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PURIFICATION AND SPECIFICATION OF BACTERIOCIN PRODUCED BY SOME *Lactobacillus* SPP. ISOLATED FROM FOOD

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ABSTRACT: A total of 20 isolates of lactic acid bacteria (LAB) were isolated anaerobically from different food sources (Domiati cheese; raw milk and mixed pickles). Three out of 20 isolates showed high inhibition of growth pathogenic bacteria by well diffusion assay method and therefore they were chosen for bacteriocin production and further studies. The selected isolates were identified based on morphological, biochemical and MALDI-TOF mass spectrometry. All of the tested species gave a score value between 2.116 to 2.165 (100%) were correctly identified by MALDI-TOF- MS to the genus and species levels. They were identified as *L. brevis*, *L. plantarum* and *L. fermentum*. The bacteriocin was purified by salt precipitation and gel chromatography methods. The molecular weight was determined by SDS-PAGE and amino acid composition was also analysed. The purified bacteriocin was characterized and found to be thermostable at temperatures up to 90°C for 30min, pH from 3 to 11 and its activity improved in the presence of Tween 80, SDS and EDTA. The contents of bacteriocin from the acidic amino acid residues aspartic + glutamic (asp+glu) were 0.74% for all tested samples. The contents of the basic amino acids argnine + lysine + hisitidine (arg + lys + his) were 0.23% for all the tested samples.

Key words: Lactic acid bacteria, bacteriocin, stability, gel chromatography, *Lactobacillus*, antimicrobial activity, amino acid.

INTRODUCTION

Lactic acid bacteria (LAB) are widely used in food industry as starter culture for fermentation. Lactobacilli have been used since decades against infectious diseases **(Bernet *et al.*, 1994)** and have been extensively studied for their ability to protect against pathogenic bacteria. These organisms have been widely used as probiotics **(Tannock, 1999; Sgouras *et al*., 2004)**.

Many of these lactic acid bacteria are known to produce antibacterial substances including bacteriocins which can inhibit the growth of several pathogenic bacteria. Bacteriocins from lactic acid bacteria are natural antimicrobial peptides or small proteins with bactericidal or bacteriostatic activity against genetically closely related species **(Klaenhammer, 1988)**. Bacteriocins can be classified broadly as those synthesized by Gram-positive and those by Gram-negative bacteria. Among those synthesized by Gram-positive bacteria, lactobacilli bacteriocins are of commercial value **(Garneau *et al*., 2002)**.

Lactobacillus bacteriocins are found within each of the four major classes. Class: I bacteriocins (antibiotics) were discovered in the *Lactobacillaceae* by **(Mortvedt *et al.*, 1991).** These bacteriocins are small membrane-active peptides (<5 kDa) containing an unusual amino acid, lanthionine. The class: II bacteriocins are less heatstable, non-lan- thionine containing and membrane-active peptides (<10 kDa). The class: III bacteriocins have been found in *Lactobacillus*, include heat labile proteins of large molecular mass. The class: IV bacteriocins are a group of complex proteins, associated with other lipid or carbohydrate moieties, which appear to be required for activity. They are relatively hydrophobic and heat stable **(Karaoğlu *et al.*, 2003).**

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Several types of bacteriocins from food-associate lactic acid bacteria have been identified and characterized **(Ravi *et al.*, 2011).** Because of the increasing demand for more natural and microbiologically safe food products, there is a need for biopreservation methods. Bacteriocins have considerable potential for food preservation, as well as for human therapy as a potential supplements or replacements for currently used antibiotics **(Fricourt *et al.*, 1994; Ogunbanwo *et al*., 2003)**.

Different bacteriocin exhibits different inhibition profile on food spoilage and pathogenic microorganisms. Therefore, they could be natural replacements for synthetic food preservatives **(Ennahar *et al.*, 1999).** In order to increase the productivity of bacteriocins, a better understanding of factors affecting their production is essential. Bacteriocin production has been reported to be affected by several factors including carbon and nitrogen sources; and fermentation conditions, such as pH, temperature and agitation **(Ennahar *et al.*, 1999)**.

