Research Article

Antioxidant Activity of Antilisterial Bacteriocns Isolated from *Paenibacillus polmyxa* and *Enterococcus faecium* GRD AA

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ABSTRACT

Anti-listerial bacteriocin producing strain was isolated, purified and characterized from fermented food source toddy and milk and found to be the genus *Paenibacillus polymyxa* and *Enterococcus faecium* GRD AA. The bacteriocins ALC101 and ALC102 were partially purified by Amberlite XAD-16 purification method and anti-listerial activity were checked. Both the bacteriocins ALC101 and ALC102 along with Nisin showed a maximum of 85% of DPPH and 85.5% of ABTS radical scavenging activity like BHT which was the standard used in the assay. The bacteriocins also showed a maximum of 87.6% Fe^{2+} reduction potential. From the results it can be concluded as bacteriocin produced by *Paenibacillus polymyxa* ALC101, and bacteriocin from *Enterococcus faecium* GRD AA ALC102 has a good antioxidant potential when compared to Nisin and BHT (positive control).

Key words: Antioxidant, Antilisterial, Bacteriocin, BHT, Radical Scavenging, Nisin.

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INTRODUCTION

Fermented food and beverages of all types have played a major role in human nutrition since the beginning of recorded human history. The intensified preservation feature of fermented foods confers a significant advantage, still crucial for food preservation in less developed societies where refrigeration is still scarce. In current societies, increasing consumer demands and preference for natural products and processes emphasizes the concept of biopreservation as a natural alternative for food preservation. This includes the use of safety-enhancing bacteria in fermented foods known as protective cultures.[1]

The new concepts of human well-being and healthy food promote scientists to search for new antibacterial compounds for food preservation. Bacteriocins and/or bacteriocin producing group Lactic Acid Bacteria (LAB) can serve as natural biopreservatives instead of synthetic preservatives. Bacteriocins are antimicrobial peptides synthesized ribosomally produced by one bacterium that are active against other bacteria either in the same species and, as with host defense peptides, cell signaling mechanisms also be involved.[2,3] Thus bacteriocins are pH sensitive, which could limit their efficacy and finally their use. Nisin is a polypeptide produced by *Lactococcus lactis* spp. It has been approved as a food additive with generally recognized as safe (GRAS) status in over 50 countries. Moreover, it is particularly effective against heat resistant bacterial spores of *Clostridium* spp. and against foodborne pathogens like *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus,* etc.[4]

Lactic Acid Bacteria (LAB) in particular, constitute the most appropriate choice for application as protective cultures, since they are found in almost all kinds of fermented foods, and having a long history of secure use and form part of the gut microflora of humans and animals. In addition, LAB produces a range of antimicrobial substances such as organic acids and bacteriocins that have been already exploited by the

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food industry. Promising LAB strains or their metabolic products have been applied in food against pathogens mainly *Listeria monocytogenes*, in dairy products such as soft and hard cheese, and meat products such as sausages and ham.[5-7] The main variance between bacteriocins and antibiotics is that bacteriocins limit their activity to strains of species, associated with the producing species especially to the strains of the same species; contrarily antibiotics, have a broad spectrum of activity and even if its activity is restricted, this doesn't show any preferential effect on closely related strains. Moreover, bacteriocins are synthesized ribosomally and produced during the initial stage of growth phase, although antibiotics are usually secondary metabolites. Bacteriocins can be used in purified or crude form or by using a product formerly fermented with a bacteriocin producing bacterial strain as a component in food processing or incorporated as bacteriocin producing starter culture strain.[3]

Free radicals and active oxygen have been recognized as important factors in the pathogenesis of several human diseases. Reaction oxygen metabolites (ROM), generated through normal reactions within the body during respiration in aerobic organisms and can cause damage to proteins, mutations in DNA, oxidation of membrane phospholipids, and modification of low density lipoproteins. Excessive amounts of ROM lead to cell damage, which in succession, promotes chronic diseases includes atherosclerosis, arthritis, diabetes, neurodegenerative diseases, and cancer. To counterpoise the oxidant molecule, the human body produces antioxidant enzymes and molecules along with the antioxidants present in the food from the biological barrier. However, in certain conditions, the defense system breaks down to protect the body against oxidative stress; and leads to the possibility of increasing antioxidant defenses is considered to be important in the maintenance of human health and disease prevention. The bacteriocins can be used as probiotics, which can improve the total antioxidant status and decrease markers of oxidative stress in healthy people.[8-10]

