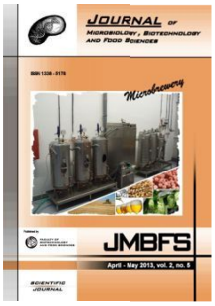

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CHARACTERIZATION OF THE PARTIALLY PURIFIED PLANTARCIN SR18 PRODUCED BY *LACTOBACILLUS PLANTARUM* SR18

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ABSTRACT

The bacteriocin bound to the cells and that secreted into the culture filtrate of *Lactobacillus plantarum* SR18 were precipitated by 75% ammonium sulphate, dialysed and further purified by Gel filtration on Sephadex G-100. Bacteriocins were purified from proteins bound to the cell of *L. plantarum* SR18 (plantarcin SR18 a) and culture filtrate proteins (plantarcin SR18 b), respectively. The SDS-PAGE of partially purified Plantarcin SR18a showed a molecular weight of 3.5 KDa. While, plantarcin SR18 b had a molecular weight of 10.3 KDa. The antibacterial activity of the tested plantarcin SR18 preparations suffered no measurable loss after 45 min at 80ºC. Whereas, At 100ºC, significant decrease in the activity of bacteriocin preparations (60- 80 %) took place by the end of 45 min. At pH ranged from 5-8, the activity of the plantarcin SR18 preparations suffered no measurable loss. Dissociating agents significantly affected the bacteriocin activity. Thus, tween 80 and mercaptoethanol increased the activity of bacteriocin preparations to 1.2-1.4 fold. Sodium dodecyl sulphate (SDS) increased the activity of the tested bacteriocin preparations by about 20%.The lowest residual activity (60%) was recorded after treatment with Triton X100 for 45 min. Protease completely inhibited the activities of all forms of plantarcin SR18 after 45 min at 37ºC.

Keywords: Plantarcin, molecular weight, stability, dissociating agents, protease

INTRODUCTION

Bacteriocins are natural peptides secreted by many varieties of bacteria for the purpose of killing other bacteria. bacteriocins from Gram-positive bacteria are generally classified according to size, structure, and modifications. **Mills et al. (2011)** identified bacteriocins as bactericidal polypeptides that are lethal against closely related species. They are small molecular weight proteins, often heat-resistant and the structural genes encoding bacteriocins are usually plasmid-linked. The structure of bacteriocins consists of bacterial peptides with specific activity against competing species in addition to carbohydrate and/or lipid moieties **(Venugopal et al., 2011).** Bacteriocins bind specifically to receptors on the surface of target cells and kill the cells by alteration of membrane-bound enzymes, disruption of membrane potential by pore formation, or by enzymatic digestion of RNA and/or DNA. The proteinaceous nature of these antimicrobial molecules as well as their natural occurrence in nature has allowed their use in foods to prevent microbial food borne diseases and bacterial food spoilage **(Nissen-Meyer et al., 2010).**

Bacteriocins were classified into two groups designated as low and high molecular weight bacteriocins. Low molecular weight forms are generally more susceptible to trypsin digestion and less sensitive to heat inactivation. The high molecular weight forms are phage related **(Jabrane et al., 1994)**. The molecular weights of a variety of bacteriocins ranges from low molecular weight form **(Du Toit et al., 2000; Sánchez et al., 2011)**, middle molecular weight **(Busarcevic et al., 2008)** and high molecular weight form **(Smarda and Benada, 2005).** Factors which affect the stability of bacteriocins include temperature, pH, enzymes and dissociating agents. Most bacteniocins can be lyophilized without great loss of activity but exceptions are known **(Houlihan and Russell, 2006).** Some bacteriocins are thermolabile **(Oh et al., 2000; Moghaddam et al., 2006; El-Shouny, 2006)**. Other bacteriocins are thermostable revealing remarkable stability over heat treatment even at the autoclaving temperature for 20 min **(Khalid et al., 1999; Ogunbanwo et al., 2003; Sarika et al., 2010).** Variations in pH value have a marked effect on the stability of bacteriocins preparations. Bacteriocin and bacteriocin like substances are stable at neutral pH and are more tolerant to acidic than alkaline pH extremes **(Gwiazdowska and Trojanowska, 2006; Joshi et al., 2006).** On the other hand, Plantaricin ASM1 from *Lactobacillus plantarum* A-1 **(Hata et al., 2010)** and bacteriocin G2 produced by *Lactobacillus plantarum* G2 **(Šeatović et al., 2011)** both showed high stability at the pH range 2–9. **Ogunbanwo et al. (2003)** and **HernÃ¡ndez et al. (2005)** reported that the dissociating agents (e.g. Sodium dodeyl sulphate, Tween 80, Triton X-100 and urea) affected the stability of some bacteriocins.

