 15mins.

Table 5: Effect of temperature on the antimicrobial activity of crude bacteriocin against *Campylobacter jejuni* and *Campylobacter coli*.

Temperature (0C)	Time	Isolate	bacteriocin activity LS 1
60	15mins	*Campylobacter jejuni*	+
Campylobacter coli	+		
80	15mins	*Campylobacter jejuni*	+
Campylobacter coli	+		
100	15mins	*Campylobacter jejuni*	-
Campylobacter coli	-		
121	15mins	*Campylobacter jejuni*	-
Campylobacter coli	-		

Key: positive= + Negative= -; LS1=*Lactobacillussonghuajiangensis*

Effect of pH Treatment on antimicrobial activity of the isolated bacteriocin-like substance

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 *Lactobacillus songhuajiangensis,* was stable at pH of 2 to 8. Its bacteriocin was able to exhibit antimicrobial activity against *Campylobacter jejuni*and *Campylobacter coli*. The bacteriocin activity was destroyed as it approached pH of 9.0-10.

Table 6: The effect of pH on the antimicrobial activity of crude bacteriocin against *Campylobacter jejuni*and *Campylobacter coli*

pH	isolate	Bacteriocin activity LS1
2	*Campylobacter jejuni*	+
Campylobacter coli	+	
4	*Campylobacter jejuni*	+
	Campylobacter coli	+
6	*Campylobacter jejuni*	+
	Campylobacter coli	+
8	*Campylobacter jejuni*	+
	Campylobacter coli	+
10	*Campylobacter jejuni*	-
	Campylobacter coli	-

Key: positive= +; Negative = -; LS1=*Lactobacillus songhuajiangensis*

Effect of Enzymes 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 *Lactobacillus songhuajiangensis*was treated with α-amylase and lipase enzyme revealing strong antimicrobial activity against *Campylobacter jejuni*and *Campylobacter coli.* While proteinase K and pepsin enzyme showed no antimicrobial activity against the two Campylobacter species.

Table 7:The effect of enzymes on the antimicrobial activity of crude bacteriocin against *Campylobacter jejuni*and *Campylobacter coli*

Enzyme	isolate	bacteriocin activity
		LS1
α -Amylase	***Campylobacter jejuni***	+
	Campylobacter coli	+
Proteinse K	*Campylobacter jejuni*	-
	Campylobacter coli	-
Pepsin	*Campylobacter jejuni*	-
	Campylobacter coli	-
Lipase	*Campylobacter jejuni*	+
	Campylobacter coli	+

Key: Positive= + Negative= -;LS1=*Lactobacillus**songhuajiangensis*

BIOASSAY

The effect of bacteriocin from *Lactobacillus songhuajiangensis*on the growth of *Campylobacter*spp is presented in Figures 2 and 3. Crude bacteriocin of *L. songhuajiangensis* inhibited the growth of the *Campylobacter* spp. significantly ($p < 0.01$) from Day 3 to Day 15, thereafter, slightly stimulated growth. This can be seen in the decrease in the absorbance from 0.710 on Day 0 to 0.081 on Day 15 for *C. Jejuni*and from 0.710 on Day 0 to 0.053 on Day 15 for *C. coli*. This represented 88% and 93% growth inhibition for the respective organisms. . However, on Day 18, there was a slight increase in growth of *C. Jejuni*as shown by an increase in absorbance from 0.081 to 0.100 (representing 24% growth stimulation) and in *C. coli*, from 0.053 to 0.085 (representing 37% growth stimulation). The one-in-two bacteriocin dilution reduced the absorbance from 0.720 on day 0 to 0.483 on day 15 in both *C. jejuni* and *C. coli.*This represented 32% and 33% growth inhibition of the organisms respectively.Thus,bacteriocin from *L. songhuajiangensis* had more inhibitory effect on *C. coli*than on *C.jejuni.*