Bacterial strains included in the genus *Paenibacillus* have been isolated from a diversity of environments, in which many of the species are pertinent to humans, animals, plants, and the environment. The majority of them are found in soil, often associated with plant roots; these groups of rhizobacteria promote plant growth and can be utilized in the field of agriculture. Numerous species of *Paenibacillus* produces antimicrobial molecules that can be used as chemotherapeutics or as pesticides, and many of them produces enzymes that can be exploited for bioremediation or to produce valuable chemicals. Many strains of *Paenibacillus* are pathogenic to honey bees or other invertebrates; mean while others are occasional opportunistic pathogens to humans. Many of these pertinent characteristics overlap within the same species.[11] *Enterococcus faecium* is a Gram positive, homofermentative, lactic acid bacteria that is a natural inhabitant of the gastro-intestinal tract. Nevertheless, they are also found in fermented foods and are frequently isolated from starter cultures and cheese producers.[12] *Enterococcus faecium* bacteriocins have gained attentions in recent years as they could be isolated easily from several fermented foods and because many of them are active against food-borne pathogens such as *Listeria monocytogenes*. *Enterococcus faecium* T136 was isolated from Spanish dry fermented sausages, which produced enterocin A and B. They were active against a wide range of Gram positive bacteria, including *Listeria* and *Staphylococci* spp. N-terminal amino acid sequencing exposed the similarity of enterocin A with pediocin family of bacteriocins whereas enterocin B showed strong homogenity to carnobacteriocin. Enterocin P inhibited most Gram positive foodborne pathogens like *Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens* and *Clostridium botulinum.*[13]

With regard to the relationship between bacteriocins and antioxidant activity, the majority of studies have focused on the antioxidant activity of intact cells, intracellular cell free extracts and cell free supernatants of LAB.[11] The aim of this study was to isolate and identify bacteriocin producing bacteria from fermented food sources and to analyze their potentiality in exerting antioxidant activity for counteracting oxidative stress in the host comparing with Nisin which is a commercially available bacteriocin.

MATERIALS AND METHODS

Screening and identification of bacteriocin producing strains: Toddy (palm sap) and milk was selected as food sources for isolating bacteriocin producing bacteria. Fermented toddy sample was collected from Palakkad district of Kerala and milk sample was collected from local market of Coimbatore, Tamil Nadu. The samples were serially diluted and plated on de Man Rogosa and Sharpe (MRS) agar plates by spread plating technique. The plates were incubated overnight at 37°C. Further screening of the isolates were done by inoculating the colonies to MRS (broth and agar) medium. A total of 30 isolate were screened from toddy and milk samples and tested its acivtity against *Listeria monocytogenes* MTCC 657 (a major foodborne pathogen). Only two of them were found to be anti-listerial and bacteriocin producing and they were

maintained in MRS agar media. The isolates were tested for cell morphology, Gram staining, catalase activity and hemolytic activity. The molecular identification of the strains were done by 16S rRNA gene amplification using polymerase chain (PCR) reactions. The strains were phylogentically identified and the gene sequence were submitted to GenBank and accession numbers were obtained.

Isolation of antilisterial bacteriocin from the strains: The production of antilisterial bacteriocin was achieved by inoculating a single colony of the isolated strain to 10 ml of production media (TSBYE, HiMedia, India) and kept for incubation for 16-18 h at 30°C without shaking.[14] The bacterial culture was centrifuged at 12000 × g for 20 min at room temperature and cell free supernatant was collected followed by incubation at 100°C for 10 min in a boiling water bath to inactivate protease. The heat inactivated cell free supernatant (crude antilisterial bacteriocin) was filtered (0.45 µm pore size filter, Millipore) and then used for further assay and purification.

Purification of antilisterial bacteriocin from the strains by Amberlite XAD-16 adsorption: Five grams of Amberlite XAD-16 was soaked in 50% isopropanol and stored at 4°C overnight. The isopropanol was removed by washing repeatedly with deionized distilled water till the smell of isopropanol disappears. Five grams of Amberlite XAD-16 was added to 250 ml of heat stabilized cell-free supernatant. This mixture was kept for incubation with shaking at room temperature for 4 hr. The mixture was then transferred to a chromatographic column and the matrix was washed with 20 ml of deionized distilled water and 20 ml of 40% (v/v) ethanol in distilled water.[15] The antilisterial bacteriocin were eluted with 20 ml of 70% (v/v) isopropanol in distilled water, followed by washing with 20 ml of absolute isopropanol. The eluted fraction of crude bacteriocin was evaporated to half of its volume and the pH were adjusted to 5.7 with 5 N NaOH. This fraction is called Amberlite fraction. The protein concentration of this fraction was estimated by measuring the absorbance at 280 nm.

