Songklanakarin J. Sci. Technol.
41 (1), 37-44, Jan. – Feb. 2019

http://www.sjst.psu.ac.th

Original Article

Growth profile and partial characterization of bacteriocin produced by *Lactobacillus plantarum* ATM11 isolated from slaughterhouse soil

Alagu Thirumurugan[1*], Rajaguru Sheela[2], and Amaresh Kumar Singh[2]

[1] *Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamilnadu, 641049 India*

[2] *Department of Biotechnology, PRIST University, Thanjavur, Tamilnadu, 613403 India*

Received: 17 June 2017; Revised: 8 September 2017; Accepted: 21 September 2017

Abstract

In this study, we report on the growth profile and partial characterization of a bacteriocin produced by *Lactobacillus plantarum* ATM11 isolated from goat slaughterhouse soil. Among eight bacteriocin-producing bacteria from 25 *Lactobacilli* isolates of goat slaughterhouse soil, the higher-yielding bacteria were selected and identified as *Lactobacillus plantarum* based on the morphology, biochemical characteristics, and partial 16S rRNA gene sequence. The physico-chemical condition effects of temperature, pH, enzymes, and detergents of bacteriocin activity were studied. The optimum temperature for bacteriocin activity was found to be 60 °C. The optimum pH for bacteriocin activity was found to be in the range of 5.0-6.0. Detergents such as sodium dodecyl sulphate (SDS), Triton X-100, and ethylene diamine tetra-acetic acid (EDTA) were influenced the bacteriocin activity, whereas urea inhibited the activity. Asparaginase and α-amylase enzymes influenced the activity, whereas protease, proteinase K, and lysozyme inhibited bacteriocin activity. The present study revealed that the action mode of the produced bacteriocin has a bacteriostatic effect against *Bacillus cereus* MTCC 1272 and *Micrococcus leuteus* MTCC 2987. Thus, it can be used as a bio-preservative agent for different foods in the future.

Keywords: bacteriocin, biochemical tests, 16S rRNA sequencing, *L. plantarum* ATM11, growth profile, physicochemical characterization

1. Introduction

The main objective of the food industry is to obtain safe food without contaminating elements that can cause diseases or can be harmful to human beings (Acuna, Morero & Bellomio, 2011). Bacteriocins produced from lactic acid bacteria (LAB) are natural antimicrobial peptides with interesting potential applications in food preservation and health care (Maria & Jayaraman, 2012). These bacteria occur naturally in several raw materials like milk, meat, and flour that are used to produce food products. In this regard, isolation and screening of microorganisms from naturally occurring processes have always been a powerful means to obtain useful cultures for scientific and commercial purposes (Vanden Berg *et al*., 1993). Thus, bacteriocins produced by lactic acid-fermenting bacteria (LFB) have received considerable attention in recent years because of their possible use as a food preservative which would result in the reduction in the use of chemical preservatives. Bacteriocins are extracellularly released peptides or protein molecules with a bactericidal or bacteriostatic mode of action against closely related species. The inhibitory spectrum of some bacteriocins also includes food spoilage and food-borne pathogenic microorganisms (Todorv & Dicks, 2005). Therefore; great attention is being drawn towards the application of natural preservative agents, such as bacteriocins, that are produced by LAB. Among the bacteriocins produced by LAB, LFB, such as *lactobacilli*,

*Corresponding author
Email address: biotechthiru@gmail.com

lactococci, leuconostocs, micrococci, pediococci, and streptococci, are active against food-borne pathogens including *Bacillus cereus*, *Clostridium perfringens*, *Staphylococcus aureus*, and *Listeria* species. Among these LFB, *Lactobacillus plantarum* is one of the most important bacteria used for the production of fermented meat, grass, and vegetable products (Kato, Matsuda & Ogawa, 1994).

Hence, identification of new and superior bacteriocin-producing strains of LFB is important since there is a growing interest in the selective application of bacteriocins with broader antimicrobial spectrums, i.e. nisin, pediocin, and lacticin, as natural bioprotective agents in food systems to reduce the incidence of bacterial food poisoning caused by *Listeria* and other pathogens (Castellano, Farias, Holzapfel & Vignolo, 2001). Also, the replacement of chemical preservatives with natural preservatives of bacteriocins is supported by increasing consumer preferences for healthy foods.

There are many reports on the isolation of bacteriocin-producing lactic acid bacteria from tempeh, cooked meat products, dosa, spoiled black olives, fermented food matters of dry sausages, goat meat products, fresh milk, and homemade feta cheese (Castro, Palavecino, Herman, Garro & Campos, 2011; Hamasaki, Fuchu, Sugiyama, & Morita, 2003; Lengkey, Balia, Togoe, Taşbac & Ludong, 2009; Martinez *et al*., 2013; Moreno *et al*., 2002; Todorv & Dicks, 2005; Oki, Rai, Sato, Watanabe & Tamang, 2011; Vijai, Jamuna & Jeevaratnam, 2004; Zhu, Zhao, Sun & Gu, 2013). In the present study, we have made an investigation on the screening of bacteriocin-producing organisms and the growth profile of bacteriocin production from *L. plantarum* ATM11 isolated from slaughterhouse soil. We also investigated the mode of action of bacteriocins.