Enzymes also affect the stability of some bacteriocins. Proteolytic enzymes as trypsin, pronase pepsin and papain abolish the activity of different bacteriocins **(Dündar, 2006; Pascual et al., 2008).** The activity of the bacteriocin produced by *Lactobacillus brevis* OG1 and that of *Lactobacillus plantarum* F1 were inactivated by proteolytic enzymes, but not by other non–proteolytic enzymes **(Ogunbanwo et al., 2003).**

This work aimed at the purification and characterization of a bacteriocin produced by *Lactobacillus plantarum* SR18. The properties of the purified bacteriocin were determined by studying its molecular weight and stability under different factors such as: temperature, pH value, dissociating agents and protease.

MATERIAL AND METHODS

Bacterial strains

The lactic acid bacterium; *Lactobacillus plantarum* SR18 obtained from yogurt was previously chosen in our laboratory as the highest bacteriocin producing bacterium. The identified strain; *Streptococcus salivarius* 5 was selected as the indicator strain and was provided from Microbiology Department, Faculty of Pharmacy, Tanta University, Egypt.

Medium

MRS agar medium was used for solid plates and MRS broth was used as a culture medium for detection and production of bacteriocin. It is based on formulation of de Man, Rogosa and Sharpe (MRS). This medium supports luxuriant growth of lactobacilli from oral, fecal, dairy and other sources **(De Man et al., 1960).** The medium composed of (g/l); peptone 10 g, beef extract 10 g, yeast extract 5 g, glucose 20 g, sodium acetate 5g, tween 80 1ml, potassium phosphate 2 g, ammonium citrate 2 g, magnesium sulfate 0.1 g, manganese sulfate 0.05 g and agar 15g for solidification. The medium was adjusted at pH 6.5.

Production of bacteriocin

Bacteriocin of *Lactobacillus plantarum* SR18 was obtained after induction of a log-phase culture with UV light. Irradiation was carried out on cells that had been grown in MRS broth at 37ºC for 6 h., centrifuged and re-suspended in 1/10 volume of saline. Portions (5 ml, containing about 5 X 10^4 cells/ ml) were irradiated in open glass Petri dishes by using T-8M, 3B ultraviolet-B lamp (8 w, 220 v & 312 nm) at a distance of 25 cm for 2 hours. The irradiated suspension was diluted 10-fold into fresh broth and incubated at 37ºC for 24 hours. The cells were removed by centrifugation and bacteriocin was assayed by the agar well diffusion method against the indicator strain; *Streptococcus salivarius* 5 **(Expert and Toussaint, 1985).**

Purification of bacteriocin

The bacteriocin bound to the cells and that secreted into the culture filtrate of *Lactobacillus plantarum* SR18 were precipitated by 75% ammomium sulphate, dialysed and further purified by Gel filtration on Sephadex G-100.

Ammonium sulphate precipitation

The bacteriocin was partially purified from two liters cultures of *Lactobacillus plantarum* SR18. Cells were grown to stationary phase in MRS broth at 37ºC in a candle jar and then collected by centrifugation (2.500 x g at 4º C, for 10 min). The culture supernatant was filter-sterilized, ultrafiltered on 1.000 cut-off memberane to concentrate 10-fold. The extracellular bacteriocin in the supernatant was directly purified but the cell bound bacteriocin was subjected to NaCl extraction.