Nisin: 100mg of Nisin (HiMedia) was solubilized in 10ml of 0.02N HCl to give the concentration of 104 IU/ml (40 IU=1g). The solution were filtered through 0.45mm filters (Whatman®, Sigma-Aldrich, India) and was stored at -4°C.[16]

Antioxidant activity of the bacteriocins

DPPH Radical Scavenging Assay: Scavenging ability of 2, 2- diphenyl-1-picryl hydrazyl hydrate (DPPH) free radical by antilisterial bacteriocins ALC101, ALC102 and Nisin were evaluated according to the method explained by.[17] The assay solution which is composed of 2.2 ml DPPH free radical (0.1mM) and 0.8 ml of the bacteriocin sample. The mixture were shaken vigorously and kept in a dark room for 30 min in room temperature. The absorbance of the sample were measured spectrophotometrically at 517 nm. Standard antioxidant BHT (Butylated hydroxyl toluene) were served as positive control (Ayigoro, 2009) and millipore water as blank control. The DPPH free radical scavenging activity of the bacteriocins ALC101, ALC102 and Nisin were calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{A_0}{1-(A_1 - A_2)} \times 100$$

Where A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample only (water instead of DPPH).[18]

$ABTS^{\cdot+}$ Radical Scavenging Assay: The antioxidant capacity of the bacterocins ALC101, ALC102 and Nisin were determined using 2,2-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid) radical cation (ABTS) method. Based on an earlier report by[19] the $ABTS^{\cdot+}$ stock solution were prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate in 10 mM phosphate buffer (PB) (pH 7) and allowed to stand in dark for 16h at room temperature prior use. Before the analysis, stock solution was dissolved in 10 mM PB (pH 7) to obtain an absorbance of 0.7 ± 0.1 AU (ABTS radical working solution) at 734 nm. The ABTS working solution (100 µL) was mixed with 1 µL of each sample, incubated for 6 min, and the absorbance was measured at 734 nm. BHT served as the positive control. The percentage inhibition of $ABTS^{\cdot+}$ radical scavenging activity was calculated using the formula:[9]

$$\text{Scavenging activity (\%)} = \frac{Abs_{\text{blank}} - Abs_{\text{sample}}}{Abs_{\text{blank}}} \times 100$$

Where Abs blank is the absorbance of the blank solution and Abs sample is the absorbance of the sample.

Reducing power assay: Antioxidant effects of antilisterial bacteriocins ALC101, ALC102 and Nisin were determined by reducing power assay method. The assay was performed according to the method of.[20] The reaction was carried out in a solution containing 2.5 ml of sample (0.05-1 mg/ml), 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) and incubated at 50°C for 20 min. Then 2.5 ml of tricholoroacetic acid (10% w/v) were added and the mixture was centrifuged for 10 min at 5000 rpm.

The supernatant (5 ml) was mixed with 0.5 ml of freshly prepared $FeCl_3$ (0.1% w/v) and the absorbance was read at 700 nm. The greater absorbance indicates a higher reducing power. BHT and deionized water served as positive control and blank control respectively.[21]

Statistical analysis: The experiment on time course and kinetics of growth were done in triplicates. All the experiments were done in independent repetitions and the data were expressed as Mean±SD. All the graphs were plotted using GraphPad Prism version 8.0 and Microsoft Excel 2013.

RESULTS AND DISCUSSION

Isolation and identification of antilisterial bacteriocin producing strains: Toddy and milk were selected as a source for isolating bacteriocin producing bacteria. From more than 30 isolates screened, two strains were found to be bateriocin producing and were designated as GRD S_1 and GRD S_6. The two strains were maintained on MRS agar. Both the strains were found to be Gram positive and anaerobic after morphological identification. The strains were then subjected to 16S rRNA sequencing and phylogenetic analysis. Microbiological test in combination with 16S rRNA gene sequencing clearly depicted the taxonomic identification of the bacterial isolate. The phylogenetic analysis clearly identified the isolates to the species level. Based on the microbiological, physiological tests and 16S rRNA phylogeny, the strain GRD S_6 showed 99% similarity with *Paenibacillus polymyxa*. The sequence was submitted to GenBank, NCBI (https://www.ncbi.nlm.nih.gov) under the accession number MH113817. The strain GRD S_1 showed 99% similarity with *Enterococcus faecium* and the sequence was submitted to GenBank under accession number MH113816.

