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Research Article

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Partial Purified, Characterization and Antibacterial Activity of Bacteriocin from *Leuconostoc Mesenteroides*

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Abstract: Lactic acid bacteria produces a variety of antibacterial compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins or bactericidal proteins as byproducts of fermentation. Bacteriocins are small proteins with bactericidal or bacteriostatic activity against genetically closely related species. Some bacteriocins are peptides consisting of only 13 to 37 amino acids. Some small bacteriocins contain unusual amino acids originating from modifications of conventional amino acids after translation. Bacteriocins are natural peptides secreted by several bacteria that exert bactericidal activity against other bacterial species. Research in this topic is promoted not only by the need to develop alternatives to antibiotics and drugs that have shown non-desirable effects, but also to the capacity of some bacteriocins to inhibit saprophytic and food-borne pathogens in food stuffs. The purification of bacteriocins produced is helpful for the knowledge of the mechanism of action, structure, and other characteristics, which helps to isolate the bacteriocin biosynthetic genes. In this study, purified bacteriocins from *Leuconostoc mesenteroides* were described. The frequently applied techniques involve ammonium sulfate salt precipitation. The results obtained through the purification of *Leuconostoc mesentroides* bacteriocins carried out its antimicrobial activity, thermostable and the effected of pH. the incubation of temperature on bacteriocin activity in terms of inhibition zones. The data recorded that the bacteriocin was active in a wide range of pH, the maximum activity was observed at pH 6.0 to 7.0, but at high pH of 8.0 the activity of the bacteriocin gradually decreased. Bacteriocin could retain its antimicrobial activity partially when there was a shift to acidic or basic range. It has been found to be thermo stable in nature as it can withstand high temperatures up to 121°C, although a partial loss in the activity was observed with a continuous increase in temperature. Bacteriocins carried out for its antimicrobial activity, thermostable and the effect of pH.

Keyword: bacteriocin, purification, characteristics, *Leuconostoc mesenteroides*, antimicrobial activity

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1. INTRODUCTION

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria that produce lactic acid in two pathways: homofermentative or heterofermentative. These bacteria are found in oral cavity, skin and human digestive system [1]. *Lactobacillus* species play an important role in gastrointestinal (GI) tract function, intestinal microbiota balance and the immune system activity by exerting a strong activity against many intestinal pathogens [2]. These activities arise from the production of some specific components such as hydrogen peroxide, organic acids, inhibitory enzymes and bacteriocins [3]. In recent years, bacteriocins have attracted significant attention because of their potential use as safe additives for food preservation [4] and other applications such as treatment of pathogenic diseases, cancer therapy and human health enhancement. Bacteriocins are a heterogeneous group of ribosomally-synthesized pore-forming antibacterial peptides. These antibacterial peptides can be produced by many labs such as *Leuconostoc, Streptococcus, Lactococcus, Pediococcus*, and *Lactobacillus* that enable these bacteria to inhibit the growth and activity of several pathogenic bacteria [5]. *Lactobacilli* have many different bacteriocins with the same activity but vary in some aspects such as mode of action, genetic origin (chromosomal or plasmid) and biochemical properties. These peptides are being tested to assess their application as narrow-spectrum antibiotics and nowadays, play an effective role in processing and packaging food and have major potential as natural preservatives[6&7]. Based on its antibacterial activity , molecular weight, genetics and chemical properties, bacteriocins are classified in one of the four major groups: Class I (small peptide inhibitors termed as lantibiotics), Class II (small heat-stable proteins that have five subclasses), Class III (large, heat labile protein and this class has two subclasses) and Class IV bacteriocins that are defined as complex bacteriocins containing lipid or carbohydrate sections [8]. Lactobacilli usually produce the highest volume of bacteriocins during the maximum cell growth, but some environmental factors such as pH, temperature, incubation period and media composition can influence the volume of bacteriocin production[9&10]. This study describes the purification and characterization of bacteriocin from *Leuconostoc mesenteroides* which has been found by extensive screening to produce a relatively high bacteriocin activity and their antimicrobial activities against pathogen microorganisms

2. MATERIALS AND METHODS

2.1 Production of bacteriocin

Leuconostoc mesenteroides was propagated in MRS broth (100 ml) seeded with slant inoculum of overnight culture and incubated for 24 h at 35°C [11]. After incubation, the whole broth was centrifuged at 10,000 rpm for 10 min and the cell-free supernatant was used as crude bacteriocin [12]. The indicator Gram positive bacterium was *S. aureus* ATCC25923, while the Gram negative bacteria were *E. coli* ATCC25422, *K. pneumonia* ATCC700603, *P. aeruginosa* ATCC27583.