2. Materials and Methods

2.1 Sample collection

Goat slaughterhouse soil was collected from Thanjavur, Tamilnadu, India. The samples were stored in the laboratory at 4 ℃ in sterile cover until they were used to isolate the bacteriocin-producing organism.

2.2 Bacterial strains and culture conditions

Bacillus cereus (MTCC 1272), *Micrococcus luteus* (MTCC 2987), and *E. coli* MTCC 433 were used as indicator microorganisms and were grown in nutrient agar medium at 37 ℃ for 24 h.

2.3 Isolation of lactic acid bacteria

For the isolation of LAB, serial dilutions of the samples were inoculated into De Man Rogosa Sharpe (MRS) agar (Hi-Media, India) by the pour plate method and incubated in aerobic condition at 35 ℃ for 48 h. After incubation, 25 colonies were randomly selected from the MRS agar plates. The isolated colonies were propagated on the same media until pure cultures were obtained. Purification of the cultures was confirmed by Gram stain. Pure colonies were again cultured on MRS agar slants and stored at 4 ℃ until use.

2.4 Identification of lactic acid bacteria

Identification of the bacteriocin-producing LAB was carried out using morphological and biochemical methods. Identification of the isolates was performed according to the criteria of Bergey's Manual of Determinative Bacteriology (7th Edition) and Amanullah *et al*. (2009). The studies included motility test, catalase test, Gram stain, Simmon's citrate agar test, and growth on mannitol salt agar, growth in MRS broth, and sugar fermentation (sucrose, maltose, mannitol, lactose, and fructose) test.

2.5 Extraction, amplification, and 16S rRNA gene sequencing

The DNA was isolated from a higher-yielding strain using a Medox kit, Chennai. The following primers 8f (5'-GAGTTTGATCATGGCTCAG-3') and 1495r (5'-CTACGGC TACCTTGTTACG-3') were used for PCR reaction. PCR (Applied Biosystem-gene amp PCR system 9700, USA) was performed under the following conditions: 95 ℃ for 5 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, annealing at 52 ℃ for 30 s, and extension at 72 ℃ for 30 s, and final extension at 72 ℃ for 10 min (Huang *et al*., 2009). The obtained PCR product was purified by a PCR purification kit (Genei, Bangalore, India), and subjected to 16S rRNA gene sequencing on a 3130XL Genetic analyzer (Applied Biosystem, USA). The sequencing data were analyzed with the BLAST (The National Centre for Biotechnology Information).

2.6 Production of bacteriocin

The isolated LAB's were grown in MRS broth (Hi-Media, India; pH 6.5) and maintained aerobically at 35 ℃ for 24 h. After incubation, the cells were removed from the growth medium by centrifugation (10,000 x *g* for 30 min at 4 ℃) and passed through 0.22 µm filters. The cell-free supernatant was adjusted to pH 6.0 using 1N NaOH and used as the crude bacteriocin.

2.7 Determination of bacteriocin activity

Bacteriocin activity was determined by the agar well-diffusion assay using the above mentioned organism as the indicator strain (Delgado, Brito, Fevereiro, Peres & Marques, 2001). Aliquots (20 µL) of the sterile supernatant were placed in 6-mm diameter wells that had been cut in Mueller-Hinton agar plates and previously seeded with the indicator organism. After 24 h of incubation, the diameters of the zones of growth inhibition were measured. The activity of cell-free supernatant was expressed in arbitrary units per milliliter (AU/mL). Unit activity of the bacteriocin was defined as 1 AU is a unit area of inhibition zone per unit volume, in this case mm^2/mL (Usmiati & Marwati, 2009). The bacteriocin activity was calculated using the following formula:

$$\text{Bacteriocin activity (AU/mL)} = \frac{\text{Lz-Ls}}{\text{V}}$$

Lz = clear zone area (mm^2), Ls = well area (mm^2)
V = volume of sample (mL)

2.8 Growth profile of bacteriocin-producing strain

The overnight culture of the bacteriocin-producing strain was inoculated into 100 mL of MRS broth and then incubated at 37 °C for 24 h. At different time intervals, the growth of cells was measured by absorbance at an optical density (OD) of 660 nm. The bacteriocin activity and pH changes were also monitored.

2.9 Effect of temperature, pH, detergents, and enzymes on bacteriocin activity

Before subjecting the bacteriocin to partial characterization, the bacteriocin-producing isolate was grown in an MRS broth for 24 h at 37 ºC. After incubation, the cultures were centrifuged at 10,000 rpm for 60 min at 4 ºC, after which the bacteriocins were precipitated with (80% saturation) ammonium sulphate and kept overnight at –20 ºC for precipitation. Following precipitation, centrifugation of the supernatants resulted in the formation of pellets which were collected and stored in phosphate buffer of pH 5.5. The precipitate was dialyzed against 20 mM potassium phosphate buffer (pH 7.0) for 12 h at 4 ºC.