Production and Purification of Antilisterial Bacteriocins: Antilisterial bacteriocin ALC101 and ALC102 production was carried out using the TGE + Tween 80 (pH 6.8) medium, which supported maximum bacteriocin production. Bacteriocin production was discovered at early exponential phase and maximized at stationary phase. Tween 80 is a non-ionic detergent, which helps in releasing bacteriocin molecules from the producer cell wall into the medium. The bacteriocin in the medium decreased with prolonged incubation period, due to self-proteolytic enzymes synthesized in the late stationary phase. The optimum pH for bacteriocin production was at 6.6 ± 0.2. Extraction of the antilisterial bacteriocin from *P. polymyxa* were achieved using Amberlite XAD-16 adsorbent, a non-ionic macro-reticular resin that adsorbs and release ionic species through hydrophobic and polar interactions.[19] By applying Amberlite XAD-16 resin to clarified cell-free culture supernatant, the antilisterial bacteriocin were selectively adsorbed, while other water-soluble components remained in the liquid phase. The bacteriocin were eluted from XAD-16 by 75% isopropyl alcohol and the fraction was evaporated to half of the volume which retain the antimicrobial activity.

Antioxidant activity of bacteriocins ALC102, ALC102 and Nisin

DPPH radical scavenging activity: The model of DPPH radical is a widely used method to evaluate the free radical scavenging activities of antioxidants. In the DPPH assay, the antioxidants are able to reduce the stable DPPH to the non-radical form. The DPPH scavenging activities of antioxidants are attributed to their hydrogen donating abilities.[21] Both the bacteriocins ALC101, ALC102, Nisin and BHT (standard) showed good radical scavenging activity [Figure 3.1]. As shown in the Figure, the scavenging capacity increased significantly in a concentration dependent manner.

ABTS radical scavenging activity: ABTS radical scavenging activity is widely used to determine the antioxidant activity of chemical components. The scavenging abilities of BHT (standard), antilisterial bacteriocins ALC101, ALC102 and Nisin against ABTS radicals are shown in Figure 3.2. The scavenging activity of ALC101, ALC102 and Nisin exhibited a concentration-dependent manner and was much lower than that of BHT at same concentrations. The maximum scavenging percentage of ascorbic acid (82%) was obtained at 6.25µg/ml concentration. However, the bacteriocins exhibited more than 80.0% ABTS radical scavenging percentage when the concentration exceeded 6.5µg/ml, and reached the maximum of

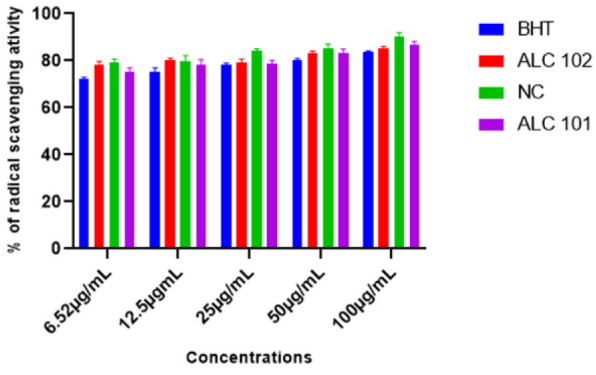

Figure 3.1: Percentage of DPPH radical scavenging activity of antilisterial bacteriocins ALC101, ALC102 and Nisin along with BHT (standard).

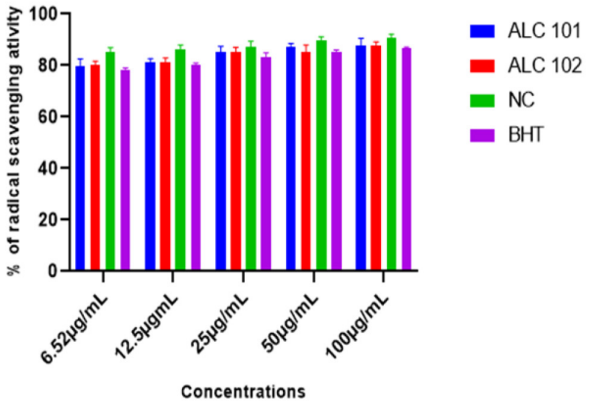

Figure 3.2: Percentage of ABTS radical scavenging activity of antilisterial bacteriocins ALC101, ALC102 and Nisin along with BHT (standard).