Therefore, the present study aimed to purify and characterize the bacteriocin produced from *Lactobacillus* spp.

MATERIALS AND METHODS

Isolation of Lactic Acid Bacteria from Different Sources

Lactic acid bacteria (LAB) were isolated from domiati cheese, raw milk and mixed pickles. The samples were collected from supermarket in Zagazig city during 2018 using sterile bottles and stored in an ice box until delivering to the laboratory of Agric. Microb. Dept., Fac. Agric, Zagazig University, Egypt for analysis. One gram/milliliter of each sample was diluted in 0.9% sterile saline solution to a final volume of 10ml and 0.1ml of each dilution was plated on selective MRS- medium (DeMan ,Rogosa-Sharpe, Oxoid, CM 361). The pH of the medium was adjusted to 6.5, using a digital electrode pH meter. Plates were incubated at 37±2°c anaerobically in jars with AnaeroGen sacks (Oxoid,UK) for 48 hr. After incubation, different colonies were randomly collected from each sample, the selected colonies were purified by streak plate technique. The purified bacterial isolates were stored in MRS broth at 4°C for further studies **(De- Man *et al*., 1960)**.

Preliminary Identification of Lactic Acid Bacteria

Morphological and biochemical characters were used to identify the most acid resistant bacteria isolates according to **Logan and De Vos (2009)**. The following tests were performed: cell morphology, Gram reaction, catalase test, growth at 10°C for 5 days and 45°C for 48 hr. in MRS- broth, salt tolerance (4% and 6.5% NaCl in MRS- broth). Sugar fermentation tests were applied using glucose, lactose, mannitol, galactose, D- cellobiose, raffinose, D-trehalose and sucrose.

MALDI-TOF-MS Profile Method

Identification of LAB had been confirmed by using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) in peptide and protein analyses.

One large colony from each of selected bacterial isolate (enough to fill about one half of a 10-µl inoculating loop) was suspended in 70% ethanol in a 1.5 ml microcentrifuge tube and loaded three times onto ground steel MALDI target according to the manufacturer′s instruction (Bruker Daltonics, Breman, Germany). Matching between experimental MALDI-TOF-MS profiles obtained from bacterial isolates and the reference MALDI-TOF-MS profiles is expressed by a BioTyper according to a Log (score) and an associated-color code (green, yellow and red). Briefly, a BioTyper log (score) exceeding 2.3 (green color) indicates a highly probable identification at the species level. A Log (score) between 2.0 and 2.3 means highly probable identification at the genus level (green color) and probable identification at the species level. A Log (score) between 1.7 and 2.0 (yellow color) implies only probable genus identification, while score value under 1.7 (red color) means no significant similarity between the unknown profile and any of those of the database. Micro Flex mass spectrometeries were performed at Academic Park, Faculty of Medicine, Alexandria, University, Egypt, according to **Biswass and Rolain (2013)** as well as **Nacef *et al*. (2016)**.

Partial Purification of Bacteriocin

A 250ml of MRS- broth was inoculated with 1% overnight inoculum of the isolate and incubated at 37°C for 18 hr. The cells were separated by centrifuging the broth at 10,000 rpm for 10min at 4°C. The crude bacteriocin substances was partially purified by salt saturation method using ammonium sulphate to precipitate out proteins. The crude bacteriocin was stirred using a magnetic stirrer with the addition of ammonium sulphate slowly till saturation point of 70% reaching at a temperature of 4°C. The precipitated proteins were separated by centrifugation at 10,000 rpm for 20min at 4°C. The protein pellet obtained was dissolved in 1M phosphate buffer of pH 7.0 **(Vera *et al*., 2012).**

Gel Chromatography

This analysis was held at Agric. Biochemistry, Dept., Fac., Agric., Zagazig Univ. Sephadex G25 was used as a column to purify (desalt) the salt precipitated protein. The gel column was pre equilibrated with 0.1M Tris HCl buffer (pH 7), 10 ml of the precipitated protein dissolved in phosphate buffer previously was added onto the column without disturbing the gel. The proteins were eluted with 0.1M Tris HCl buffer (pH 7) and sample fractions of 1ml were collected in eppendoff tubes by setting the flow rate to 15 ml/hr. **(Ravi *et al.*, 2012).**