The dialyzed bacteriocin (500 µL) was exposed to various heat treatments at 30 °C, 40 °C, 60 °C, 80 °C, and 100 °C for 30 min and 121 °C for 15 min. A sample maintained at room temperature (35±2 °C) was considered as a control. Aliquot volumes of each fraction were then removed. Similarly, the effect of pH was tested with the bacteriocin (pH 6.5, 50 µL), mixed with 100 µL of buffer (0.1 M potassium phosphate pH 4.0, pH 5.0, pH 6.0, pH 7.0, pH 8.0, and pH 9.0) and the sample adjusted to pH 6.5 was considered as a control. The samples were then incubated for 1 h and the bacteriocin activity against *Bacillus cereus* was determined.

Triton X-100, Tween 80, sodium dodecyl sulphate, and Tween 80 surfactants were added to the bacteriocin at a concentration of 0.1 mL or 0.01 g/mL of bacteriocin solutions. These preparations were incubated at 30 °C for 60 min. Similarly, α-amylase, asparaginase, lysozyme, protease, and proteinase-K each at a final concentration of 1 mg/mL were tested on the bacteriocin preparation. Untreated bacteriocin was considered as a control. They were then incubated at room temperature for 2 h, and bacteriocin activity against *Bacillus cereus* was determined. Assays were performed in triplicates and standard error was found to be within 5% in all experiments.

2.10 Mode of action of bacteriocin

To study the mode of action of bacteriocin against the indicator organism, the antibacterial compound (5 mL) of neutralized culture supernatant was added at the fourth hour to 25 mL of growing cells of indicator organisms in the early exponential phase. The OD_{600} was measured at appropriate intervals using UV-visible spectroscopy (Ivanova *et al*., 1998; Todorov & Dicks, 2009). Similarly, samples were taken every two hours in order to determine colony forming units (CFU mL^{-1}) for each strain. The number of CFU mL^{-1} was determined by the agar plate count method.

3. Results and Discussion

3.1 Isolation and identification of bacteriocin-producing organism

Most food processing industries are focusing more on natural preservatives due to possible health hazards with the consumption of foods preserved by chemical preservatives which has brought a significant change in the attitude of people towards such foods. Therefore, there is a great need for effective and safer natural preservatives to meet the demand. This is one of the few reports of bacteriocin produced by LAB isolated from a meat environment, particularly from goat slaughterhouse soil. A total of 25 bacterial isolates were obtained from the meat environment. Microscopic identification, Gram stain, and biochemical tests supported the characterization of *Lactobacilli*. From 25 isolates, 8 isolates were able to produce bacteriocin. The higher-yielding organisms were screened based on the potential of bacteriocin production by the agar well diffusion method. A comparison of antimicrobial activity of bacteriocin values are tabulated in Table 1. A higher yielding-strain was further identified as *Lactobacillus plantarum* based on biochemical characteristics (Table 2) and partial 16S rRNA gene sequence. The partial 16S rRNA gene sequence analysis showed a similarity of both reverse primers as well as forward primer with existing *L. plantarum.* Thus, the isolated bacteriocin-producing organism was confirmed as *L. plantarum*. The obtained sequences were submitted to the National Centre for Biotechnology Information and assigned the accession number JQ934539.

Table 1. Screening of bacteriocin-producing isolates against indicator organisms.

Bacterial isolates	Indicator organisms					
	Bacillus cereus MTCC 1272		*Micrococcus luteus* MTCC 2987		*E. coli* MTCC 433	
Isolates	a	A	a	A	a	A
1	14.3±0.5	3224.04	11.26±1.6	1383.38	10.6±1.3	824.18
2	14.4±0.31	3528.00	11.4±0.52	1458.00	11.0±0.23	1250.00
3	12.3±1.3	2022.48	11.36±0.73	1436.48	12.1±0.9	1860.50
4	11.4±1.2	1240.44	12.26±0.21	1959.38	11.16±0.62	1331.28
5	12.6±0.6	1800.00	10.96±0.32	1230.08	10.23±1.3	894.64
6	11.3±0.52	1265.04	10.33±0.41	937.44	10.6±0.75	824.18
7	10.4±0.43	894.64	12.16±0.63	1897.28	11.3±1.8	1265.04
8	10.2±0.21	812.04	11.23±0.7	1367.64	10.93±1.3	1215.24

Values are expressed as mean±SD; a - Diameter of zone of inhibition (mm); A - Bacteriocin activity (AU/mL).

Table 2. Morphological and biochemical characteristics *L. plantarum* ATM11.

Morphological and biochemical characteristics	Observations
Colony morphology	White, shiny, smooth colonies
Gram stain	Gram-positive, rods
Growth in MRS broth	Uniform turbidity
Catalase	Negative
Motility	Non-Motile
Growth on mannitol salt agar	Negative
Simmon's citrate agar test	Negative
Carbohydrate test (sucrose, maltose, mannitol, lactose, and fructose)	Positive

The antimicrobial activities of the eight isolates of LAB and their degree of inhibition against food-spoiling isolates were studied. From a total of 25 lactic acid bacteria, the culture supernatants of eight isolates yielded zone of inhibition when tested against the indicator organisms. The diameters of the inhibition zones ranged from 10 to 14 mm. The highest diameter (14.4 mm with 3528 AU/mL) was recorded for the culture supernatants of isolate 2 against *Bacillus cereus* and the smallest of 10.23 mm with 894.64 AU/mL for isolate 6 against *E. coli*. Among the eight bacteriocin-producing organisms, isolate 2 was selected for further study since it showed the maximum activity against indicator organisms. Bacteriocins have already been reported to be inhibitory against several other bacteria (Karthikeyan & Santosh, 2009). Thus, possession of bacteriocin by *L. plantarum* is an indication that the bacteria can be used as a probiotic and as a bio-preservative agent.