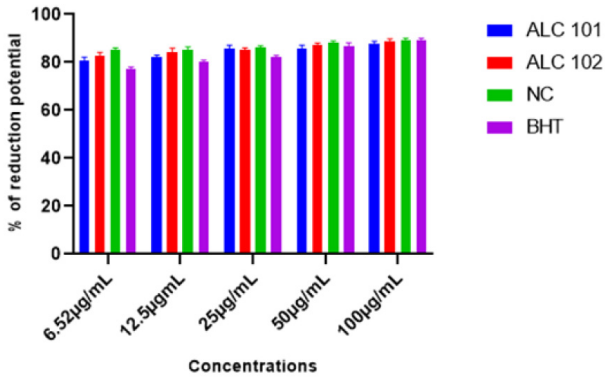

Figure 3.3: Percentage of reduction potential of antilisteral bacteriocins ALC101, ALC102 and Nisin along with BHT (standard).

85.5% at the concentration of 100µg/ml. These results indicated that bacteriocins had scavenging activity on 2, 2-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) radical.

Reducing power: The reducing power or reduction potential of a compound may serve as a significant indicator of its capacity of antioxidant activity. In this assay, antioxidants are able to reduce Fe^{3+}/Ferricyanide complex to its ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Prussian blue at 700 nm. The reduction potential bacteriocin purified from *Paenibacillus polymyxa* ALC101, from *Enterococcus faecium* GRD AA ALC102 and Nisin long with BHT (standard) increased with increase of concentrations (Figure 3.3).

DISCUSSION

Paenibacillus polymyxa (formerly known as *Bacillus polymyxa*) has attracted considerable interest because of its great biotechnological potential in different industrial processes and in sustainable agriculture. The genus *Paenibacillus* was created by[11] to accommodate the former 'group 3' of the genus *Bacillus*. It comprises over 30 species of facultative anaerobes and endospore forming, neutrophilic, periflagellated heterotrophic, low G+C Gram positive bacilli. The name this fact, in Latin 'Paene' means almost, and therefore the *Paenibacillus* is almost a *Bacillus*. Comparative 16S rRNA sequence analyses revealed that rRNA group 3 bacilli represents a phylogenetically distinct group and exhibit high intragroup sequence relatedness and is only remotely related to *Bacillus subtilis*; the type species of the genus *Bacillus*. The taxon contains various species such as *Bacillus alvei, Bacillus amylolytics, Bacillus azotofixans, Bacillus gordonae, Bacillus larvae, Bacillus macquariensis, Bacillus polymyxa, Bacillus pulvifaciens* and *Bacillus validus*. The bacteriocin like compound produced by *Paenibacillus polymyxa* is effective against a wide range of Gram positive and Gram negative bacterial species including foodborne pathogens. It posseses bacteriocin like properties such as proteinaceous nature (sensitive to proteases), sensitivity to organic solvents and chelators, heat stability (up to 10 min at 90°C) and instability in acidic and alkaline conditions. *Paenibacillus* species are known to produce at least two of the three class bacteriocins, being lantibiotics and pediocins. Lantibiotics, also known as Class I bacteriocins, contain the non-coded amino acid lanthionine and are typically active against Gram positive pathogenic bacteria.[22,23] *Enterococcus faecium* is a Gram positive, homofermentative, lactic acid bacteria that is a natural inhabitant of the gastro intestinal tract. Nevertheless, they are also found in fermented foods and are frequently isolated from starter cultures and cheese producers *Enterococcus faecium* bacteriocins have gained attentions in recent years as they could be isolated easily from several fermented foods and because many of them are active against foodborne pathogens such as *Listeria monocytogenes*.[24] *Enterococcus faecium* T136 was isolated from Spanish dry fermented sausages, which produced enterocin A and B. Enterocin P inhibited most of Gram positive foodborne pathogens like *Listeria monocytogens, Staphylococcus aureus, Clostridium perfringenes* and *Clostridium botulinum*. Most bacteriocins from enterococci are classified as Class II bacteriocins; small, heat stable, non-lantibiotics. These are further divided into three sub groups: (II a) listeria-active peptides, (II b) poration complexes consisting of two proteinaceous peptides for activity and (III c) thiol-activated peptides requiring reduces cysteine residues for activity.[25,13]