Molecular Weight Determination of Protein by SDS PAGE

This analysis was held at Agric. Biochemistry, Dept., Fac., Agric., Zagazig Univ., An amount of bacteriocin from lactic acid bacteria (10 mg) was dispersed in 1ml SDS 10% with 100 µl β-mercaptoethanol for 15 min with vortexing every 5 min. The extract was centrifuged at 10,000 rpm for 10 min **(Osman *et al.*, 2018).** A mixture of 20 µl extract and 20 µl of SDS-loading sample buffer (SDS 4%, β-mercaptoethanol 3%, glycerol 20%, Tris HCl 50 mM pH 6.8 and bromophenol blue traces), was heated at 96°C for 3 min and 10 µl aliquot (per lane) was electrophoresed by SDS-PAGE according to **(Laemmli, 1970).**

Bacteriocin Assay

The antibacterial activity of the bacteriocin isolated from *Lactobacillus* spp. was determined using the well diffusion method as described by **Ivanova *et al.* (2000)** against 2 pathogenic bacteria Gram positive *Staphylococcus aureus* ATCC 43300 and Gram negative *E. coli*, which was kindly provided by the Dept. of Microbiology, Faculty of Medicine, Alex. Univ. 50 µl of the bacteriocin were placed in 5-mm diameter wells that had been cut in nutrient-agar plates previously seeded with the indicator bacteria. The plates were incubated at 37°C for 24 hr. After incubation, the diameter of zone of growth inhibition was measured.

Stability of Bacteriocin at Different pH and Temperature Degrees

Effect of Ph level

To determine the effect of pH, 0.5 ml of purified bacteriocin was added into 4.5 ml of nutrient- broth at different pH values (3 to 11) and incubated for 30 min at 37°C. Each of the bacteriocin samples treated at different pH values was assayed against indicator bacteria by well diffusion method **(Motta and Brandelli, 2002; Sharma and Gautam, 2007)**.

Effect of different temperatures

Purified bacteriocin (0.5 ml) was added into 4.5 ml of nutrient- broth in the test tube. Each test tube was then overlaid with paraffin oil to prevent evaporation and then incubated at different temperatures (30, 40, 50 and 60, 70, 80, 90 and 100°C) for 30 min. The preparations containing nutrient- broth (4.5 ml) and bacteriocin (0.5 ml) in test tubes were plugged with nonabsorbent cotton and covered with aluminum foil and kept in an autoclave at 121°C for 15 min to check its activity at very high autoclaving temperature. The bacteriocin activity of above different heat-treated was measured by well diffusion method **(Sharma *et al*., 2006)**

Effect of surfactants

The effect of surfactants on the bacteriocins was tested by adding SDS, EDTA and Tween 80 individually (0.5% *V/V* final concentration), to bacteriocins and untreated bacteriocin preparation was used as positive control. All samples were incubated at room temperature for 2 hr., then tested for residual antibacterial activity by well diffusion method **(Sivaramasamy *et al.*, 2014).**

Amino Acid Analyzer

This experiment was held at Regional Centre for Food and Feed, Research Center, Ministry of Agriculture. Total amino acids composition in lyophilized crude extract of bacteriocin of *L. brevis*, *L. plantarum* and *L. fermentum* were determined by amino acid analyzer apparatus model "Eppendorf LC3000" **(Simpson *et al.*, 1976)** using the following steps: Bacteriocin crude extract from production media, 10 ml of sample were taken and centrifuged at 10,000 rpm for 10 min. the supernatant was collected and lyophilized. A known weight 0.2 g from lyophilized crude extract of bacteriocin of each sample was received 10 ml 6 N hydrochloric acid in a sealing tube, and then placed in oven at 110°C for 24 hr. Hydrolysates were transferred quantitatively into a porcelain dish and the hydrochloric acid was then evaporated to dryness at 50-60°C on a water bath. Distilled water (5 ml) was added to the hydrolysate and then evaporated to dryness to remove the excess of hydrochloric acid and finally the residue was dissolved in 10 ml distilled water and filtrate through 0.45 Mm filter. The filtrate was dried under vacuum with a rotary evaporator, then 10 ml of distilled water were added and the samples were dried a second time. One ml of 0.2 N sodium citrate buffer at pH 2.2 was added and the samples were stored frozen in a sealed vial until separation of amino acids by amino acid analyzer [Column: hydrolysate column Eppendorf LC 3000 (250 × 4.6)]. The temperature of amino acid analyzer was 47°C; Sample: 20 µl; Buffer system: Sodium acetate, buffer A (pH 3.3), buffer B (pH 3.6), buffer C (pH 4.3) and buffer D (pH11.0); Flow rate: 0.2 ml/min.). Ninhydrin was used for the detection of amino acids at 440 nm for proline and 570 nm for the other amino acids through an oxidative decarboxylation reaction. The peak area and percentage of each amino acid were calculated by computer software AXXIOM CHROMATOGRAPHY- 727.