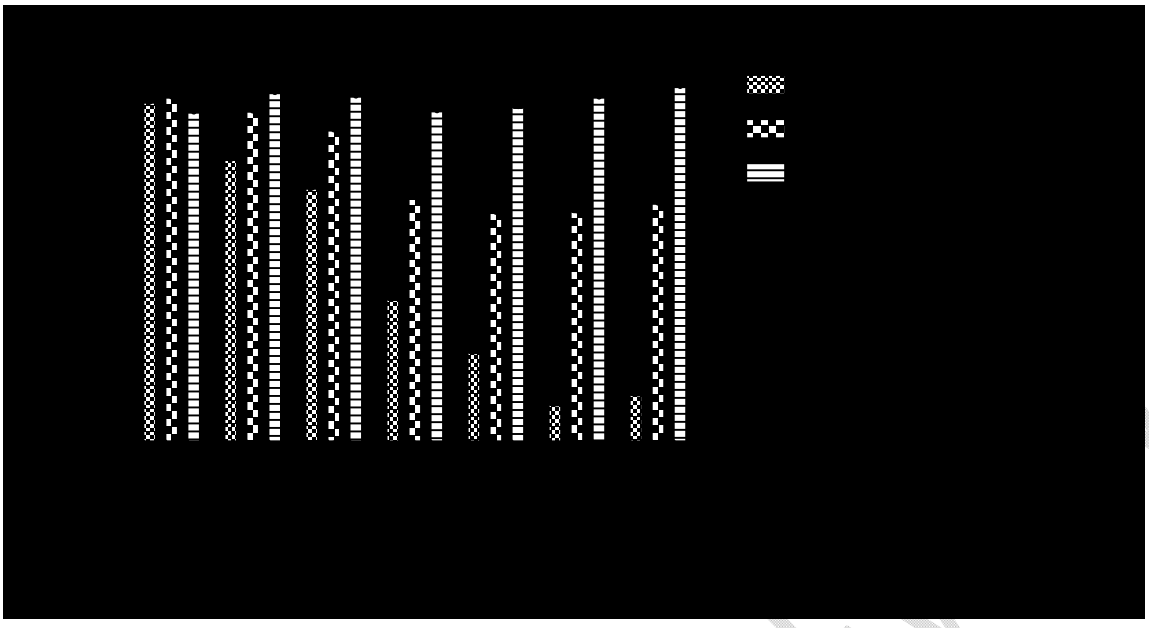

Fig 2:Growth of *Campylobacter jejuni*in the presence of bacteriocin from *Lactobacillus songhuajiangensis* in raw pap

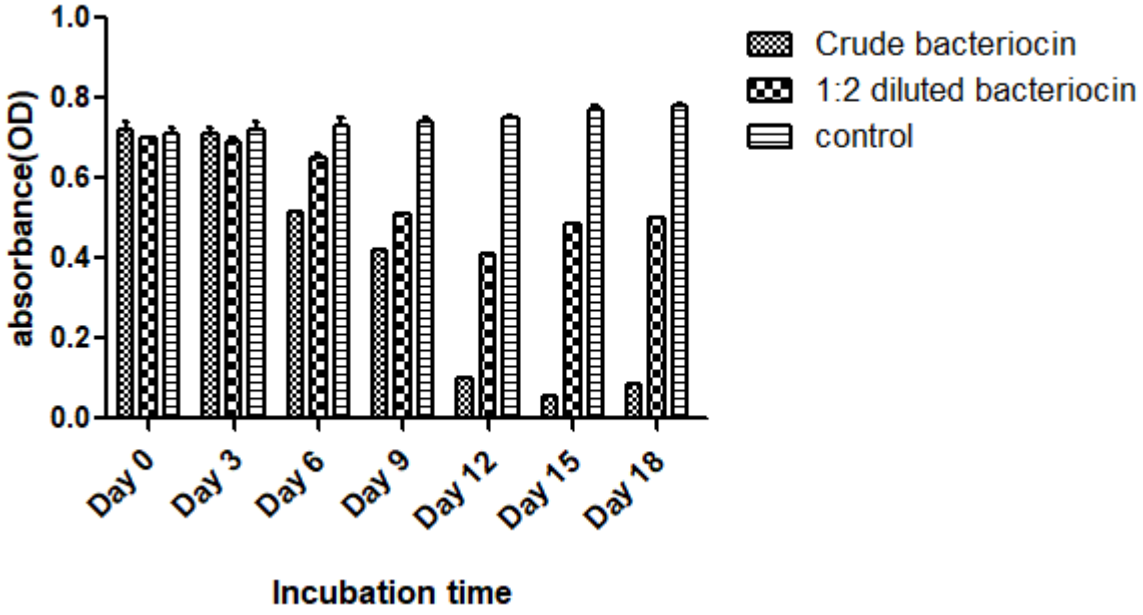

Fig 3: Growth of *Campylobacter coli* in the presence of bacteriocin from *Lactobacillus songhuajiangensis* in raw pap

Molecular Size of Bacteriocin

Results presented in Plate 1 showed the molecular size of the bacteriocins produced from *Lactobacillus songhuajiangensis.* It was observed that the molecular weight of the bacteriocin produced by the *Lactobacillus songhuajiangensis* falls at the 2.6KDa band in a SDA-PAGE analysis. Hence,grouping it as a class I bacteriocin group.

