
EXTRACTION AND CHARACTERIZATION OF BACTERIOCIN FROM CUCUMBER (*CUCUMIS SATIVUM*) EXTRACT

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Abstract

Lactic acid bacteria are a group of Gram positive non – sporulating rods or *Cocci*, which produce lactic acid as one of the main fermentation products of the metabolism of carbohydrate. Bacteriocins are ribosomally synthesized extra cellularly released low molecular mass peptide. The present study aimed to isolate lactic acid bacteria (LAB) extract bacteriocin from cucumber (*Cucumis sativum*) extract. LAB was identified using conventional biochemical test and by sugar fermentation using API50CHL as *Lactobacillus plantarum* and *Lactobacillus acidophilus*. Bacteriocin extracted from the isolated LAB was tested for antibacterial activity on *S. cereus* and *E. coli* highest activity zone of inhibition of 9.3±0.33mm on *Staphylococcus aureus* was obtained. Characterization of the bacteriocin extract shows that they are thermo stable (40°C – 121°C) active over a wide range of pH (3 – 11) inactivated by the enzyme trypsin and addition of sodium dodecylsulfate (SDS), Tween 80 and urea reduced the antibacterial activity, while addition of EDTA enhanced the antibacterial activity.

Key words: Bacteriocin, Cucumber, Characterization, Lactic Acid Bacteria (LAB), *Staphylococcus aureus*.

INTRODUCTION

Bacteriocins are ribosomally synthesized extracellularly released low molecular mass peptide or proteins. They are proteinous toxins that are active against other bacteria either of the same specie (narrow spectrum) or across genera (broad spectrum). They are generally recognized as “natural” compounds able to influence the safety and quality of foods (Zavala *et al.*, 2007). Bacteriocins are antimicrobial compounds that are produced by many different bacterial specie including lactic acid bacteria (LAB) (Cleveland *et al.*, 2001). Some bacteriocin produced by LAB inhibit not only closely related specie, but are also effective against food borne pathogens such as *Listeria monocytogenes* and many Gram positive food spoilage causing microorganisms (Svetoslav, 2009). For these reason, bacteriocins can be used to confer a preservative activity to foodstuff. Current trends have shown that consumers are particularly aware of the health concerns regarding food additives and health benefits of “natural” and “traditional” foods, processed with no added chemical preservatives, these foods are becoming more and more attractive to consumers (Zavala *et al.*, 2007). Food preservation is carried out to maintain the quality of raw material and physico – chemical properties as well a functional quality of the product whilst providing safe and stable products (Jeevarantnam *et al.*, 2004). Preservation aims at either to eliminate or reduce the growth of potential spoilage or pathogenic organisms in food and also extend its shelf life. The consumption of food that has been formulated with chemical preservatives has also increased consumer concern to demand for more natural and minimally processed foods (Cleveland *et al.*, 2001). Since food safety has become an increasingly important international concern, the application of anti-microbial peptides from Lactic Acid Bacteria (LAB) that target food pathogens without toxic or adverse effects to consumers has received great attention.

The US cost of foodborne illness associated with *Campylobacter jejuni*, *Clostridium perfringens*, *Escherichia coli* 0157: H7, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Toxoplasma gondii* is between \$ 6.6 and \$ 34.9 billion (WHO, 2016). Outbreaks of emerging pathogens such as *L. monocytogenes* have prompted the food industry, the public, and the government to question the adequacy of current methods of food preservation (Bumpres, 2010). As a result, there has been a great interest in naturally produced antimicrobial agents.

This work is therefore set-up with the aim of isolating lactic acid bacteria from cucumber (*Cucumis sativum*) extracting bacteriocin from the isolated LAB and characterizing the extracted bacteriocin.

MATERIALS AND METHODS

Collection and Processing of Samples

Fresh visibly disease free *Cucumis sativum* (Cucumber) was purchased from Rimi Market, Kano Municipal LGA, Kano State. Further confirmation and authentication of *Cucumis* was carried out at the Herbarium of the Department of Plant Biology, Bayero University, Kano. They were immediately transported to the laboratory. Ten grams (10g) of the samples were homogenized for 5 minutes in 90ml of sterile normal saline solution (NaCl 8.5g/l) and serial dilution was made up to 10^{-5} dilution (Todorov and Dicks, 2005).