RESULTS AND DISCUSSION

Isolation and Preliminary Screening of Isolated LAB

A total of 20 isolates were isolated from domiati cheese, raw milk and mixed pickles as shown in Table 1. Most of them were characterized as Gram positive, catalase negative and non spore forming bacteria. From the preliminary screening only 3 isolates out of 20 were selected based on their high activity for inhibition of pathogenic bacteria. These 3 isolates were then identified using phenotypic and biochemical methods and subjected to *in vitro* characterization to purify bacteriocin from them.

Identification of the Selected Isolates

Morphological and biochemical characteristics were used to identify the 3 selected isolates (Table 2) according to **(Logan and De Vos, 2009).** Gram positive and catalase negative isolates were considered as presumptive LAB. All the isolates were Gram positive, catalase negative and non-spore forming, negative for gas production from glucose. According to morphological and biochemical tests the isolates R3 and M5 grew at 10°C except isolate D2 which grew at 45°C. All isolates tolerated 4% of NaCl concentration. Concerning growth at 6.5% NaCl, strain R3 folerated it but M5 and D2 did not. All the isolates fermented lactose and sucrose which showed various fermentation levels to other carbohydrates. Based on these results, isolates R3 tend to be *Lactobacillus brevis*, M5 *Lactobacillus plantarum* and *D2 Lactobacillus fermentum*.

Direct Identification of the Tested Bacteria Using MALDI-TOF-MS

The above mentioned isolates were identified at Academic Park Fac. Medicine Alex. Univ., Egypt, using MALDI-TOF-MS. (matrix-assisted lazer desorption ionization-time of flight mass spectrometry). This strategy is becoming a method of choice for determining the genus, species and even subspecies of bacterial isolates **(Carbonnelle *et al.*, 2012; Dušková *et al.*, 2012).** Also, this strategy is achievable for other microorganisms (*e.g.* yeasts, fungi,) from various sources **(Chalupová *et al.*, 2014)**. Using this advanced method, the identification was confirmed and the prospective strains were identical with their numbers as conserved in the International Cultural Center for Microorganisms. The score values for the bacterial isolates are shown in Table 3. All of the isolates showed a score value between 2.116

Table 1. Number of LAB isolates and their sources

Product abbreviation	No. of isolated LAB	Total percentage
Domiati cheese (D)	6	30
Raw milk (R)	7	35
Mixed pickles (M)	7	35
Total	20	100%

Table 2. Some morphological and biochemical characteristics of the selected LAB isolates

Criteria / Isolate	Presumptive name	Gram reaction	Endospore formation	Catalase test	Growth at Temp. °C		Growth at NaCl (%)		Gas from glucose	Acid from carbohydrates							
					10	45	4	6.5		Glucose	Lactose	Mannitol	Galactose	D- Cellobiose	Raffinose	D-Trehalose	Sucrose
R 3	*L. brevis*	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+
M 5	*L. plantarum*	+	-	-	+	-	+	-	-	+	+	-	-	+	+	-	+
D 2	*L. fermentum*	+	-	-	-	+	+	-	-	+	+	+	+	-	+	-	+