2.2 Partial purification of bacteriocin

Ammonium salt precipitation methods were used for this purpose [13]. The cell free culture supernatant (crude bacteriocin) of *Leuconostoc mesenteroides* was saturated with various concentrations of solid ammonium sulfate 60, 70 and 80% in 250 ml Erlenmeyer flasks after stirring on a magnetic stirrer. It was kept undisturbed at 4°C overnight stored. The pellet was collected after centrifugation at 10,000 rpm at 4°C for 30 min. The pellet was dissolved in 25ml of sodium phosphate buffer (0.05 M, pH 7.0) and dialyzed against the same buffer at 4°C overnight [12].

2.3 Bacteriocin assay

Agar well diffusion procedure described by Zhang et al [14], was used to determine the production of bacteriocin in the culture supernatant using, Gram positive bacteria -*S. aureus* ATCC25923, and the Gram negative bacteria - *E. coli* ATCC25422, *K. pneumonia* ATCC700603, *P. aeruginosa* ATCC27583. Inhibition of bacterial growth was measured as inhibition zone diameters (mm).

2.4 Characterizations of partial purified bacteriocin

2.4.1 Heat stability

A 5-mL aliquot of partial purified bacteriocin in different test tubes was overlaid with paraffin oil to prevent evaporation and then heated at 30, 40, 50, 60, 70, 80, 90 and 100°C for 10 and 20 mins respectively, and the effect of autoclaving condition also tested and validated. The heat-treated bacteriocin samples were then assayed for bacteriocin activity as described earlier.

2.4.2 Effect of different pH on partial purified bacteriocin activity

A 5-mL aliquot of partial purified bacteriocin was placed in test tubes and the pH values of the contents were adjusted to 3, 4, 5, 6, 7, 8, 9 and 10 individually, using either diluted NaOH or HCl (1 M NaOH or 1 M HCl solution). After allowing the samples at room temperature for 2 h the antimicrobial activities of bacteriocins were assayed as described earlier [15].

2.4.3 Effect of inhibitors enzyme on partial purified bacteriocin activity

Trypsin and pepsin concentrations (1.0 mg/ml) at pH=7 were added to the purified bacteriocin and incubated for 2 h at 35°C. After incubation, the bacteriocin activities were determined by using well diffusion method as described by [8].

3. STATISTICAL ANALYSIS

Results are presented as the mean of three or four replicates ± standard error (SE). The statistical analyses were carried out using SPSS program (version 22). Data obtained were analyzed statistically to determine the degree of significance using one way (ANOVA) at probability level $P \leq 0.05$ levels of significans.

4. RESULTS AND DISCUSSION

4.1 Partial purification of bacteriocin

Ammonium sulfate was used to precipitation bacteriocin that was produced by *Leuconostoc mesentroides*. The bacteriocin extraction was done at 70% saturation of ammonium sulfate and suspended in 0.05 mmol sodium phosphate buffer, pH=7.0. Finally, the results of the pellets weight from 100 ml

were 1.50 gm (Figure 1). On the other hand, various concentrations of solid ammonium sulfate 60 and 80% ammonium sulfate didn't give the result. The result of partial purification for precipitation of bacteriocin by 70% ammonium sulfate was in agreement with Oppegard [16] whose purification bacteriocin produced by *Lactobacillus plantarum* isolated from cow milk used (70%) ammonium sulfate and stored at 4 °C to precipitate out the proteins. The optimal bacteriocin recovery was achieved by including ammonium sulfate precipitation. This agreed with the findings of Ivanova [17].

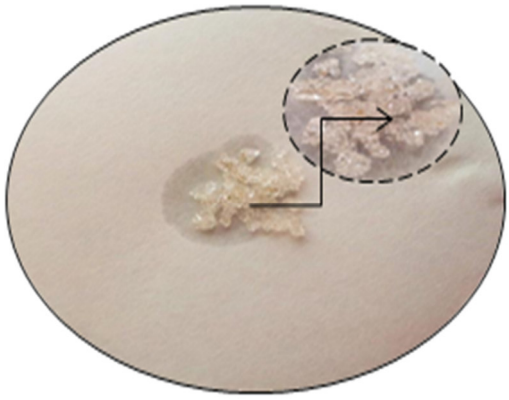

Fig 1. The pellets formatted by ammonium sulfate precipitation from crude bacteriocin of *Leuconostoc mesenteroides*