Isolation of Lactic Acid Bacteria (LAB)

Serially diluted sample was plated by pour plate method on De Man Ragosa Sharpes (MRS) agar, in triplicate. The plate was incubated anaerobically in an anaerobic jar for 48 to 72 hours. Distinct white and creamy colonies were sub - cultured and well isolated colonies were selected and transferred into MRS broth for 24 hrs (Emerenini *et al.*, 2013).

Identification of LAB

The isolates were characterized using Gram staining, indole test, oxidase test, catalase test and sugar fermentation presented below:

Gram Staining: It is carried out and described by (Cheesebrough, 2006).

Biochemical Tests were carried out to confirm the identity of the isolate based on the standard protocols describe by (Sandle, 2016). They include catalase test, oxidase test, indole, methyl red.

Sugar Fermentation Using API 50 CHL

Two representative isolates were selected. The selected two (2) isolates were further confirmed for production of acids from carbohydrates and related compounds by the use of API 50 CHL system (Biomérieux® SA, France). All LAB identification procedures were conducted in accordance with manufacturer's instructions.

Extraction of Crude Bacteriocin

The isolates were each inoculated into 250ml of De-Man Ragosa Sharpe (MRS) broth (Hi media Laboratory Pvt Ltd. India) (pH-6.0) at 5% inoculum (12.5ml) of overnight culture at 35°C for 48hrs after incubation. The cells were removed from the growth medium by centrifugation 10,000rpm for 15minutes Yang *et al.* (2014).

Determination of Physical, Chemical and Biochemical Properties of the Extracted Bacteriocin

Bacteriocin Activity at Various pH and Temperature

The method described by Yang *et al.* (2014) was adopted. The bacteriocin preparation was treated at various pH values (3 – 11) using NaOH and HCL and then incubated at 30°C for 3hrs. Their pH was adjusted to 7.0 and the solutes were assayed for antibacterial activity. Heat stability was examined by treating the bacteriocin preparation at temperatures of 30, 40, 50, 60, 70, 80, 90, 100 and 121°C for 15minutes. The bacteriocin activity of the different heat treated bacteriocin was measured by agar well diffusion (Balciunas *et al.*, 2013).

Bacteriocin Activity at Various Concentration of Enzyme and Detergents

The bacteriocin extract was treated with different concentration (0.1µg/ml, 1.0µg/ml) of the enzyme trypsin (Hi Media Laboratory Pvt Ltd. India). The detergents that will be tested are sodium dodecyl sulphate (SDS). Tween 80, Tritone X – 100, EDTA and Urea at concentrations of 0.1%, 1.0%, 2.0% and 5.0% each (Hi media laboratory Pvt. Ltd. India). All the treated samples and untreated crude bacteriocin (control) was incubated at 30°C for 5hrs and tested for antibacterial Yang *et al.* (2014) and Todorov, (2009).

Statistical Analysis

Data were presented as mean and standard deviation. Mean variation of the variables were analysed using one- way analysis of variance (ANOVA). LPCBE and LACBE variation were analysed using independent Student's T-test. Significance difference was declared at 5% significance level (P<0.05). Statistical Package for the Social Science (SPSS) version 20.0 was used for data analyses.

RESULTS AND DISCUSSION

Lactic acid bacterial isolates appeared on MRS agar as small smooth flat white colonies and cream raised colonies. The lactic acid bacteria isolated in this work from cucumber (*Cucumis sativum*) is an indication of its presence in vegetables as reported by Muccillia and Restuccia, (2015).

Gram staining and biochemical test reveals them as gram positive rods, indole, catalase and oxidase negative, non – motile and do not form spores. Similar finding were reported by Chen *et al.* (2025).

Characterization of the isolates using sugar fermentation from cucumber and designated C₁ and C₂ isolates as *L. plantarum* and *L. acidophilus* t 99.9%, 99.7% respectively using API WEB. This similar to the work of Sharpe (2009) who isolated lactic acid strains from fresh cut vegetables. In a study on selection and biochemical characterization of LAB strains isolated from salad dressing(Desislava *et al.*,2014; Roza *et al.*, 2025; Kaya, 2025) found the biochemical profiles of the isolates using API550CHL and after subsequent softant processing with different reliability as *L. pentosus* (98.3%), *L. plantarum* (91.6%), *L. plantarum* (99.7%) and *L. plantarum* (99.95%).