3.2 Growth profile of *L. plantarum* ATM11

The growth profile of the bacteriocin-producing strain was studied along with the bacteriocin and pH changes were also monitored. In the control experiment, the cell density of *L. plantarum* ATM11 increased from 0.06 to 1.579 (OD_{600nm}) during 48 h of growth at 37 ºC. The pH of the medium decreased from 6.3 to 4.76 over the same period. Low levels of bacteriocin activity (approximately 50 AU/mL) were detected after 5 h of growth in MRS broth with maximal activity (3698 AU/mL) after 18 h (Figure 1). This suggests that the peptide is a primary metabolite. According to Parente & Ricciadi (1994), most bacteriocins show a more or less sharp decrease in activity at the end of the exponential growth phase. However, a decrease in activity has not been observed for all bacteriocins from LAB even after prolonged incubation. Similar to the above report, this strain also showed decreased activity at the end and the pH also came down. This report is in agreement with the findings of Foo *et al.* (2003) which suggests that the increase of biomass cells and organic acid production are the main reasons for the lower pH. The lactic acid and acetic acids produced by *L. plantarum* strains during its metabolite production augment the growth of the producer cells (Foo *et al*., 2003; Savadogo, Ouattara, Bassole & Traore, 2006). The maintenance of the acidic pH of the metabolites is supplied by the lactic and acetic acids which are the major contributors to the acidic environment (Brashears,

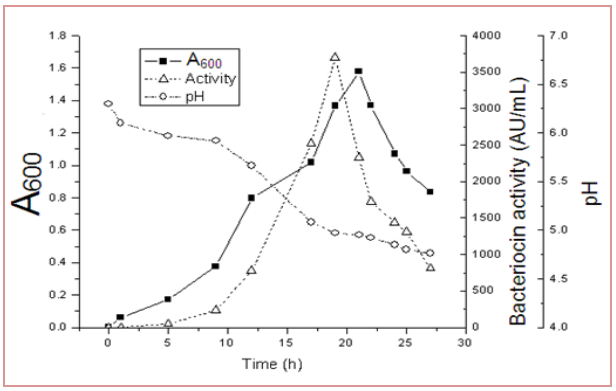

Figure 1. Bacteriocin productions during the growth of *L. plantarum* ATM11 in MRS broth at 37 °C. The optical density (absorbance at 600 nm) and pH of culture were measured at different time intervals. The bacteriocin activity was also assayed and expressed as AU/mL.

Jaroni & Trimble, 2003). Based on these results, the optimum incubation temperature, culture pH for bacteriocin production, and maximal absorbance for *L. plantarum* ATM11 were determined to be pH 6.5 at 37 °C.

3.3 Effect of temperature, pH, detergents, and enzymes on bacteriocin activity

Bacteriocin production is strongly dependent on pH, source of nutrients, and temperature as claimed by Todorov & Dicks (2004). Therefore, it is necessary to maintain temperature, pH, and nutrient sources in bioprocesses particularly for cell growth and product formation. Accordingly, the dialyzed supernatant obtained from 80% ammonium sulphate precipitate of bacteriocin activity was tested with different temperatures at 30 °C, 40 °C, 60 °C, 80 °C, and, 100 °C for 30 min and 121 °C for 15 min at 15 psi. The results showed that bacteriocin activity increased as the temperature increased and was more stable even at 100 °C and 121 °C. A comparison of antimicrobial activities at different temperatures is graphically represented in Figure 2. Furthermore, the maximum arbitrary unit was measured as 4167.84 AU/mL at 60 ºC and the minimum activity was measured as 1280.18 AU/mL at 30 °C. The results revealed that the bacteriocins in this study were thermostable.

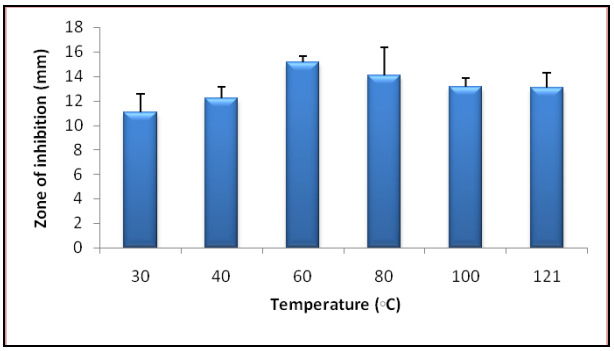

Figure 2. Effect of temperature on bacteriocin activity against *Bacillus cereus*.