4.2 Determination of inhibitory spectrum

Crude bacteriocin and partial pure bacteriocin activity were assayed by the agar-well diffusion method. The susceptibilities of various Gram-positive and Gram-negative bacteria to growth inhibition by the bacteriocin produced by *Leuconostoc mesenteroides* dissolved in buffer (Sodium phosphate buffer 0.05M; pH 7) are presented in (Table 1). It shows the activity of crude bacteriocin (in terms of inhibition zone diameter) from 21.66 to 23.66 mm. On the other hand, the mean diameter of inhibition zone by pure bacteriocin ranged between 25 to 27.66 mm (Figure 2**)**. It was found that pure bacteriocin from *Leuconostoc mesenteroides* was higher and efficient in production of bacteriocin than crude bacteriocin (Figure 3). High level of significance was obtained with the partial pure bacteriocin. By comparison, leucocin A-UAL 187 from *Leuconostoc gelidum* purification was conducted by an ammonium sulfate precipitation followed by three steps of chromatography [18]. On the other hand Vaughan [19] reported that inhibitory activity of bacteriocin isolated from malted barley was precipitated from cell free supernatant using (40%) ammonium sulfate saturation, and resuspended in 2 mmol sodium phosphate buffer, pH=6.0 and purified using chromatography. Bacteriocin was partially purified by ammonium sulfate precipitation (80% saturation) from *Leuconostoc mesenteroides* 406 isolated from Mongolian fermented mare's milk [20]. The culture supernatant was concentrated by ammonium sulfate precipitation (60%) of *Leuconostoc mesenteroides* [21].

Table 1. Determination of inhibitory spectrum of crude bacteriocin and partial pure bacteriocin production by *Leuconostoc mesenteroides*.

Kind of bacteriocin	Indicator microorganisms			
	***P.aeruginosa* ATCC27583**	***S.aureus* ATCC25923**	***E.coli* ATCC25422**	***K.pneumonia* TCC700603**
	Mean diameter of inhibition zone (mm)			
Crude bacteriocin	22.66	21.66	22.33	23.66
Partial pure bacteriocin	26.00	25.00	26.33	27.66

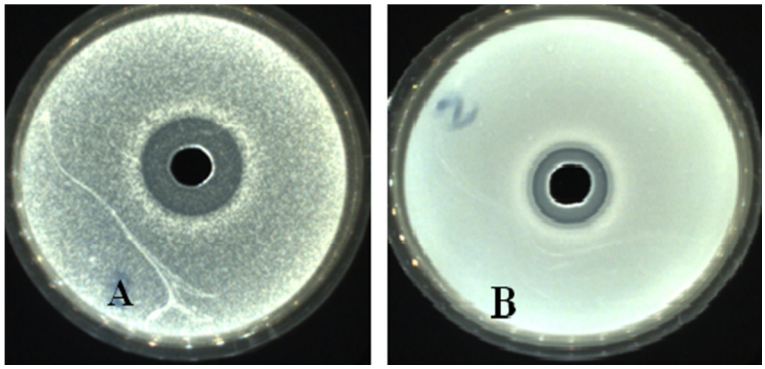

Fig 2. Determination of inhibitory spectrum of the partial pure bacteriocin (A) and crude bacteriocin (B) production by *Leuconostoc mesenteroides* against *S.aureus* ATCC25923

4.3 *Effect of different temperature on the activity of the partial purified bacteriocin*

The effects of different temperature on bacteriocin activity were determined using *P.aeruginosa* ATCC27583, *S.aureus* ATCC25923, *E.coli* ATCC25422 and *K.pneumoniae* ATCC700603 as indicator microorganism. A volume of 5 mL of bacteriocin in different test tubes was overlaid with paraffin oil to prevent evaporation and then inoculated at 30, 40, 50, 60, 07, 80, 90 and 100 °C for 10 and 20 min respectively. The effect of autoclaving condition was also tested. The data represented that, (Table 2) shows the incubation of temperature on bacteriocin activity in terms of inhibition zones. It has been found to be thermo stable in nature as it can withstand high temperatures up to 121°C, although a partial loss in the activity was observed with a continuous increase in temperature. Partially purified bacteriocin was found to be stable at 30°C - 40°C for 20 min, The maximum antimicrobial activity was observed at 30 °C and 40°C for 20 min.

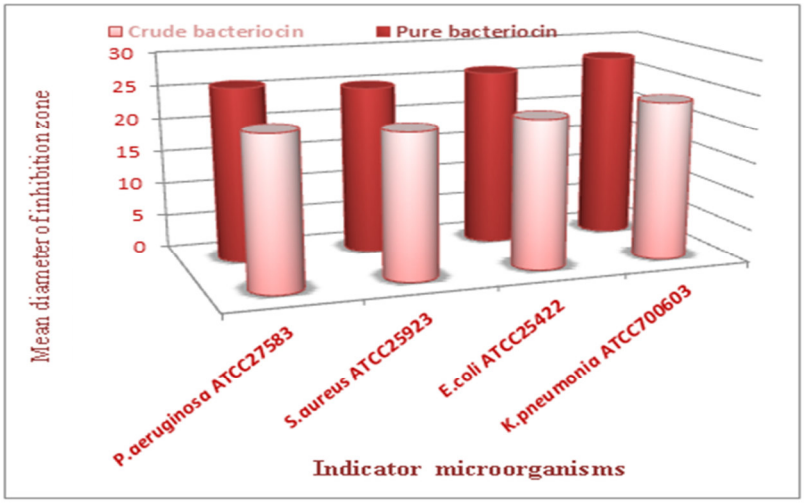

Fig 3. Determination of inhibitory spectrum of crude bacteriocin and partial pure bacteriocin production by *Leuconostoc mesenteroides*