L.: Lactobacillus R3: Isolate from raw milk M5: Isolate from mixed pickles D2: Isolate from domiati cheese

Table 3. Rate of classification results as determined by Bruker Daltonik MALDI Biotyper

Isolate code	Analyte Name	Organism(best match)	ScoreValue
R3	**(++) (A)**	*Lactobacillus brevis* DSM 2647 DSM	2.122
M7	**(++) (A)**	*Lactobacillus plantarum* DSM 2601 DSM	2.116
D2	**(++) (A)**	*Lactobacillus fermentum* DSM 12341 DSM	2.165

- Category A= species consistency (2.300-3.000). DSM: Deutsche Sammlung von Mikroorganismen.

to 2.165 (100%) and were correctly identified to genus and species levels. All the tested bacterial strains were type strains that are included in the Bruker Database, and all spectrum scores were greater than 2.0. Thus, all of the tested LAB were correctly identified to genus and species levels with biotype software score values **(Bizzini *et al.*, 2010; Wang *et al.*, 2013)** greater than 2.0, and all of them had high degree of precision.

Purification of bacteriocin

The purification methods by using well diffusion assay method produced was summarized in Table 4. During the three stage of purification, a significant increase in bacteriocin purity and yield was observed. The susceptibilities of various Gram positive (*Staph. aureus*) and Gram negative (*E. coli*) bacteria to growth inhibition by bacteriocin produced by *L. brevis*, *L. plantarum*, *L. fermentum* were presented in Table 4. It shows antibacterial activity against *E. coli* and *Staph. aureus*. Among these, highest growth inhibition was recorded against *E. coli* and moderate inhibition was observed against *Staph. aureus*. Similarity, bacteriocin from *L. brevis* showed high inhibition activity against *E. coli* and *Staph. aureus* in the three stages of purification. Bacteriocin from *L. plantarum* was found to be high active against pathogenic *E. coli* and *Staph. aureus*. Also bacteriocin from *L. fermentum* had high activity against *E. coli* and *Staph. aureus* in the stage of gel filteration. These results indicated that the presence of bacteriocin produced by the isolated lactic acid bacteria is responsible for their antimicrobial activity **(Ravi *et al.*, 2012).**

Molecular Weight of Protein by SDS PAGE

The SDS-PAGE of bacteriocin produced from lactic acid bacteria are shown in Figure 1.The molecular weight was investigated by SDS-PAGE which showed a single band with an estimated molecular weight of 30 KDa in comparison with protein marker.

Stabitity of Bacteriocin at Different pH Values and Temperature

pH and temperature have an important role in cell growth as well as bacteriocin production. Heat stability is considered an important character not only that makes them attractive in food industry, but also, it is an important factor in classifying bacteriocins. If a bacteriocin is considered as a bio preservative, it should be stable at a wide range of pH to overcome the effects of acids and alkalis in food and also heat stable which is an important criteria, since it should withstand the effect of heat during pasteurization, it should also retain its activity for a longer period which in turn will increases the shelf life to the preserved food.

Effect of pH

Bacteriocin was active in wide range of pH, but the maximum activity was observed at pH 5, 6, 7 and 8 (Table 5). Bacteriocin could retain its antimicrobial activity partially when there was a shift to acidic or basic range. Stability of bacteriocin in different pH scale is a limiting factors for recommending its use in food items. Bacteriocin produced by *L. brevis*, *L. plantarum* and *L. fermentum* retained their antibacterial activity in an acidic pH of 5 and 6, also in a basic pH range of 7 and 8, while less activation occurred at pH 3,4,10 and 11. These results are in a good agreement with those obtained by **Sankar *et al.* (2012), Radha and Tallapragada (2015), Seema *et al.* (2015) and Sahar *et al.* (2017).**

Effect of temperature

Different heat treatments were given to the bacteriocin to check its stability. The stability was assessed by noting the diameter of zone of inhibition after heat treatments. From Table 6, it can be noted that the bacteriocin produced by *L. brevis*, *L. plantarum* and *L. fermentum* was stable at temperatures ranging between 30°C to 80°C for period of 30 min which shows that the bacteriocin is thermostable in nature as it can withstand high temperature up to 121°C for 15 min. Thermostability of bacteriocin at high temperature makes it feasible to used foods which needs to be pasteurised before consumption. Highest activity was observed when it was heated at 60°C for 30 min. and retained its activity up to 80° C for 30 min. which shows that it is able to withstand pasteurization which is related to its molecular structure composed of small peptides with no tertiary structure as found by **Parada *et al.* (2007)** which is an important characteristic of a bio preservative.