Similar studies already reported a number of bacteriocins were produced by *Lactococcus* and *Lactobacillus* species (Todorov, Nyati, Meincken & Dicks, 2007). In addition, Sarika, Lipton & Aishwarya (2010) have observed that the bacteriocin GP1 produced by *L rhamnosus* had remarkable stability after heat treatment even at the autoclaving temperature for 20 min. According to a report by Sifour, Tayeb, Haddar, Namous & Assaoui (2010), heat stability of *L. plantarum* F12 at 100 °C is important if the bacteriocin is used as a food preservative since many procedures of food preparation involve a heating step. Similarly, *L. plantarum* ATM11 producing bacteriocin can be used as a food preservative even in the heating step of food preparation.

Dialyzed bacteriocin was treated at different pH levels from 4.0 to 9.0. The results showed that the maximum activities obtained at pH 5.0 and 6.0 were 3329.28 AU/mL, 1230.08 AU/mL, respectively. Minimum activities were observed at pH 4.0, 7.0, and 8.0 but no activity was observed at pH 9.0. A comparison of the antimicrobial activity values are graphically represented in Figure 3. With respect to the effect of pH on bacteriocin, Sung-Mee Lim (2010) already reported similar results to the present study. The stability of heat resistance up to 121 °C and its activity over the different pH values from 2.0 to 10.0 is a common feature of class IIa bacteriocins (Fimland, Johnsen, Dalhus & Nissen-Meyer, 2005). From the above evidence, bacteriocin produced from *L. plantarum* ATM11 may also belong to class-IIa bacteriocins.

The detergents used to study the inhibitory agents were sodium dodecyl sulphate (SDS), Tween 80, Tritone X-100, EDTA, and urea. Sodium dodecyl sulphate (SDS), EDTA, and Tritone X-100 stimulated bacteriocin activity, whereas Tween 80 offered slight stimulation. In contrast, bacteriocin activity was strongly inhibited by urea. Similarly, urea inhibited the bacteriocin produced by *Lactobacillus lactis* (Rajaram, Manivasagan, Thilagavathi & Saravanakumar (2010). A comparison of antimicrobial activity is graphically represented in Figure 4. Similar results were reported by Moghaddam, Sattari, Mobarez & Doctorzadeh (2006).

The protein nature of the antimicrobial compounds was verified by treatment with enzymes. The enzymes used to study the inhibitory effects were proteinase K, α-amylase, protease, asparaginase, and lysozyme. Complete inactivation or significant reduction in activity was observed after adding protease, proteinase K or lysozyme to the bacteriocin which confirmed the proteinaceous nature of the active agent (Figure 5). However, α-amylase and asparaginase did not cause inactivation. This result suggested that the peptide was not glycosylated or its activity was not dependent on glycosylation (Kwaadsteniet, Fraser, Van Reenen & Dicks, 2006).

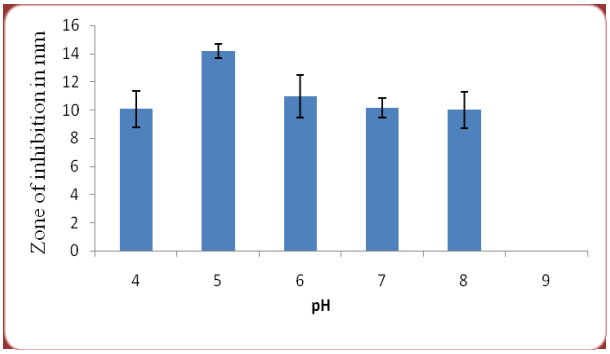

Figure 3. Effect of pH on bacteriocin activity against *Bacillus cereus*.

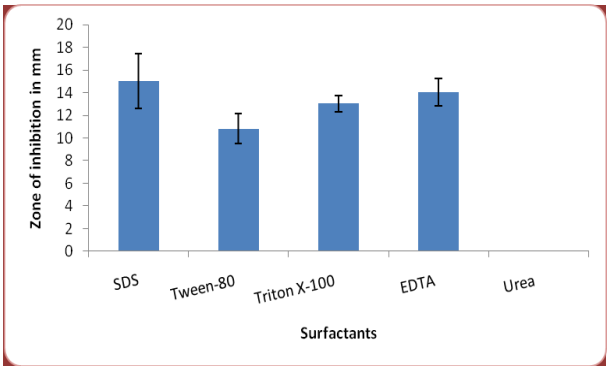

Figure 4. Effect of detergents on bacteriocin activity against *Bacillus cereus*.

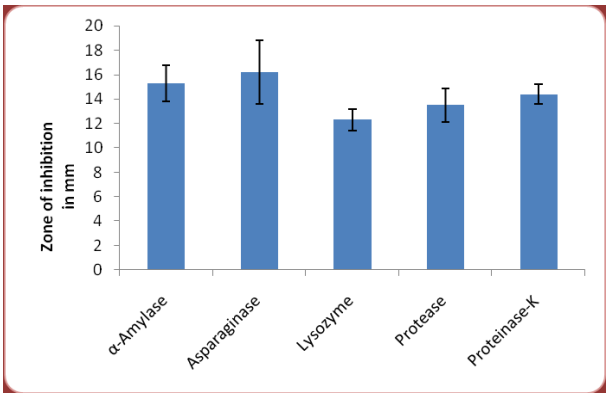

Figure 5. Effect of enzymes on bacteriocin against *Bacillus cereus*.