Extraction of cell-bound bacteriocin

The bacteriocin activity in the culture was also found in the cell pellet. The extracellular nature of the bacteriocin permitted the extraction of activity without disruption of the cells by simply suspending the cell pellet in 1 liter of 0.05 M KPO_4, pH 7.0, containing 1 M NaCl. The cells were mixed with the extractant for 1h, and the suspension centrifuged at 10.000 x g for 20 min. The milky supernatant fluid was decanted and sterilized by shaking with 5 ml of chloroform. Usually, over 90% of the activity in the cell pellet was solubilized by this procedure. Residual bacteriocin could be solubilized by simply repeating the NaCl extraction **(Foulds, 1972).**

Both cell bound and culture supernatant bacteriocins were treated with ammonium sulphate to 75% saturation with constant stirring under cooling. The preparation were then left overnight at 4º C and the precipitate was collected by centrifugation at 16.300 g for 20 min, re-dissolved in 5 ml of 0.05M phosphate buffer (pH 7.2) and stored in a refrigerator, until used **(El-Shouny, 2006).**

Gel filtration chromatography

A column of 2.5×39 cm was filled with Sephadex G- 100 gel (Pharmacia fine chemical company) bed dimensions 2 x 40 cm previously swollen for 28 h at 4ºC. The column was then washed with 0.1 M phosphate buffer (pH 7.0), containing 0.02% (w/v) sodium azide at least overnight and preferably for 3 to 4 days. A sample of 2 ml of the crude ammonium sulphate precipitated bacteriocin was applied to the Sephadex G-100 column. The column was eluted with 0.1M phosphate buffer (pH 7.0) containing 0.02% (w/v) sodium azide at a flow rate of 18 ml/h and 3 ml fractions were collected in a number of separate test tubes using LKB 2070 altroroc fraction collector. Eluates were collected and assayed for protein content at 280 nm in a Shimadzu UV-visible recording spectrophotometer uv-240 (Pharmacica, sweeden). Protein concentration for each fraction was determined by the method of **Lowry et al. (1951)** with bovine serum albumin as a standard. The bacteriocin activity of each fraction was determined by the agar well diffusion assay method and expressed as arbitrary units/ml. The active fractions were stored at -20ºC until used **(Martirani et al, 2002).**

Bacteriocin assay

Agar well diffusion assays were used for qualitative and quantitative determinations of bacteriocins in solution. Two fold serial dilutions (10 µl) of the bacteriocin solution prepared from the selected isolate *L. plantarum* 18 were pipetted in agar wells of a plate seeded with the indicator strain; *S. salivarius* 5 (10^7 CFU/ml). The plates were incubated for 24 h at 37 ºC in presence of 5% CO_2. Bacteriocin titers were expressed in arbitrary units (AU); an AU was the reciprocal of the highest serial twofold dilution causing visible growth inhibition of the indicator strain per milliliter. Thus bacteriocin activity was calculated in AU/ml **(Hsich et al., 1996).**

$$\text{Bacteriocin activity (AU/ml)} = \frac{\text{The highest dilution provided inhibition zone} \times 1000}{\text{Volume µl}}$$

Molecular weight determination

The proteins were separated by discontinuous SDS polyacrylamide gel electrophoresis using 12% separating acrylamide gel and vertical polyacrylamide gel electrophoresis apparatus (Pharmacia, Sweeden). The molecular weights of the tested bacteriocin components were determined on the basis of the mobility through polyacrylamide gel as compared with the mobility of known molecular size proteins present in the standard sample containing 45, 30, 21.5, 6.5 and 2.5 KDa proteins which were used as molecular weight standards **(Laemmli, 1970).** The protein samples were injected into the gel and then electrophoresis was carried out for 3 h at 30 ºC.

Stability of bacteriocin

The stability of bacteriocin was detected by studing different factors affecting the activity of the bacteriocin preparations: Crude culture filtrate (C.C.F.), ammonium sulphate precipitate (A.S.P) and purified bacteriocin.

Thermal stability

Portions of 100 µl of the bacteriocin preparations were incubated in a water bath at 40, 60, 80 or 100ºC. At incubation times of 30 and 45 min, 20 µl portions were used for the determination of the residual bacteriocin activity using the agar well diffusion method at 37ºC **(Joshi et al., 2006).**

pH stability

Aliquots of 100 µl of bacteriocin preparations were adjusted to pH 3–10 using predetermined microvolume of citrate, phosphate and borax- NaOH buffers. The adjusted bacteriocin preparations were held for 90 min at 4ºC, readjusted to pH 6.5 and their residual activity were determined at the optimal temperature and pH by the agar well diffusion method **(Abo-Kamar, 1992).**

Effect of dissociating agents

Aliquots of 100 µl of the bacteriocin preparations in Eppendorf tubes were separately mixed with each of the following dissociating agents at the final concentrations indicated Tween 80 (0.1%), Triton X 100 (0.1%), sodium dodecyl sulphate (1 %), and mercaptoethanol (0.2%). The tubes were incubated at 37ºC for 45 min. For each agents used, two tubes served as controls. The first tube contained bacteriocin in buffer solution instead of the dissociating agent and the second contained dissociating agent in buffer solution alone **(El-Shouny, 2006).** The residual activity of bacteriocin was determined as mentioned before.