Table 4. Bacteriocin activivty of LAB strains, against Gram positive and Gram negative bacteria using well diffusion assay method

Purification stage / LAB	Culture supernatant (crude)		Partial purification		Gel filteration	
	E. coli Diameter inhibition zone (mm)*	*Staph. aureus* Diameter inhibition zone (mm)	*E. coli* Diameter inhibition zone (mm)	*Staph. aureus* Diameter inhibition zone (mm)	*E. coli* Diameter inhibition zone (mm)	*Staph. aureus* Diameter inhibition zone (mm)
L. brevis	21	17	25	25	29	29
L. plantarum	19	17	22	21	26	23
L. fermentum	17	16	20	19	26	25

L.: Lactobacillus E.: Esherichia Staph.: Staphylococcus *: Inhibition zone around the well < 5mm: No inhibition
5-10 mm: Low inhibition 11-20: Moderate inhibition 20-30 mm: High inhibition

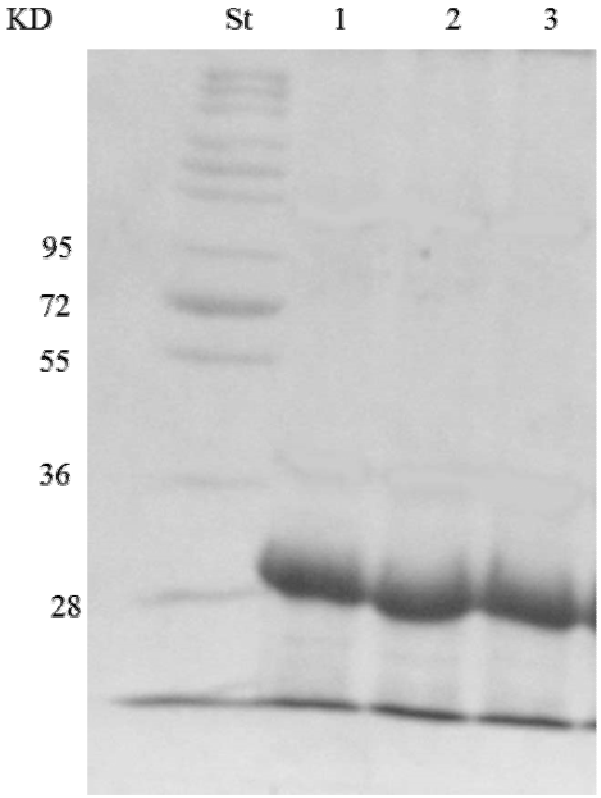

Fig. 1. SDS-PAGE of bacteriocin produced from *L. brevis* (lane 1), *L. plantarum* (lane 2) and *L. fermentum* (lane 3)

Table 5. Stability of bacteriocin produced by LAB at different pH against two pathogenic bacteria tested by well diffusion assay method

Bacteria / pH	Diameter of the inhibition-zone (mm) * against *E. coli*			Diameter of the inhibition-zone (mm) against *Staph. aureus*		
	L. brevis	*L. plantarum*	*L. fermentum*	*L. brevis*	*L. plantarum*	*L. fermentum*
3	4	3	3	3	4	4
4	5	6	4	5	6	6
5	22	23	22	23	21	21
6	29	26	26	29	23	25
7	27	25	25	28	22	23
8	24	21	20	23	20	19
9	14	15	14	15	15	14
10	8	7	7	8	7	8
11	5	4	5	5	5	4

L. :Lactobacillus E.: Esherichia Staph.: Staphylococcus *: Inhibition zone around the well < 5mm: No inhibition
5-10 mm: Low inhibition 11-20: Moderate inhibition 20-30 mm: High inhibition