Recently, a number of bacteriocins produced by enterococci have been reported and information about them, for example, their inhibitory spectra and their pH and enzyme stabilities is available for their purification and the cloning of DNA related to bacteriocin production.[26] Numerous purification strategies have been reported for bacteriocin with varying degrees of success, which may be attributable to the extremely heterogeneous nature of bacteriocin. The purification methods commonly employed include ammonium sulphate precipitation, ion-exchange chromatography, hydrophobic chromatography coupled with cation-exchange column chromatography, reverse-phase high-performance liquid chromatography (RP-HPLC), Amberlite XAD- 2, Sephadex G-25 gel filtration, ultrafiltration and gel permeation chromatography and ethanol precipitation. Each and every purification method has its own downsides, which includes issues with low yield and purity, price, and the requirement for a skilled worker. Among these methods tested, the Amberlite XAD-16 method found to be the most appropriate in partially purifying the sample, especially since it is used in the previously reported purification scheme of the NKR-5-3 enterocins.[27]

In vitro experiments showed that the studied strains of microorganisms reduce the amount of liposomes in suspensions, exposed to induced oxidation, lipid peroxidation products; hence, the mechanism of antioxidant activity of bacteriocin producing micro-organisms is the inhibition of lipid peroxidation metabolites that make up cell membranes, which is probably determined by the binding of free radicals and the inhibition of the lipoxygenase enzyme.[28] Free radicals are formed uncontrollably in biological processes and may cause several functional disorders, such as mutagenesis, carcinogenesis, circulatory disturbances and ageing problems. These disorders are mostly due to the strike of oxidative agents on membrane lipids, intracellular proteins, enzymes carbohydrates and DNA in cells and tissues. Therefore, the solicitation of lactic acid bacteria (LAB) which can exhibit both probiotic and antioxidant activity can be contemplated as worthy plan of action to overcome this problem. In point of fact, many methods have been applied for the determination of antioxidant activity of fermented products.[29] DPPH is commonly used assay to evaluate antioxidant activity in biological systems. DPPH is a free radical, capable of accepting an electron or hydrogen radical to become a stable diamagnetic molecule. Commonly, DPPH radical scavenging activity by *Lactobacillus* spp. is a dose-dependent activity. In addition, the antioxidant activity of LAB may vary depending on the initial concentration of the micro-organisms.[30-32] Reactive oxygen species (ROS) are formed inside our body due to external and internal factors and are found to be responsible for many diseases when produced in excess. Antioxidant compounds have an important role in sequestering ROS from body that can decrease the risk of multiple chronic degenerative diseases, for instance cancer, Alzheimer, cataract and coronary heart disease. Now a days, substitutes of natural antioxidant compounds from bio-resources are attempted and developed to obtain compounds which is specific, better activity and safer.[33,34]

CONCLUSION

In our study, we isolated, identified the bacteriocins from *Paenibacillus polymyxa* strain and *Enterococcus faecium*. The bacteriocins ALC101 and ALC102 isolated from the strains were partially purified and characterized. The antioxidant efficiency was evaluated using DPPH, ABTS and reducing power methods. The bacteriocins ALC102 and ALC102 showed good activity of DPPH and ABTS radical scavenging activity, reducing power in a concentration dependent manner. From our study it is clear that the bacteriocins ALC101 and ALC102 are good candidates having antioxidant potential when compared to the activity of Nisin.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest

ABBREVIATIONS

LAB: Lactic acid bacteria; **GRAS:** Generally recognized as safe; **Rom:** reactive oxygen metabolites; **PCR:** Polymerase chain reaction; **MRS:** de Man Rogosa Sharpe; **DPPH:** 2, 2- diphenyl-1-picryl hydrazyl hydrate; **ABTS:** 2,2-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid); **BHT:** Butylated hydoxy toluene.

REFERENCES

1. Stiles ME, Holzapfel WH. Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol. 1997 Apr 29;36(1):1-29. doi: 10.1016/s0168-1605(96)01233-0, PMID 9168311.
2. Kaškonienė V, Stankevičius M, Bimbiraitė-Survilienė K, Naujokaitytė G, Šernienė L, Mulkytė K, *et al*. Current state of purification, isolation and analysis of bacteriocins produced by lactic acid bacteria. Appl Microbiol Biotechnol. 2017 Feb 1;101(4):1323-35. doi: 10.1007/s00253-017-8088-9, PMID 28070665.