Effect of protease

The effect of protease enzyme on the bacteriocin was determined by mixing 100 µl of the bacteriocin with 100 µg/ml of enzyme and incubated at 37ºC for 45 min. The residual bacteriocin activity was determined by the assay method as usual **(El-Shouny, 2006).**

Statistical analysis

The obtained results were statistically analyzed using the two ways analysis of variance (ANOVA) to determine the degree of significance for the variations between the treatments, F test and the LSD at 0.05 level was calculated for treatment means and their interactions. All of the statistical methods were according to the method described by **(Bishop, 1983).** Each value presented in the tables is the means of three readings ±the standard deviation (SD).The values are highly significant (*) at $P<0.001$, significant at $P=0.001$ and Insignificant at $P> 0.001$. The least significant difference is abbreviated as LSD.

RESULTS

Isolation and purification of the active bacteriocin

The bacteriocin bound to the cells and that secreted into the culture filtrate of *Lactobacillus plantarum* SR18 were precipitated by 75% ammomium sulphate, dialysed and further purified by Gel filtration on Sephadex G-100 (Figures 1 & 2). Bacteriocins were purified from proteins bound to the cell of *L. plantarum* SR18 (plantarcin SR18 a) and culture filtrate proteins (plantarcin SR18 b), respectively. Gel filtration resulted in two peaks for the precipitated cell bound proteins. The large peak showed killing activity (plantarcin SR18 a) but the small proteins did not (Fig. 1). From two peaks obtained for culture filtrate proteins, only one peak showed bacteriocin activity (plantarcin SR18 b) that further characterized (Fig. 2).

Molecular weight estimation of plantarcin SR18

The malecular weight of bacteriocin was estimated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described in materials and methods. The results presented in fig. 3 revealed the marker proteins in lane 1, plantarcin SR18a in lane 2 and plantarcin SR18b in Lane 3. The appearance of single bands in lane 2 and 3 referred to purity of the tested plantarcin SR18 samples. Cell-bound bacteriocin of *L. plantarum* SR18 (plantarcin SR18a) showed a molecular size of 3.5 KDa (lane 1). Whereas bacteriocin partially purified from the culture filtrate (plantarcin SR18 b) had a molecular weight of 10.3 KDa (lane 2).

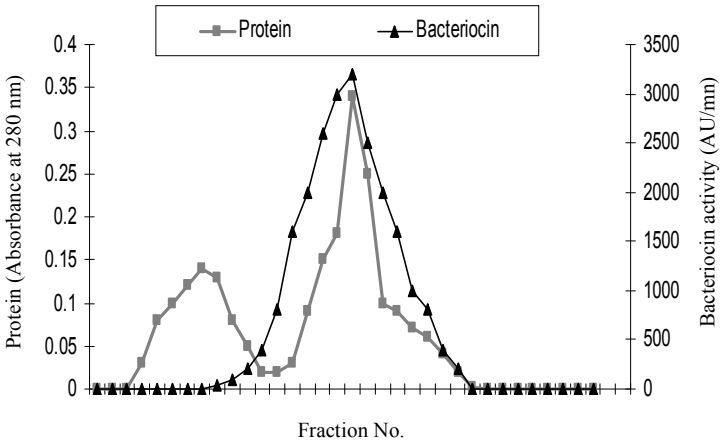

Figure 1 Gel filtration of bacteriocin SR18a from *Lactobacillus plantarum* SR18 on Sephadex G-100 chromatography