3.4 Mode of action of bacteriocin

The mode of action of the bacteriocin produced by *L. plantarum* ATM11 was studied. The addition of bacteriocin (pH 6.0) to a 3-hour-old culture of *M. luteus* and *B. cereus* (OD_{600nm}) at the fourth hour resulted in growth inhibition for the next 11 hours (Figure 6a and b). Viability loss was accompanied by a decrease of the OD of the culture of *M. luteus* (Figure 6c). It revealed an indication of cell lysis after the treatment. The effectiveness of the antibacterial activity decreased throughout the incubation period in bacteriocin treated indicator organisms. The results suggested that the mode of action of bacteriocin may be considered to be bacteriostatic. Higher concentrations might be necessary in order to achieve a bacteriocidal effect as reported by Ivanova *et al.* (1998). Similar results were reported for the mode of action of BacUB9 on the strain BGHN14 which was identified as bacteriostatic (Tolinački *et al*., 2010).

4. Conclusions

In conclusion, this is one among the reports of bacteriocin production by *L. plantarum* strain ATM11 that was isolated from goat slaughter house soil. The isolated bacteriocin-producing organism was identified as *L. plantarum* strain based on the morphology, biochemical test, and

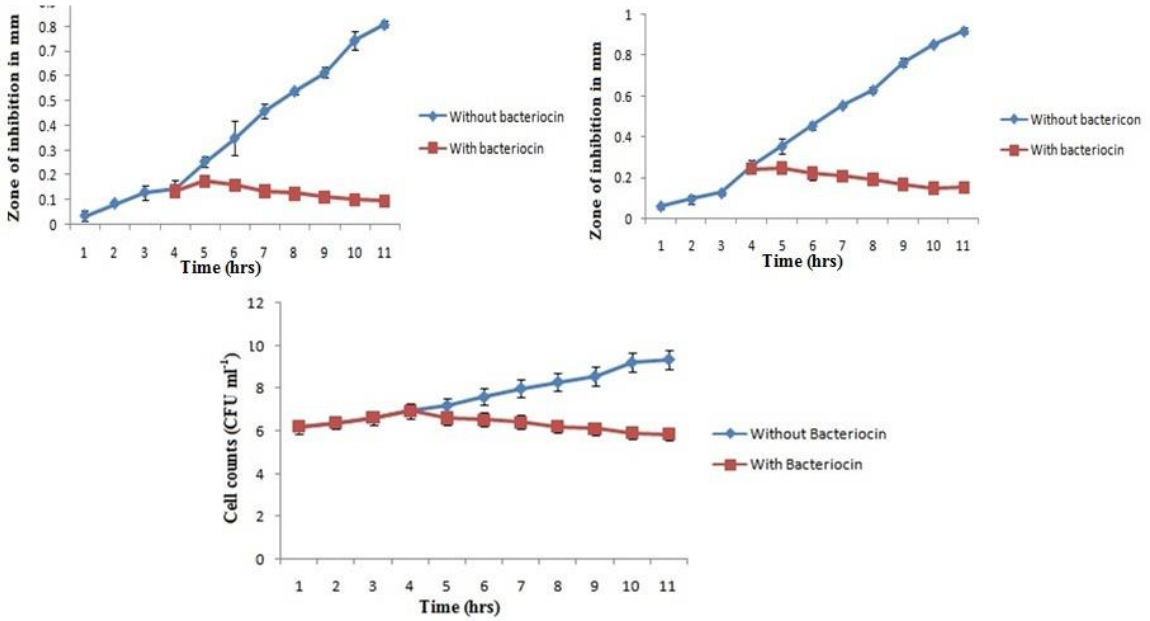

Figure 6. Mode of action of bactriocin against a) *Micrococcus luteus* b) *Bacillus cereus* and c) measurement of colony forming units (CFU mL^{-1}) of *Micrococcus luteus*.

16S rRNA sequencing. The bacteriocin produced by *L. plantarum* ATM11 growth profile and the conditions were studied. Bacteriocin activity (3698 AU/mL) was exhibited at pH 6.5 and at 37 ^{o}C after 18 h. The bacteriocin that was produced was subjected to physical-chemical characterization. It was found to be thermostable and its mode of action confirmed a bacteriostatic effect. From the physical-chemical characterization studies, the bacteriocin of *L. plantarum* ATM11 might belong to the class-IIa group and a study with mass spectrometry is needed for confirmation. Therefore, in the future this bacteriocin may find application as a bio-preservative in the food industry.

Acknowledgements

We thank the management for their support and encouragement throughout the research work.