3. Liu L, O'conner P, Cotter PD, Hill C, Ross RP. Controlling *Listeria monocytogenes* in cottage cheese through heterologous production of enterocin A by *Lactococcus lactis*. J Appl Microbiol. 2008 Apr;104(4):1059-66. doi: 10.1111/j.1365-2672.2007.03640.x, PMID 18005345.
4. Maragkoudakis PA, Mountzouris KC, Psyrras D, Cremonese S, Fischer J, Cantor MD, *et al*. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against *Listeria monocytogenes* and *Salmonella enteritidis*. Int J Food Microbiol. 2009 Apr 15;130(3):219-26. doi: 10.1016/j.ijfoodmicro.2009.01.027, PMID 19249112.
5. Prosekov AY, Dyshlyuk LS, Milentyeva IS, Sykhikh SA, Babich OO, Ivanova SA, *et al*. Antioxidant and antimicrobial activity of bacteriocin-producing strains of lactic acid bacteria isolated from the human gastrointestinal tract. Prog Nutr. 2017 Apr;19(1):67-80.
6. Samelis J, Sofos JN, Kendall PA, Smith GC. Fate of Escherichia coli O157:H7, *Salmonella typhimurium* DT 104, and Listeria monocytogenes in fresh meat decontamination fluids at 4 and 10 degrees C, [*Salmonella typhimurium*]. J Food Prot. 2001 Jul;64(7):950-7. doi: 10.4315/0362-028x-64.7.950, PMID 11456202.
7. Foulquié Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. Int J Food Microbiol. 2006 Jan 15;106(1):1-24. doi: 10.1016/j.ijfoodmicro.2005.06.026, PMID 16216368.
8. Serafini M, Del Rio D. Understanding the association between dietary antioxidants, redox status and disease: Is the total antioxidant capacity the right tool? Redox Rep. 2004 Jun 1;9(3):145-52. doi: 10.1179/135100004225004814, PMID 15327744.
9. Ji K, Jang NY, Kim YT. Isolation of lactic acid bacteria showing antioxidative and probiotic activities from kimchi and infant feces. J Microbiol Biotechnol. 2015;25(9):1568-77. doi: 10.4014/jmb.1501.01077, PMID 25951843.
10. Benbettaïeb N, Debeaufort F, Karbowiak T. Bioactive edible films for food applications: Mechanisms of antimicrobial and antioxidant activity. Crit Rev Food Sci Nutr. 2019 Nov 30;59(21):3431-55. doi: 10.1080/10408398.2018.1494132, PMID 29999405.
11. Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. Current knowledge and perspectives of Paenibacillus: A review. Microb Cell Fact. 2016 Dec;15(1):203. doi: 10.1186/s12934-016-0603-7, PMID 27905924.
12. Belgacem ZB, Abriouel H, Omar NB, Lucas R, Martínez-Canamero M, Gálvez A, *et al*. Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. Food Control. 2010 Apr 1;21(4):462-70. doi: 10.1016/j.foodcont.2009.07.007.
13. Losteinkit C, Uchiyama K, Ochi S, Takaoka T, Nagahisa K, Shioya S. Characterization of bacteriocin N15 produced by *Enterococcus faecium* N15 and cloning of the related genes. J Biosci Bioeng. 2001 Jan 1;91(4):390-5. doi: 10.1263/jbb.91.390, PMID 16233010.
14. Holzapfel WH, Geisen R, Schillinger U. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int J Food Microbiol. 1995 Jan 1;24(3):343-62. doi: 10.1016/0168-1605(94)00036-6, PMID 7710912.
15. Backialakshmi S, Meenakshi RN, Saranya A, Jebil MS, Krishna AR, Krishna JS, *et al*. Biopreservation of fresh orange juice using antilisterial Bacteriocins101 and antilisterial Bacteriocin103 purified from *Leuconostoc mesenteroides*. J Food Process Technol. 2015;6(8).
16. Mahdavi M, Jalali M, Kermanshahi RK. The effect of nisin on biofilm forming foodborne bacteria using microtiter plate method. Res Pharm Sci. 2009 May 10;2(2):113-8.
17. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr;181(4617):1199-200. doi: 10.1038/1811199a0.