As show in Figure (4), the loss in the activity was observed at 50°C followed by 60°C, 70°C, 80°C and 90°C for 10 min it reached 21, 19, 18, 16 and 14 mm against K.pneumoniae ATCC700603 respectively. Some of bacteriocins produced by LAB, for example, bacteriocins produced by *L. acidophilus* LF221 or lacticin NK24 from *Lactococcus lactis* could at least partially preserve their activity, even after heating at 100 °C for 30 min (22). Heat resistance is the major characteristic of many bacteriocins and bacteriocin-like compounds produced by LAB and can vary dramatically ranging from 60 °C or 100 °C for more than 30 min (e.g. lactocin 27, lactocin S, carno bacteriocins A and B) to autoclaving at 121 °C for 15-20 min (e.g. lactacin B, lactacin F, nisin etc.)[23]. Many of bacteriocins produced by LAB, particularly the ones of class I and class II, were described as small hydrophobic proteins containing little tertiary structure, which explains their heat stability. Other factors contributing to heat stability of the bacteriocin of LAB are stable cross-linkages, a high glycine content and occurrence of strongly hydrophobic regions [23]. However, after incubation for 15 min at 121 °C, partially loss of activity took place, the diameter of inhibition zone was 11 mm against *K.pneumoniae* ATCC700603, while at the same condition, only 8mm of activity could be retained against *Escherichia coli*. The inhibitory compound produced by *Leuconostoc mesentroides* was considered to be heat stable at 30 to 40 °C for 20 min but partial stable at 50 to 90 °C for 10 min. Bacteriocin produced by *Leuconostoc mesentroides* was considered to be the same condition, as the activity remained constant after heating at 121°C for 15 minutes. Earlier studies revealed that bacteriocins produced by *L. paracaseii, L. lactis, L. plantarum* and *L. pentosus* remained active after heating till 121°C for 20 min [24]. 40% loss of activity of *L.mesenteroides* E131 bacteriocin was determined after incubation at higher temperatures (90 and 100°C) and the pure bacteriocin of *L sakei* 1154 under sterilization conditions retained 40% of the initial activity after 15 min of heat treatment. Indeed, other bacteriocins isolated from *L. mesenteroides* or *L.sakei* has similar features. Mesenteric 52 is stable at pH range 4.5-7.0 and resistant at 100°C for 15 min [25], mesenteric 5 is resistant at 100°C for 30 min [21], and sakacin P is resistant at 100°C for 20 min[22].

Table 2. Effect of different temperature on the activity of the partial purified bacteriocin

T°	Indicator microorganisms Mean diameter of inhibition zones (mm)							
	P.aeruginosa ATCC27583		*S.aureus* ATCC25923		*E.coli* ATCC25422		*K.pneumoniae* ATCC700603	
	Time (after 10 min , after 20 min)							
	10min	20min	10min	20min	10min	20min	10min	20min
30	26.00	26.00	24.66	23.33	25.33	25.33	27.00	26.66
40	25.33	25.00	24.00	23.33	25.00	24.66	26.33	26.00
50	21.66	20.66	20.66	20.00	21.00	19.66	22.66	21.66
60	20.00	18.66	19.00	18.33	19.33	18.00	20.66	19.66
70	17.66	16.66	17.00	16.33	16.33	15.66	18.33	17.00

80	15.66	15.33	15.33	14.66	14.66	14.00	16.66	16.00
90	14.66	14.00	14.00	13.66	13.33	12.66	15.00	14.33
100	12.66	11.66	11.66	10.66	11.33	10.33	13.33	12.00
121		10.33			8.66		8.33	10.66