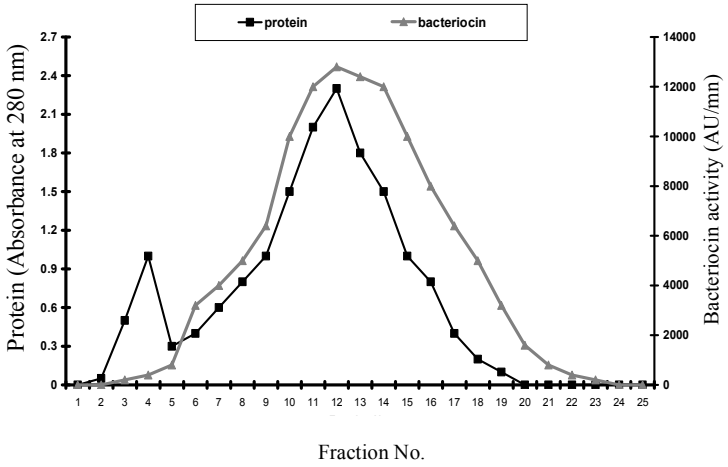

Fig. 2 Gel filtration of bacteriocin SR18b from *Lactobacillus plantarum* SR18 on Sephadex G-100 chromatography

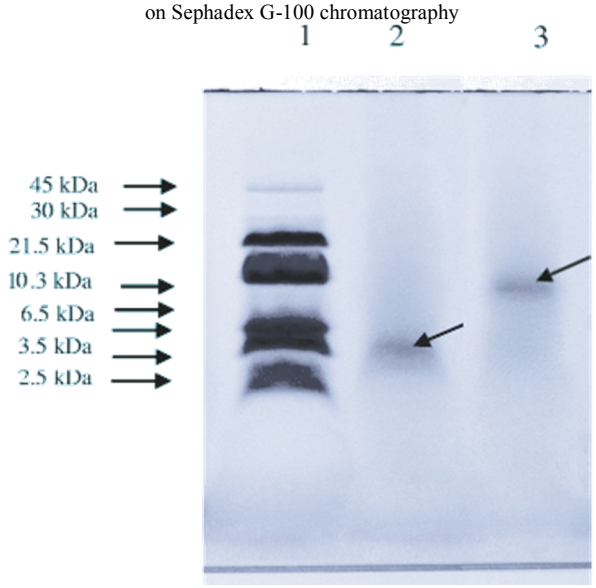

Figure 3 SDS-PAGE of partially purified plantarcin SR18a and SR18b proteins prepared from *Lactobacillus plantarum* SR18. Lane 1: Marker proteins, lane 2: bacteriocin SR18a, Lane 3: bacteriocin SR18b

Factors affecting the activity of plantarcin SR18 preparations

Thermal stability

The thermal stability of bacteriocin preparations of *Lactobacillus plantarum* SR18 is shown in Table (1). The antibacterial activity of the plantarcin SR18 preparations (crude culture filtrate, ammonium sulphate precipitate and the purified plantarcin SR18b) suffered no measurable loss during a period of 30-45 min at temperature between 37 and 80ºC. Whereas, At 100ºC the bacteriocin preparations remained fully active during the first 30 min of exposure but significant decrease in the activity (60- 80 %) took place by the end of 45 min.

pH stability

The activity of plantarcin SR18 preparations evaluated after 90 min of exposure at 4ºC in the presence of buffers having different pH values in the range 1-10 and the results obtained are shown in table (2). At pH ranged from 5-8, the antimicrobial activity of the plantarcin SR18 preparations suffered no measurable loss. Significant decrease in the stability of plantarcin SR18 was observed at pH ranged from (3-4) and (9 -10). Crude and purified preparations kept at least 75% of their original activity at this range of pH values.

Effect of dissociating agents

From table (3), it could be observed that dissociating agents significantly affected the activity of bacteriocin preparations. It was noticed that tween 80 and mercaptoethanol increased the activity of bacteriocin preparations of crude culture filtrate, ammonium sulphate precipitate and the purified bacteriocin to 1.2-1.4 fold. Sodium dodecyl sulphate (SDS) increased the activity of the bacteriocin preparations under test by about 20%.The lowest residual activity (60%) was recorded after treatment with Triton X100 for 45 min.

Effect of protease

The data shown in Table (3) revealed the sensitivity of the crude and purified forms of plantarcin SR18 obtained from *L. plantarum* SR18 to protease. The activities were completely inhibited by protease after 45 min at 37ºC.

Table 1 Thermal stability of plantarcin SR18 preparations of *Lactobacillus plantarum* SR18

Temperature ºC	C.C.F.	A.S.P.	S.R. 18 b
	Residual activity (%)		
30 min. incubation			
40	100±2	100±3	100±1
60	100±5	100±4	100±0
80	100±2	100±5	100±5
100	100±4	100±3	100±1
45 min. incubation			
40	100±1	100±2	100±4
60	100±3	100±1	100±5
80	100±1	90±3	100±2
100	80±2	60±1	80±2

Table 1 continue Two ways analysis of C.C.F.