Table 6. Stability of bacteriocin producd by LAB at different heat treatments against two pathogenic bacteria tested by well diffusion assay method

Temperature (°C) / Bacteria	Diameter of the inhibition-zone (mm) * against *E. coli*			Diameter of the inhibition-zone (mm) * against *Staph. aureus*		
	L. brevis	*L. plantarum*	*L. fermentum*	*L. brevis*	*L. plantarum*	*L. fermentum*
30°C, 30 min.	29	26	26	29	23	25
40°C, 30 min.	28	26	26	29	23	25
50°C, 30 min.	25	22	23	26	21	23
60°C, 30 min.	24	21	23	26	20	21
70°C, 30 min.	21	19	19	22	20	19
80°C, 30 min.	18	16	17	20	18	17
90°C, 30 min.	11	12	12	14	12	13
100°C, 30 min.	8	7	8	9	8	7
Autoclaving (121° C, 15 min.)	5	4	4	6	5	5

L. :Lactobacillus E.: Esherichia Staph.: Staphylococcus *: Inhibition zone around the well < 5mm: No inhibition
5-10 mm: Low inhibition 11-20: Moderate inhibition 20-30 mm: High inhibition

The bacteriocin produced from *L. brevis* was found to be stable from pH 5 to 8 and thermostable up to 70°C with a partial loss in activity at 80°C. *L. plantarum* was found to be active at wide pH range from 5 to 8 and active at wide range of temperatures from 30°C to 70°C for 30 min. Bacteriocin produced by *L. fermentum* was stable up to 70°C for 30 min. and pH 5-8 which showed higher inhibition activity. These results confirm those obtained by **Sankar *et al.* (2012), Radha and Tallapragada (2015), Seema *et al*. (2015) and Sahar *et al.* (2017)**.

Effect of Surfactants and Detergents on Bacteriocin Activity

Surfactants and detergents were used to study the bacteriocin activity profile. The MRS- broth was supplemented with SDS, EDTA and Tween 80 (0.5% *V/V* final concentration). From Table 7, it is noted that Tween 80 (0.5% *V/V*) improved bacteriocin activity produced from *L. brevis*, *L. plantarum* and *L. fermentum* and similar results were obtained with EDTA and SDS. Tween 80 supported the maximum bacteriocin activity of 33mm inhibition zone by *L. brevis* against *E. coli*. These results are in line with those found by **Sahar *et al.* (2007); Ogunbanwo *et al.* (2003)** as well as **Radha and Tallapragada (2015)**.

Amino Acids Composition of the Bacteriocins Produced by *L. brevis*, *L. plantarum* and *L. fermentum*

The amino acids composition of lactic acid bacteria (*L. brevis*, *L. plantarum* and *L. fermentum*) obtained by gel chromatography are listed in Table 8**.** The contents of the hydrophobic amino acids residues (Pro, Gly, Ala, Val, Ile, Leu, Phe) are 0.92%, 0.83% and 0.92% for *L. brevis*, *L. plantarum* and *L. fermentum*, respectively. The contents of the acidic amino acid residues (Asp + Glu) are 0.74% for all tested samples while the contents of the basic amino acids (Arg + Lys + His) are 0.23% for all tested samples. The antibacterial properties of proteins are dependent on their interaction with the bacterial cell wall and membranes *via* hydrophobic and electrostatic interaction **(Hancock, 2004)**. Positively charge protein electrostatically bind to lipopolysaccharides on the outer membrane of gram negative bacteria or lipoteichoic acids on the surfaces of gram positive bacteria **(Glinel *et al*., 2012)**.

Conclusion

Lactobacillus spp. are non-pathogenic, useful lactic acid bacteria. They produce secondary metabolites termed bacteriocins which are used as anti-microbial agents, which inhibited the growth of both Gram-positive and Gram-negative pathogenic thus exhibiting broad spectrum inhibition. The produced bacteriocin could withstand varied temperatures and pH treatments which is a novel characteristic of a protein to be explored as a food preservative (bio preservative). It was found to be stable at higher temperatures which makes it feasible to be used in liquid and solid foods which needs to be pasteurised before consumption, for example ready to eat foods. The bacteriocin can be used as a bio preservative in different foods from highly acidic foods such as fruit juices to vegetables which are alkaline.