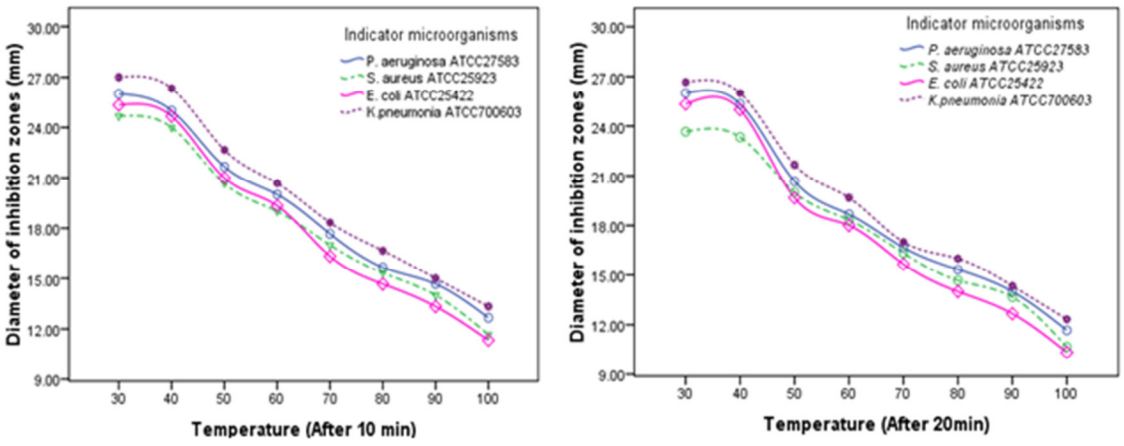

Fig 4. Effect of different temperature on the activity of the partial purified bacteriocin effect of different pH values on the activity of the partial purified bacteriocin

A 5-mL aliquot of purified bacteriocin was taken in test tubes and the pH values of the contents were adjusted to 3–10 individually. To determine the pH stability of bacteriocin, pH values of the bacteriocin were adjusted within the range of 3 to 10 by HCl or NaOH. The data recorded in (Table 3 and Figure 5) showed that the bacteriocin was active in a wide range of pH, the maximum activity was observed at pH 6.0 to 7.0, but the high pH=8.0, the activity of the bacteriocin gradually decreased. Bacteriocin could retain its antimicrobial activity partially when there was a shift to acidic or basic range. Bacteriocin was stable at acidic and neutral pH values (from pH 4 to pH 7). This differs from other bacteriocins produced by *Lactococcus lactis* which are unstable at neutral pH value[26], but similar to bacteriocin S 50 produced by *Lactococcus lactis* subspecies diacetylactis which is active in the pH range 2 to 11[27]. Many bacteriocins are most active at low pH .[28] Stability of bacteriocin at different pH scale is a limiting factor for recommending its use in food items. Bacteriocins produced by *Leuconostoc mesentroides* retained their antimicrobial activity in pH range of 5.0 to 7.0, while decrease occurred at pH 3.0 and 10.0. The high and loss of pH causes the gradually decreased of bacteriocins activity. Bacteriocins differ greatly with respect to their sensitivity to inactivation by changes in pH and temperature. Many of the bacteriocins and bacteriocin-like substances produced by lactic acid bacteria are only stable at acid and neutral pH [23] and are inactivated even at a pH above 8.0 (e.g. nisin, lacto strepcins, pediocin AcH, leucocin A-UAL 187). Bacteriocins produced by *L. plantarum* and *L. brevis* OGI retained their antimicrobial activity in an acidic pH range of 2.0 to 6.0, while inactivation occurred at pH 8.0 to 12.0 [29]. *L. mesenteroides* E131 bacteriocin retains activity at a broad range of pH (4.0-9.0), the stability at low pH is very important for its potential application in foods, such as fermented meat products, in which acidic conditions prevail. Most European fermented sausages have final pH values ranging from 4.8 to 5.0[30].

Table 3. Effect of different pH values on the activity of the partial purified bacteriocin

pH	Indicator microorganisms			
	P.aeruginosa **ATCC27583**	***S.aureus*** **ATCC25923**	***E.coli*** **ATCC25422**	***K.pneumoniae*** **ATCC700603**
	Mean diameter of inhibition zone (mm)			
3	14.66	13.66	14.33	15.33
4	16.33	14.33	14.66	16.66
5	21.33	19.33	20.00	22.00
6	25.33	24.66	25.33	25.66
7	24.33	24.00	24.66	25.33
8	19.00	16.00	17.00	19.66
9	16.33	14.66	15.66	16.66
10	14.66	12.66	13.00	15.00

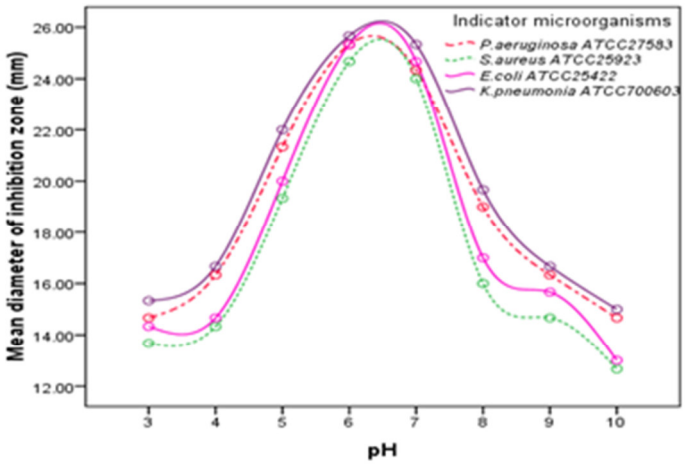

Fig 5. Effect of different pH values on the activity of the partially purified bacteriocin effect of inhibitors enzyme on the partial purified bacteriocin activity