Source	DF	F Value	P
Incubation period	1	18.75*	0.0005
Tempreture	3	18.75*	0.0001
Incubation period x Tempreture	3	18.75*	0.0001
Least Significant Difference		3.3726	

Two ways analysis of A.S.P.

Source	DF	F Value	P
Incubation period	1	101.35*	0.0001
Tempreture	3	58.11*	0.0001
Incubation period x Tempreture	3	58.11*	0.0001
Least Significant Difference		14.782	

Two ways analysis of S.R.18b

Source	DF	F Value	P
Incubation period	1	15.79*	0.0011
Tempreture	3	15.79*	0.0001
Incubation period x Tempreture	3	15.79*	0.0001
Least Significant Difference		14.782	

Legend: The initial activity of crude culture filtrate (C.C.F.) was 12800 AU/ml.
Ammonium sulphate ppt. (A.S.P.) was 6400 AU/ml.
Purified bacteriocin (SR18b) was 25600 AU/m.l
Each value is the mean of three readings ± SD.
*Highly significant at P < 0.001.

Table 2 pH stability of plantarcin SR18 preparations of *Lactobacillus Plantarum* SR18

Buffered pH	C.C.F.	A.S.P.	SR 18b
		Residual activity (%)	
3	80±2	70±1	90±3
4	90±2	85±1	95±3
5	100±3	100±2	100±4
6	100±2	100±12	100±1
7	100±4	100±3	100±5
8	100±2	100±5	100±4
9	90±1	70±2	80±3
10	80±2	70±1	75±1
F value	40.99 *	115.30*	27.91*
P	0.0001	0.0001	0.0001
L.S.D.	5.7186	5.7186	7.8191

Legend: The initial activity of crude culture filtrate (C.C.F.) was 12800 AU/ml.
Ammonium sulphate ppt. (A.S.P.) was 6400 AU/ml.
Purified bacteriocin (SR18b) was 25600 AU/ml.
Each value is the mean of three readings ± SD.
*Highly significant at P < 0.001.

DISCUSSION

In this study, *Lactobacillus plantarum* was grown under its optimum conditions for maximum production of bacteriocin which tested against the indicator strain *Streptococcus salivarius* 5. The molecular weight of the obtained purified plantarcin SR18 was 3.5 KDa for plantarcin SR18a and 10.3 KDa for plantarcin SR18 b. Similar results were obtained by the cell-free supernatant bacteriocin ST13BR produced by *Lactobacillus plantarum* ST13BR which had a molecular mass of approximately 10 kDa **(Todorov and Dicks, 2004).**

Table 3 Effect of dissociating agents and protease on plantarcin SR18 preparations of *Lactobacillus plantarum* SR18b

Additives	C.C.F	A.S.P.	SR 18b
		Residual activity (%)	
Tween 80	140±7	120±5	140±5
Triton X100	100±2	60±1	70±2
S.D.S.	120±4	100±1	100±4
Mercaptoethanol	140±6	110±5	100±3
Protease	0±0	0±0	0±0
F value	485.71*	698.08*	755.56*
P	0.0001	0.0001	0.0001
L.S.D.	11.858	8.3451	8.5041

Legend: The initial activity of crude culture filtrate (C.C.F.) was 12800 AU/ml.
Ammonium sulphate ppt. (A.S.P.) was 6400 AU/ml.
Purified bacteriocin (SR18b) was 25600 AU/ml.
Each value is the mean of three readings ± SD.
*Highly significant at P < 0.001.

Lactobacillus plantarum ST194BZ produced two bacteriocins, viz. ST194BZ (a) of 3.3 kDa and ST194BZ (b) of 14.0 kDa **(Todorov and Dicks, 2005).** While *Lactobacillus acidophilus* 30SC produced bacteriocin with molecular weight of 3.5 kDa **(Oh et al., 2000).** In this concern, the molecular weight was about 2.5 kDa for both garviecin L1-5 **(Villani et al., 2002)** and *Lactobacillus plantarum* TF711**(HernÃ¡ndez et al., 2005)** but strain *Lactobacillus sakei* R1333 produced a 3811Da bacteriocin **(Todorov et al., 2010).** Plantaricin ASM1 (PASM1) was produced by *Lactobacillus plantarum* A-1 with molecular mass 5045.7 Da **(Hata et al., 2010).**