Table 7. Effect of surfactants and detergents on the activity of bacteriocin produced by LAB strains, which showed inhibition zones of two pathogenic bacteria using well diffusion assay method

Bacteria / Detergents	Diameter of the inhibition-zone (mm) aganist *E. coli*			Diameter of the inhibition-zone (mm) aganist *Staph. aureus*		
	L. brevis	*L. plantarum*	*L. fermentum*	*L. brevis*	*L. plantarum*	*L. fermentum*
SDS	31	28	29	30	28	28
EDTA	32	28	29	31	29	28
Tween 80	33	29	30	32	29	29

L. :Lactobacillus E.: Esherichia Staph.: Staphylococcus *: Inhibition zone around the well < 5mm: No inhibition
5-10 mm: Low inhibition 11-20: Moderate inhibition 20-30 mm: High inhibition

Table 8. Comparison of amino acids composition of the bacteriocins produced by *L. brevis*, *L. plantarum* and *L. fermentum*

Amino acid g/100 ml / LAB	Aspartic (ASP)	Therionine (THR)	Serine(SER)	Glutamic(GLU)	Glycine (GLY)	Alanine (ALA)	Valine (VAL)	Isoleucine (ILE)	Leucine(LEU)	Tyrosine(TYR)	Phenylalanine (PHE)	Hisitidine (HIS)	Lysine (LYS)	Argnine (ARG)	Proline(PRO)	Cystine(CYS)	Methionine
L. brevis	0.14	0.08	0.10	0.60	0.11	0.12	0.13	0.10	0.17	0.08	0.10	0.04	0.13	0.06	0.26	---	---
L. plantarum	0.14	0.08	0.10	0.60	0.11	0.13	0.13	0.10	0.17	0.08	0.11	0.04	0.13	0.06	0.26	---	---
L. fermentum	0.14	0.08	0.10	0.60	0.11	0.13	0.13	0.10	0.17	0.08	0.12	0.04	0.14	0.06	0.26	---	---

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تنقية ومواصفات البكتريوسين المنتج بواسطة بعض أنواع جنس اللاكتوباسلس المعزولة من الأغذية

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تم عزل ٢٠ عزله من بكتيريا حامض اللاكتيك لاهوائيا من مصادر غذائية مختلفة وهى (الجبن الدمياطى- اللبن الخام - المخللات)، تم اختيار ٣ عزلات فقط من ال ٢٠ عزله والتى أظهرت أعلى نشاط ضد البكتيريا المرضية بواسطة اختبار well diffusion assay methods وهى التى تم اختيارها لإنتاج البكتريوسين، العزلات المختارة تم تعريفها مورفولوجيا وبيوكميائيا وكذلك عن طريق تطبيق MALDI-TOF MS والذى تم بواسطته تعريف العزلات إلى مستوى الجنس والنوع، وكانت هذه الأنواع هي*L. brevis, L. fermentum, L. plantarum* والتى تم عزل وتنقيه البكتريوسين منها، تم تنقيه البكتريوسين باستخدام طريقة Salt precipitation ثم التفريد على جيل كروماتوجرافي وقياس الوزن الجزئيي بواسطة SDS-PAGE وتحليل الأحماض الأمينة، ولقد وجد أن البكتريوسين المعزول ثابت لدرجات الحرارة العاليه والتى قد تصل إلى ٩٠°م لمده ٣٠ دقيقه وكذلك مقاوم لمدى عالى من الـ pH من ٣ إلى ١١ وتم تعزيز نشاط البكتريوسين بإضافة EDTA وSDS و80 Tween، وعند تحليل الأحماض الأمينية للبكتريوسين وجد أن جميع العينات تحتوى على أحماض أمينية حمضية (الأسبارتك والجلوتاميك) بنسبة ٠,٧٤% وكذلك مجموعة من الأحماض الأمينية القاعدية (الأرجنين والليسين والهيستادين) بنسبة ٠,٢٣%.

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