Bacteriocin extracted from the isolated strains namely; *L. plantarum* (C₁) and *L. acidophilus* (C₂) is referred to as bacteriocin *Lactobacillus plantarum* crude bacteriocin extract (LPCBE) and *Lactobacillus acidophilus* crude bacteriocin extract (LAPBE). It has been reported by several researchers that LAB produces bacteriocin – like substances (Yang *et al.*, 2012).

Preliminary bioassay reveals higher zone of inhibition recorded with *S. aureus* which is a gram positive organisms (Table 1) statistically there was significant difference between the means of zones of inhibition at 55% level. Marwa *et al.* (2015) reported that the inhibitory effectiveness of bacteriocin was higher on gram positive bacteriocin than gram negative bacteria.

The effect of pH and heat treatment on the stability of the crude bacteriocin taken at different pH value, shows that it was stable over a wide range pH (Table 2) highest zone of inhibition was observed at (pH 2, 4, 10 and 11) Marwa *et al.* (2015) reported in a similar study that bacteriocin organisms. However, the findings of this work does not conform with that of Senbagam *et al.* (2013) who extracted bacteriocin whose activity was deactivated at extreme pH of 12. The wide range of pH tolerance indicates that such bacteriocin may be useful in acidic as well as non – acid foods. Bacteriocin remains stable over a wide range of temperature after treatment for 15 minutes. The observed stability across a range of pH and temperature condition support earlier findings that LAB bacteriocin possesses robust physicochemical properties suitable for food preservation application Chen *et al.* (2025); Roza *et al.*, (2025) Table 3. The bacteriocin remains stable after treatment at temperature of 100 and 121°C.

Earlier studies revealed certain bacteriocins are thermostable in nature and can withstand high temperature upto 121°C (Ravi *et al.*, 2012). Temperature stability is important if the bacteriocins are to be used as a food preservative, become many procedure of food preparation involve heating. The examined bacteriocin exhibited strong heat stability which means it could be placed within the heat stable low molecular weight group of bacteriocin. Lactocin NK 24 produced by *L. lactic*, lost 87.5% of its activity after 30 minutes at 100°C and was completely inactivated after 15 minutes at 121°C Lee and Pack, (2001) statistically, there is significant difference between the means of temperature and pH at 5%.

Table 1: Preliminary Bioassay of the Crude Bacteriocin against Test Organism

Test Organisms	Extract/Mean Zone of Inhibition (mm)	
	LPCBE	LACBE
<i>S. aureus</i>	8.60±0.033	9.00±0.57
<i>E. coli</i>	6.60±0.33	6.30±0.33

Key: Values are mean of three different reading ± standard error zone diameter of well = 4mm

LPCBE = *Lactobacillus plantarum* strain DSM10667 crude bacteria extract

LACBE = *Lactobacillus acidophilus* strain NBRC 13951 crude bacteriocin extract

P > 0.05

Table 2 Effect of pH on the Antibacteria Activity of the Crude Bacteriocin Extract against some Test Organisms

pH	Extract/Organisms/Mean Zone of Inhibition (mm)			
	LPCBE		LACBE	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
3	9.60±0.33	9.00±0.00	9.30±0.33	9.00±0.00
4	9.60±0.33	8.00±0.00	8.60±0.33	6.60±0.67
5	8.00±0.57	7.30±0.33	8.30±0.33	7.30±0.33
6	8.30±0.33	6.60±0.33	7.30±0.33	7.60±0.33
7	8.30±0.33	6.60±0.33	7.30±0.33	6.30±0.33
8	8.00±0.57	7.30±0.33	7.60±0.33	7.30±0.33
9	9.60±0.33	8.00±0.57	8.60±0.33	7.30±0.66
10	9.60±0.33	8.60±0.33	8.60±0.33	7.30±0.33
11	9.30±0.33	8.30±0.33	9.30±0.33	8.60±0.33
Control	8.30±0.33	6.60±0.33	9.00±0.57	6.30±0.33

Table 3: Effect of Heat on the Antibacterial Activity of the Crude Bacteriocin Extract Against some Test Organism