References

Acuna, L., Morero, R. D., & Bellomio, A. (2011). Development of wide-spectrum hybrid bacteriocins for food biopreservation. *Food Bioprocess Technology, 4*(6), 1029-1049. doi:10.1007/s11947-010-0465-7

Amanullah, M. S., Alam, R. N., Subarna, R., Bateen, Huque, K. S., & Sultana, A. (2009). Feeding Lactobacilli as probiotic and proportion of *Escherichia coli* in the intestine of calves. *Bangaladesh Vetrinarian*, *26* (1), 17 - 22. doi:10.3329/bvet.v26i1.4627

Brashears, M. M., Jaroni, D., & Trimble, J. (2003). Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. *Journal of Food Protection*, *66*(3), 355-363. doi:10.4315/0362-028X-66.3.355

Castellano, P., Farias., M. E Holzapfel, W., & Vignolo, G. (2001). Sensitivity variations of *Listeria* strains to the bacteriocins, lactocin 705, enterocin CRL35 and nisin. *Biotechnology Letters*, *23*(8), 605–608. doi: 10.1023/A:1010320808989

Castro, M. P., Palavecino, N. Z., Herman, C., Garro, O. A., & Campos, C. A. (2011). Lactic acid bacteria isolated from artisanal dry sausages: Characterization of antibacterial compounds and study of the factors affecting bacteriocin production. *Meat Science, 87*(4): 321-329. doi:10.1016/j.meatsci.2010.11.006

Delgado, A., Brito, D., Fevereiro, P., Peres, C., & Marques, J. F. (2001). Antimicrobial activity of *L. plantarum* isolated from a traditional lactic acid fermentation of table olives. *Le Lait*, 81(1), 203-215. doi:10.1051/lait:2001124

Fimland, G., Johnsen, L., Dalhus, B., & Nissen-Meyer, J. (2005). Pediocin-like antimicrobial peptides (class IIa bacteriocins) and their immunity proteins: Biosynthesis, structure, and mode of action. *Journal of Peptide Sciences*, *11* (11), 688–696. doi:10.1002/psc.699

Foo, H. L., Loh., T. C, Law, F. L., Lim, Y. Z., Kufli, C. N., & Rusul, G. (2003). Effects of feeding Lactobacillus plantarum I-UL4 isolated from Malaysian Tempeh on growth performance, faecal flora and lactic acid bacteria and plasma cholesterol concentrations in postweaning rats. *Journal of Food Science and Biotechnology*, *12*(4), 403-408.

Hamasaki, Y., Fuchu, H., Sugiyama, M., & Morita, H. (2003). Behavior of psychrotrophic lactic acid bacteria isolated from spoiling cooked meat products. *Applied Environmental Microbiology*, *69*(6), 3668–3671. doi:10.1128/AEM.69.6.3668-3671.2003

Huang, Y., Luo, Y., Zhai, Z., Zhang, H., Yang, C., Li, H. T. Z., . . . Hao, Y. (2009). Characterization and application of an anti-Listeria bacteriocin produced by Pediococcus pentosaceus 05-10 isolated from Sichuan Pickle, a traditionally fermented vegetable product from China. *Food Control*, *20*(11), 1030–1035. doi:10.1016/j.joodcont.2008.12.008

Ivanova, I., Miteva, V., Stefanova, T. S., Pantev, A., Budakov, I., Danova, S., . . . Boyaval, P. (1998). Characterization of a bacteriocin produced by *Streptococcus thermophilus* 81. *International Journal of Food Microbiology*, *42*(3), 147–158. doi:10.1016/S0168-1605(98)00067-1

Karthikeyan, V., & Santosh, S. W. (2009). Isolation and partial characterization of bacteriocin produced from *Lactobacillus plantarum*. *African Journal of Microbiological Research*, *3*(5), 233-239.

Kato, T., Matsuda, T., & Ogawa, E. (1994). Plantaricin 149, a bacteriocin produced by *Lactobacillus plantarum* NRIC 149. *Journal of Fermentation Engineering*, *77*(3), 277–282. doi:10.1016/0922-338X(94)90234-8

Kwaadsteniet, M. D., Fraser, T., Van Reenen, C. A., & Dicks, L. M. T. (2006). Bacteriocin T8, a novel class IIa sec-dependent bacteriocin produced *by Enterococcus faecium* T8, isolated from vaginal secretions of children infected with human immunodeficiency virus. *Applied Environmental Microbiology*, *72*(7), 4761–4766. doi:10.1128/AEM.00436-06

Lengkey, H. A. W., Balia, R. L., Togoe, I., Taşbac, B. A., & Ludong, M. (2009). Isolation and identification of lactic acid bacteria from raw poultry meat. *Biotechnology in Animal Hunbandry*, *25*, 1071-1077.

Maria, M., & Jayaraman, S. (2012). Detection of heat stable bacteriocin from Lactobacillus acidophilus NCIM 5426 by liquid chromatography/mass spectrometry. *Indian Journal of Science and Technology*, *5*(3), 2325 - 2332.