All bacteriocins compounds are of protein nature [31]. To identify this nature of the antimicrobial substances, the action of proteolytic enzymes (trypsin and *pepsin*) was tested; it shows the activity of bacteriocin produced by *Leuconostoc mesentroides* was fully inactivated by the enzyme trypsin and *pepsin* against all four indicator microorganisms, which indicates their proteinaceous nature (Figure 6).

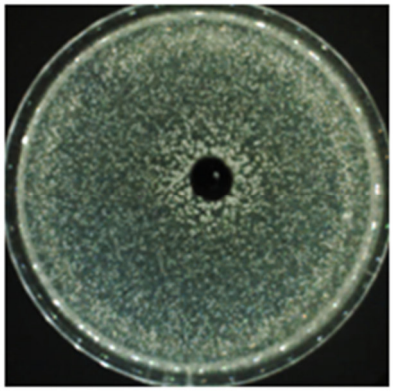

Fig 6. Effect of partial purified bacteriocin treated with trypsin against *K. pneumoniae* ATCC700603

In this study, the sensitivity of bacteriocin produced by *Leuconostoc mesenteroides* from all four indicator microorganisms was fully inactivated by the enzyme trypsin and pepsin, which indicates their proteinaceous nature. It is well known that bacteriocins produced by LAB are very often sensitive to trypsin, while the sensitivity to other enzymes varies [32]. These data were similar with Halil[33] who observed that, bacteriocin of *Leuconostoc mesenteroides* subsp. *cremoris* was treated by a variety of enzymes (trypsin -pepsin) to verify the protein nature of the inhibitor substance. The inhibitory activity of the bacteriocin was inhibited by all the proteases employed. These data clearly showed that the antimicrobial substance is of proteinaceous nature, containing cleavage-sites suitable for the mentioned proteases. Characterization of the proteinaceous inhibitor confirmed that the antimicrobial agent produced by *Leuconostoc mesenteroides* subsp. *cremoris* was a bacteriocin according to the criteria outlined by Tagg [34]. There has been a few bacteriocins studied in literature like leuconocin S produced by *Leuconostoc mesenteroides* [35]. Leuconocin S bacteriocin produced by *Leuconostoc mesenteroides* isolated from retail lamb and has a broad spectrum of activity. It was active against *L. monocytogenes* and *S. aureus.* [36]

5. CONCLUSION

The application of bacteriocins as biopreservatives for vegetab -le food matrices started approximately 20 years ago. In these years, a lot of studies have focused on the inhibition of spoilage and/or human pathogen bacteria by bacteriocins and their application appeared as a good alternative to chemical compounds and antibiotics. Furthermore, it can be concluded that in addition to the traditional hurdle technology represented by low temperature and vacuum packaging, the exploitation of bacteriocin gentic cultures, as well as their pure bacteriocins holds a great potential for extension of shelf-life and improvement of microbiological safety of vegetable raw materials and final products.

6. AUTHOR CONTRIBUTION STATEMENT

Mrs. Zahra Al-Hassan Mohammad Al-Mahmoudi conceptualized and gathered the data with regard to this work. Dr.Salha Hassan Mastour Al-Zahrani and Enas Nabil Danial analyzed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the methodology and results and contributed to the final manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