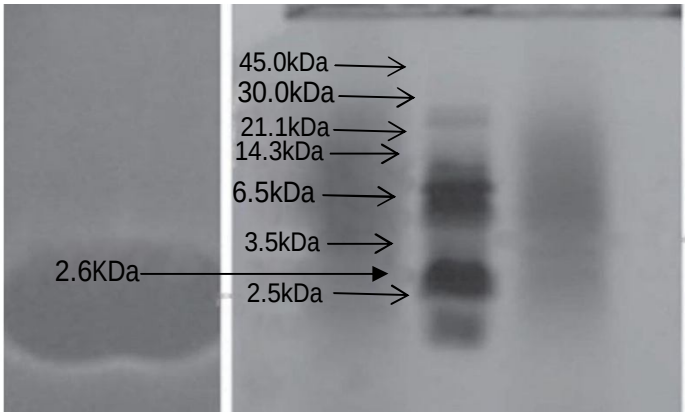

Plate 1: 2.6KDA SDS-PAGE gel of partial baceriocin of *Lactobacillussonghuajiangensis strain.*

Discussion

The bacteriocin like substances(BLIS) from *Lactobacillussonghuajiangensis* is an interesting antimicrobial compounds because of their ability to inhibit Campylobacter species. Thus, the bacteriocin was tested for antimicrobial activity against *Campylobacter jejuni* and *Campylobacter coli*. The highest inhibitory activity was exhibited against *Campylobacter coli* than in C*ampylobacter jejuni*by bacteriocin producing *L.songhungiansis.* The results of the present studies has shown that the bacteriocin can be inhibitory against *Campylobacter* sp. [24].From the study, in the bio assay, the bacteriocin producing *L.songhuajiangensis* was able to inhibit *Campylobacter jejuni* and *C. coli* with a decrease in the absorbance from 0.710 on Day 0 to 0.081 on Day 15 for *C. Jejuni*and from 0.710 on Day 0 to 0.053 on Day 15 for *C. coli*. This represented 88% and 93% growth inhibition for the respective organisms. Two Lactobacillus strains, *L. plantarum* and *L. paraplantarum*, displayed the ability to prevent human gut infection by food borne pathogens such as *Listeria*, *Campylobacter*spp and *Escherichia* spp., by preventing their adhesion to intestinal human cells [25]. Bacteriocin compound may serve as natural substitute for chemical preservatives to enhance shelf-life of food [26].

In the study, the heat treatment at 60^0C and 80^0C, of bacteriocin producing *Lactobacillus songhuajiangensis*was stable and able to inhibit the food borne pathogen while at 100^0C and 121^0C antimicrobial activity of bacteriocin producing LAB was inactivated. This could be as a result of the proteinaceous nature of the BLIS, which are easily affected by high temperature[27]. According to Todorov and Dicks [28], he reported that bacteriocin production was strongly dependent on temperature, pH and nutrient source.

The pH can enhance the antimicrobial activity of LAB. The pH of the bacteriocin producing LAB isolated was active at a pH range of between 2 and 8 against both Campylobacter species. It was observed that the *L. sonnghuajiangensis* showed no activity against both speciesof Campylobacter after treating with proteinase k and pepsin while amylase and lipase enzyme did not affect the antimicrobial activity.Class I bacteriocins are naturally more resistant to proteases than class II bacteriocins due to undergoing extensive PTMs [29]. The use of SDS-PAGE gel revealed the molecular weight of the bacteriocin, with a band of 2.6 kDa was detected. Much of the interest in structure/function analysis of LAB-producing bacteriocins is driven by their potential applications. The properties of the bacteriocins studied, like the inhibition of pathogenic strains, their stability over a wide pH range, heat resistance makes them promising agents in food preservation. Further studies on food systems and more purification steps are needed for the practical application of isolated bacteriocins.

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