Temperature (%)	Extract/Organisms/Mean Zone of Inhibition (mm)			
	LPCBE		LACBE	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>E. coli</i>
40	6.60±0.33	6.00±0.00	7.60±0.33	6.30±0.33
50	7.00±0.57	6.30±0.33	6.30±0.33	6.00±0.00
60	7.30±0.33	6.60±0.33	7.00±0.00	6.30±0.33
70	6.60±0.33	6.30±0.33	7.30±0.00	6.60±0.33
80	6.60±0.33	6.30±0.33	7.70±0.33	6.30±0.33
90	6.60±0.33	6.60±0.33	7.60±0.33	6.60±0.33
100	7.30±0.33	6.60±0.33	7.60±0.33	6.00±0.00
121	8.60±0.33	7.30±0.33	10.00±0.00	6.60±0.33
Control	8.30±0.33	6.60±0.33	9.00±0.00	6.30±0.33

Table 4: Effect of Enzyme trypsin on the Antibacterial Activity of the Crude Bacteriocin

Extracts/Conc.	Test Organisms/Mean Zone of Inhibition	
	<i>S. aureus</i>	<i>E. coli</i>
LPCBE 0.1	4.0	4.0
1.0	4.0	4.0
Control	8.3	6.6
LACBE 0.1	4.0	4.0
1.0	4.0	4.0
Control	9.3	6.0

P> 0.05 No significant difference values are mean $\pm$ standard error zone of well = 4mm

The effect of the proteolytic enzyme trypsin on the antibacterial activity of LPCBE and LACBE at different concentration of 0.1mg/ml and 1.0mg/ml on *S. aureus* and *E. coli* shows zone off inhibition of 4mm only which equals to the diameter of well (Table 4). There were no zones of inhibition indicating that the antimicrobial activity has been destroyed completely. Corsetti *et al.* (2004) reported that bioactivity of bacteriocin extracted from milk was completely destroyed by trypsin and repsin enzymes. The effect of the proteolytic enzyme confirms the protenous nature of bacteriocin (Rari *et al.*, 2012).

The effect of sodium dodecylsulfate (SDS) at different concentrates on the bioactivity of the extracted bacteriocin studied in this research shows that at low concentration of SDS activity is decreased wile at high concentration of 2.0% and 5.0% activity was completely lost Kaktchman *et al.* (2012) reported similar findings of reduced activity by SDS and urea. In this studies effect of urea also showed decreased activity at low concentration while activity was completely lost at high concentration.

The tween 80 obtained in this work shows no significant difference on the bioactivity by zone of inhibitory indicating non sensitivity of the tween 80. Kaktcham, (2012) reported that partially purified bacteriocin was not sensitivity of partially purified bacteriocin was not sensitive to Nacl, Tween 80, tween 20 and triotone X – 100. Addition of EDTA on the bioactivity of the bacteriocin showed enhanced activity. Higher zones of inhibition were obtained. This finding conforms with that Sharma *et al.* (2011) and Kaktcham (2012) who reported that addition of EDTA to bacteriocin R75 and LP65H repeatedly enhances their bioactivity. Many other bacteriocin produced by *L. plantarum* strains were found to be stable to the above factors. However plataricin C₁₉ produced by *L. plantarum* C₁₉ lost its activity after treatment with SDS and Tritone X – 100 (Chopra *et al.*, 2015).

Conclusion

This study revealed the presence of *Lactobacillus* in cucumber (*Cucumis sativum*) which were confirmed using biochemical test and API50CHL techniques. The physical, chemical and biochemical properties show that the bacteriocin is stable over a wide range of temperature and pH. The antibacterial activity of the bacteriocin was completely lost by the addition of Trypsin enzyme tween 80 does not have any effect on the bioactivity.

Recommendations

- The bacteriocins stability heat and pH suggest potential application as a natural biopreservative in mildly processed and acidic foods.
- Sensitivity to enzymes and denaturing agents indicates a proteinaceous nature, warranting further purification molecular characterization and formulation stability studies

Acknowledgments

This research was possible through the financial assistance of TETFund (TETF/DR&D/CE/POLY/KANO/RG/2025/VOL.I) given to the corresponding author Dr. Halima Bashir Ahmad. Immeasurable thanks go Mal. Idris Bala and Umar Sani Dorayi of Microbiology Department, Bayero University, Kano who assisted the laboratory analysis.

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