8. REFERENCES

1. Mohankumar A, Murugalatha N. Characterization and antibacterial activity of bacteriocin producing Lactobacillus isolated from raw cow milk sample. Int J Biol. 2011;3(3):128. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.988.83&rep=rep1&type=pdf
2. Nadeen G. Mostafa, E.M. Gewaily, Fatma I. El-Zamik and A.S. Al. Purification and specification of bacteriocin produced by some lactobacillus spp. Isolated from food. Zagazig J. Agric. Res. 2019;46(2):489-500. Available from: https://zjar.journals.ekb.eg/article_33403_900663550e8c4445d98b432b8e5a594b.pdf
3. Ghanbari M, Jami M, Kneifel W, Domig KJ. Antimicrobial activity and partial characterization of bacteriocins produced by lactobacilli isolated from Sturgeon fish. Food Control. 2013;32(2):379-85. doi: 10.1016/j.foodcont.2012.12.024
4. Diop MB, Dubois-Dauphin R, Tine E. Bacteriocin producers from traditional food products. Biotechnol Agron Soc Environ 2007;11(4):275-81. Available from: https://orbi.uliege.be/handle/2268/40541
5. Sure KP, Kotnis PV, Bhagwat PK. Production and Characterization of a Bacteriocin Produced by Lactobacillus Viridescence (NICM 2167). Brazilian Arch Biol and Technol. 2016;59. Available from: http://www.scielo.br/scielo.php?pid=S1516-89132016000100407&script=sci_arttext
6. Stoyancheva G, Marzotto M, Dellaglio F, et al. Bacteriocin production and gene sequencing analysis from vaginal Lactobacillus strains. Arch Microbiol. 2014;196(9):645-53. Available from: https://link.springer.com/article/10.1007/s00203-014-1003-1
7. Omar NB, Abriouel H, Lucas R, Martínez-Cañamero M, Guyot JP, Gálvez A. Isolation of bacteriocinogenic Lactobacillus plantarum strains from ben saalga, a traditional fermented gruel from Burkina Faso. Int J Food Microbiol 2006;112(1):44-50. doi: 10.1016/j.ijfoodmicro.2006.06.014
8. Todorov, S. D. and Dicks, L. M. T. Comparison of two methods for purification of plantaricin ST31, a bacteriocin produced by *Lactobacillus plantarum* ST31. Enzyme and Microbial Technology, 2004;. 36(1) 318-326.
9. Alessandra De G., Federica B., Matilde F., Paola F., Guido S. and Patrizia Di G. Identification of a bacteriocin-like compound from Lactobacillus plantarum with antimicrobial activity and effects on normal and cancerogenic human intestinal cells AMB Expr. 2019;9(1):88. Available from: https://link.springer.com/article/10.1186/s13568-019-0813-6
10. Oh S, Kim SH, Worobo RW. Characterization and purification of a bacteriocin produced by a potential probiotic culture, Lactobacillus acidophilus 30SC. J Dairy Sci. 2000;83(12):2747-52. doi: 10.3168/jds.S0022-0302(00)75169-1
11. Danial N. Enas, Al-Zahrani H.M. Salha and Al-Mahmoudi M. Zahra. Biotechnological studies for reusable production of bacteriocin using LAB immobilized cells. Res J Pharm Biol Chem Sci. 2017;8(2):781-6.
12. Ogunbanwo, S.T., Sanni, A.I. and Onilude, A.A. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OGI. African Journal of Biotechnology. 2003;2(8):219-27. Available from: https://www.ajol.info/index.php/ajb/article/view/14770
13. Yang, R., Johnson, M.C. and Ray, B. A Novel method to extract large amounts of bacteriocins from lactic acid bacteria, Appl Environ Microbiol. 1992;58(10):3355-9. Available from: https://aem.asm.org/content/58/10/3355.short
14. Zhang, H., Cui, Y., Zhu, S., Feng, F., and Zheng, X. (2010) Characterization and antimicrobial activity of a pharmaceutical micro emulsion. International Journal of Pharmaceutics. 2010;395(1-2):154-60. doi: 10.1016/j.ijpharm.2010.05.022
15. Ten Brink, B., Minekus, M., Vander Vossen, J. M. B. M., Leer, R. J. and Huis, J.H.J. Antimicrobial activity of *lactobacilli:* preliminary characterization and optimisation of production of acidocin B, a novel bacteriocin produced by *L.acidophilus* M46. Journal of Applied Bacteriology. 1994;77:140-8.
16. Oppegard C, Kjos M, Veening JW, Nissen-Meyer J, Kristensen T. A putative amino acid transporter determines sensitivity to the two-peptide bacteriocin plantaricin JK. Microbiology. 2016;5(4):700–8. doi: 10.1002/mbo3.363
17. Ivanova, I., Todorov, S., Gotcheva, B., Dousset, X. Onno, B. Influence of growth medium on bacteriocin production in *Lactobacillus plantarum* ST31. Biotechnology & Biotechnological Equipment. 2000;14(1):50-5. doi: 10.1080/13102818.2000.10819062
18. Hastings, J. W., Sailer, M., Johnson, K., Roy, K. L., Vederas, J.C. and Stiles, M.E. Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*, J Bacteriol. 1991;173(23):7491-500. doi: 10.1128/jb.173.23.7491-7500.1991
19. Vaughan, A., Eijsink, V.G.H., O'Sullivan, T.F., O'Hanlon, K. and Van Sinderen, D. An analysis of bacteriocins produced by lactic acid bacteria isolated from malted barley. J Appl Microbiol. 2001;91(1):131-8. doi: 10.1046/j.1365-2672.2001.01365.x
20. Takayuki, A., Kazushi, H., Kensuke, A., Hiroyuki, N. and Taku, M. Production of bacteriocin by *Leuconostoc mesenteroides* 406 isolated from Mongolian fermented mare's milk, airag, Animal Science Journal. 2012;83(10):704-11. doi: 10.1111/j.1740-0929.2012.01010.x
21. Daba, H., Pandian, S., Gosselin, J.F., Simard, R.E., Huang, J. and Lacroix, C. Detection and activity of bacteriocin produced by *Leuconostoc mesenteroides*, Appl Environ Microbiol. 1991;57(12):3450-5. doi: https://aem.asm.org/content/57/12/3450.short
22. Lee, N.K. and Paik, H.D. Partial characterization of lacticin NK24, a newly identified bacteriocin of *Lactococcus lactis* NK24 isolated from Jeot-gal. Food