The stability of the purified bacteriocin could be affected by different factors. The antibacterial activity of the bacteriocin preparation under investigation was found to resist heating for 45 min at 40-80 ºC and only for 30 min at 100 ºC. Heat stability of bacteriocin could be due to the formation of small globular structures and the occurrence of strongly hydrophobic regions, and stable cross-linkage **(Lim and Dong 2009).** The high stability of this plantarcin SR18 after heating at 100ºC for 45 min, place it within heat stable low molecular weight group of bacteriocins. This quality of the bacteriocin makes it superior in processed food stuffs where high heat is applied. This is in agreement with the results obtained by **Abo-Amer (2007)** who reported that the activity of the antimicrobial substance of *Lactobacillus plantarum* AA135 isolated from Egyptian home-made yogurt was resistant to heat at 121ºC for 30 min. Similar results was shown by **Hata et al. (2010)** for plantaricin ASM1 the bacteriocin produced by *Lactobacillus plantarum* A-1. **HernÃ¡ndez et al. (2005)** also found that antimicrobial substance produced by *Lactobacillus plantarum* TF711 was stable to heat, as it retained more than 70% residual activity after treatment at 100°C for 30 min. However, heat sterilization led to its complete inactivation.

The stability of bacteriocin under different pH values seems to differ according to the producer organism. In the present study, the bacteriocin of *Lactobacillus plantarum* SR18 displayed a complete stability at pH 5-8. Higher or lower pH values resulted in a significant decrease in the activity, but it still keeping at least 75% of their original activity at this range of pH values. Similar stability pattern was obtained by *Lactobacillus plantarum* AMA-K isolated from Amasi, a Zimbabwean fermented milk product where the optimal adsorption of bacteriocin AMA-K (75%) to *Listeria* sp. were recorded at pH 7.0. Lower levels of pH (3.5) resulted in reduction of the adsorption of bacteriocin AMA-K to this *Listeria* species to 50% **(Todorov, 2008).** On the other hand, **Hata et al., (2010)** said that plantaricin ASM1 produced by *L. plantarum* A-1 was stable in a wide range of pH (1-12). **HernÃ¡ndez et al. (2005)** recorded that antimicrobial activity of *L. plantarum* TF711D was highest when the pH of the supernatant was between 1 and 8, more than 75% activity was maintained up to pH 11 and it was completely inactivated above pH 11.

In the present study, it was noticed that tween 80 and mercaptoethanol significantly increased the activity of bacteriocin preparations to 1.2-1.4 fold. Sodium dodecyl sulphate (SDS) increased the activity of the bacteriocin preparations under test by about 20%. Where, Triton X100 showed significant decrease in the residual activity to be about (70%). The increment of bacteriocin activity in the crude forms caused by the mentioned dissociating agents might be attributed to an inhibition of possible proteolytic effects obtained from the crude culture of the plantarcin SR18 in the concentration used. **Ogunbanwo et al. (2003)** concluded that the exposure of the bacteriocin of *Lactobacillus plantarum* F1 to surfactants (SDS, Tween 80 and Triton X-100) resulted in an increase in the bacteriocin titre by at least one to two fold dilutions. Similar results were obtained by **Todorov (2008)** for bacteriocin of *L. plantarum* AMA-K.

In this study, the plantarcin SR18 preparations completely lost its antibacterial activity by treatment with protease enzyme. Such a result was previously reported for similar plantarcins and for other bacteriocins by a number of investigators **(El-Shouny, 2006; Todorov et al., 2007; Karthikeyan and Santosh, 2009; Kumar et al., 2010).** This indicates that the plantarcin used in this study are actually protein in nature. On the other hand, **Khalil et al. (2009)** concluded that in case of the bacteriocin of *Bacillus megaterium* 19, the proteolytic enzymes failed to modify the antimicrobial activity of the bacteriocin, which is not unusual and might be due to the presence of unusual amino acids in the bacteriocin structure, or cyclic N-and/ or C- terminally blocked peptides.

CONCLUSION

Depending on the proven stability and safety of the herein obtained plantracin from the non-pathogenic bacterium *L. plantarum* SR18, the purified plantarcin SR18 would be investigated as food preservative and as a therapeutic agent. Further confirmatory studies in this concern are recommended.

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