18. Shen Q, Shang N, Li P. *In vitro* and *in vivo* antioxidant activity of *Bifidobacterium animalis* 01 isolated from centenarians. Curr Microbiol. 2011 Apr;62(4):1097-103. doi: 10.1007/s00284-010-9827-7, PMID 21132298.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999 May 1;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3, PMID 10381194.
20. Sharpe MD, Mustard RA, Finley RR, Rutledge FS, Sibbald WJ. Failure of therapy with 2, 3-dihydroxybenzoic acid to modify the course of sepsis-induced lung injury. J Appl Physiol (1985). 1990 Nov 1;69(5):1893-902. doi: 10.1152/jappl.1990.69.5.1893, PMID 2272983.
21. Prabhaa S. Vb P. Probiotic efficiency of lactic acid bacteria from milk treated against gastro intestinal pathogens. Int J Cur Res Eng Sci Tech. 2018;1(1):17-23.
22. Yuan H, Zhang W, Li X, Lü X, Li N, Gao X, *et al*. Preparation and *in vitro* antioxidant activity of κ-carrageenan oligosaccharides and their over sulfated, acetylated, and phosphorylated derivatives. Carbohydr Res. 2005 Mar 21;340(4):685-92. doi: 10.1016/j.carres.2004.12.026, PMID 15721341.
23. He Z, Kisla D, Zhang L, Yuan C, Green-Church KB, Yousef AE. Isolation and identification of a *Paenibacillus polymyxa* strain that coproduces a novel lantibiotic and polymyxin. Appl Environ Microbiol. 2007 Jan 1;73(1):168-78. doi: 10.1128/AEM.02023-06, PMID 17071789.
24. Strompfová V, Marciňáková M, Simonová M, Gancarčíková S, Jonecová Z, Sciranková L, *et al*. *Enterococcus faecium* EK13—an enterocin A-producing strain with probiotic character and its effect in piglets. Anaerobe. 2006 Oct 1;12(5-6):242-8. doi: 10.1016/j.anaerobe.2006.09.003, PMID 17071114.
25. Park SH, Itoh K, Fujisawa T. Characteristics and identification of enterocins produced by *Enterococcus faecium* JCM 5804T. J Appl Microbiol. 2003;95(2):294-300. doi: 10.1046/j.1365-2672.2003.01975.x, PMID 12859761.
26. Cosentino S, Fadda ME, Deplano M, Melis R, Pomata R, Pisano MB. Antilisterial activity of nisin-like bacteriocin-producing *Lactococcus lactis* subsp. lactis isolated from traditional Sardinian dairy products. J Biomed Biotechnol. 2012 Jan 1;2012:376428. doi: 10.1155/2012/376428, PMID 22536018.
27. Mills S, Stanton C, Hill C, Ross RP. New developments and applications of bacteriocins and peptides in foods. Annu Rev Food Sci Technol. 2011 Apr 10;2:299-329. doi: 10.1146/annurev-food-022510-133721, PMID 22129385.
28. Cao CC, Feng MQ, Sun J, Xu XL, Zhou GH. Screening of lactic acid bacteria with high protease activity from fermented sausages and antioxidant activity assessment of its fermented sausages. CyTA J Food. 2019;17(1):347-54. doi: 10.1080/19476337.2019.1583687.
29. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol. 2011 Aug;48(4):412-22. doi: 10.1007/s13197-011-0251-1, PMID 23572765.
30. Madhava Naidu MM, Sulochanamma G, Sampathu SR, Srinivas P. Studies on extraction and antioxidant potential of green coffee. Food Chem. 2008;107(1):377-84. doi: 10.1016/j.foodchem.2007.08.056.
31. Soares JR, Dinis TC, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis. Free Radic Res. 1997 Jan 1;26(5):469-78. doi: 10.3109/10715769709084484, PMID 9179593.
32. Wang AN, Yi XW, Yu HF, Dong B, Qiao SY. Free radical scavenging activity of *Lactobacillus fermentum in vitro* and its anti-oxidative effect on growing–finishing pigs. J Appl Microbiol. 2009 Oct;107(4):1140-8. doi: 10.1111/j.1365-2672.2009.04294.x, PMID 19486423.
33. Del Río LA. ROS and RNS in plant physiology: An overview. J Exp Bot. 2015 May 1;66(10):2827-37. doi: 10.1093/jxb/erv099, PMID 25873662.
34. Alwendawi SA. *In vitro* assessment the potential antioxidant and antitumor activities of *Bifidobacterium* derived bacteriocins. Int J Drug Deliv Technol. 2019 Jan 21;9(2):207-16. doi: 10.25258/ijddt.9.2.15.

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