Microbiol. 2001;18(1):17-24. doi: 10.1006/fmic.2000.0368

23. De Vuyst, L. and Vandamme, E.J. Nisin, Antibiotic Produced by *Lactococcus lactis* subsp. *Lactis* Properties Biosynthesis Fermentation and Applications, Bacteriocins of Lactic Acid Bacteria, Microbiology Genetics and Applications. 1994;151-221.
24. Papathanasopoulos, M.A., Krier, F., Junelles, A.M.R., Lefebvre, G., Le Caer, J.P., Von Holy, A. and Kumar, V. and Indira Hastings, J.W. Multiple bacteriocin production by Leuconostoc mesenteroides TA33a and other Leuconostoc, Weissella strains, Current Microbiology. 1997;35(6):331-5. Available from: https://link.springer.com/article/10.1007/s002849900264
25. Mathieu, F., Suwandhi, I., Rekhif, N., Milliere, J.B. and Lefebvre, G. Mesenteric 52, a bacteriocin produced by *Leuconostoc mesenteroides* ssp *mesenteroides* FR52. Journal of Applied Bacteriology. 1993;74(4):372- 9. doi: 10.1111/j.1365-2672.1993.tb05141.x
26. Varadaraj, M.C., Devi, N., Keshava, N. and Manjrekar, S.P. Antimicrobial activity of neutralized extracellular culture filtrates of lactic acid bacteria isolated from a cultured Indian milk product "Dahi", International Journal of Food Microbiology. 1993;20(4):259-67. doi: 10.1016/0168-1605(93)90170-L
27. Kojic, M., Svirchevic, J., Banina A. and Topisirovic L. Bacteriocin – producing strain of *Lactococcus lactis* subsp. *diacetylactis* S50, Applied Environmental Microbiology1991;57(6):1835-7. Available from: https://aem.asm.org/content/57/6/1835.short
28. Mortvedt-Abildgaard, C.I., Nissen-Meyer, J., Jelle, B., Grenov, B., Skaugen, M. and Nes, I.F. Production and pH-dependent bactericidal activity of lactocin S, a lantibiotic from *Lactobacillus sakei* L45. Appl Environ Microbiol. 1995;61(1):175-9. Available from: https://aem.asm.org/content/61/1/175.short
29. Bromberg, R., Moreno, I., Lopez Zaganini, C., Regina Delboni, R., and De oliveria, J. Isolation of bacteriocin-producing Lactic Acid Bacteria from meat and meat products and spectrum of inhibitory activity. Braz J Microbiol. 2004;35(1-2):137-44.
30. Lücke, F.K. Fermented sausages, In: Microbiology of fermented foods, Edited by: Wood, B., J., B. London: Elsevier. 1985;41-83.
31. Porto MCW, Kuniyoshi TM, Azevedo POS, Vitolo M, Oliveira RPS. Pediococcus spp: an important genus of lactic acid bacteria and pediocin producers. Biotechnol Adv. 2017;35(3):361–74. doi: 10.1016/j.biotechadv.2017.03.004
32. Jack, R.W., Tagg, J.R. and Ray, B. Bacteriocins of gram-positive bacteria. Microbiol. Mol. Biol. Rev. 1995;59:171-200. Available from: https://mmbr.asm.org/content/59/2/171.short
33. Halil, D. Characterization and purification of a bacteriocin produced by *L.mesenteroides* subsp. *cremoris*, Dr. Thesis, Middle East Technical University, Turkey. 2006
34. Tagg, J.R., Dajani, A.S. and Wannamaker, L.W. Bacteriocins of gram positive bacteria. Bacteriol Rev. 1976;40(3):722-56. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC413978/
35. Lewis, C.B., Sun, S. and Montville, T.J. Production of an amylase sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. Applied Environmental Microbiology 1992;58(1):143-9. Available from: https://aem.asm.org/content/58/1/143.short
36. Gagliano, V.J. and Hinsdill. R.D. Characterization of a *Staphylococcus aureus* bacteriocin. J Bacteriol. 1970;104(1):117-25. Available from: https://jb.asm.org/content/104/1/117.short