Martinez, R. C. R., Wachsman, M., Torres, N. I., Leblanc, J. C., Todorov, S. D., Gombossy, B. D., & Franco, D. M. (2013). Biochemical, antimicrobial and molecular characterization of a noncytotoxic bacteriocin produced by *Lactobacillus plantarum* ST71KS. *Food Microbiology*, *34*(2), 376-381. doi:10.1016/j.fm.2013.01.011

Moghaddam, M. Z., Sattari, M., Mobarez, A. M., & Doctorzadeh, F. (2006). Inhibitory effect of yogurt Lactobacilli bacteriocins on growth and verotoxins production of enterohemorrhgic *Escherichia coli* O157:H7. *Pakistan Journal of Biological Sciences*, *9*(11), 2112-2116

Moreno, M., Leisner, J. J., Tee, L. K., Ley, C., Radu, S., Rusul, G., Vancanneyt, M., & Luc de Vuyst. (2002). Microbial analysis of malaysian tempeh, and characterization of two bacteriocins produced by isolates of *Enterococcus faecium*. *Journal of Applied Microbiology*, *92*(1), 147-157. doi:10.1046/j.1365-2672.2002.01509.x

Oki, K., Rai, A. K., Sato, S., Watanabe, K., & Tamang, J. P. (2011). Lactic acid bacteria isolated from ethnic preserved meat products of the Western Himalayas. *Food Microbiology*, *28*(7), 1308-1315. doi:10.1016/j.fm.2011.06.001

Rajaram, G., Manivasagan, P., Thilagavathi, B., & Saravana kumar, A. (2010). Purification and characterization of a bacteriocin produced by *Lactobacillus lactis* isolated from marine environment. *Advances in Food Science and Technology*, *2*(2),138-144.

Sarika, A. R., Lipton, A. P., & Aishwarya, M. S. (2010). Bacteriocin production by a new isolate of Lactobacillus rhamnosus GP1 under different culture conditions. *Advances in Food Science and Technology*, *2*(5), 291-297.

Savadogo, A., Ouattara, C. A. T., Bassole, I. H. N., & Traore, S. A. (2006). Bacteriocins and lactic acid bacteria - A mini review. *African Journal of Biotechnology*, *5*(9), 678-683.

Sifour, M., Tayeb, I., Haddar, H. O., Namous, H., & Assaoui, S. (2010). Production and characterization of bacteriocin of *Lactobacillus plantarum* f12 with inhibitory activity against *Listeria monocytogenes*. *The Online Journal of Science and Technology*, *2*, 55-61.

Sung-Mee Lim (2010). Cultural conditions and nutritional components affecting the growth and bacteriocin production of *Lactobacillus plantarum* KC21. *Food Science and Biotechnology*, *19*(3), 793-802. doi:10.1007/s10068-010-0111-1

Todorov, S., & Dicks, L. M. T. (2005). Optimization of bacteriocin ST311 production by *E.faecium*, isolated from spoiled black olives. *Journal of Microbiology*, *43* (4), 370-374.

Todorov, S. D., & Dicks, L. M. T. (2004). Comparison of two methods for purification of plantaricin ST31, a bacteriocin produced by *Lactobacillus plantarum* ST31. *Enzymes and Microbial Technology*, *35*(1), 318-326. doi:10.1590/S1517-83822004000100026

Todorov, S. D., & Dicks, L. M. T. (2009). Bacteriocin production by *Pediococcus pentosaceus* isolated from marula (Scerocarya birrea). *International Journal of Food Microbiology*, *132*(2-3), 117–126. doi:10.1016/j.ijfoodmicro.2009.04.010

Todorov, S. D., Nyati, H., Meincken, M., & Dicks, L. M. D. (2007). Partial characterization of bacteriocin AMA-K, produced by *Lactobacillus plantarum* AMA-K isolated from naturally fermented milk from Zimbabwe. *Food Control*, *18*(6), 656–664. doi:10.1016/j.foodcont.2006.03.003

Tolinački, M., Kojić, M., Lozo, J., Terzić-vidojević, A., Topisirović, L., & Fira, D. (2010). Characterization of the bacteriocin-producing strain *Lactobacillus paracasei subsp*. paracasei BGUB9. *Archives of Biological Sciences*, *62*(4), 889-899.

Usmiati, S., & Marwati, T. (2009). Selection and optimization process of bacteriocin production from *Lactobacillus sp. Indonesian Journal of Agriculture*, *2*(2), 82-92.

Vanden Berg, D. J. C, Smits A., Bot, B., Ledeboer, M., Kersters, K., Verbakel, J. M. A., & Theo Verrips, C. (1993). Isolation, screening and identification of lactic acid bacteria from traditional food fermentation processes and culture collections. *Food Biotechnology*, *7*(3), 189-205. doi:10.1080/08905439309549857

Vijai, P., Jamuna, M., & Jeevaratnam, K. (2004). Isolation and characterization of bacteriocin producing lactic acid bacteria from a south Indian special dosa (Appam) batter. *Journal of Culture Collection*, *4*(1): 53-60.

Zhu, X., Zhao, Y., Sun, Y., & Gu, Q. (2014). Purification and characterisation of plantaricin ZJ008, a novel bacteriocin against *Staphylococcus spp*. from *Lactobacillus plantarum* ZJ008. *Food Chemistry*, *165*(15), 216–223. doi:10.1016/j.foodchem.2014.05.034