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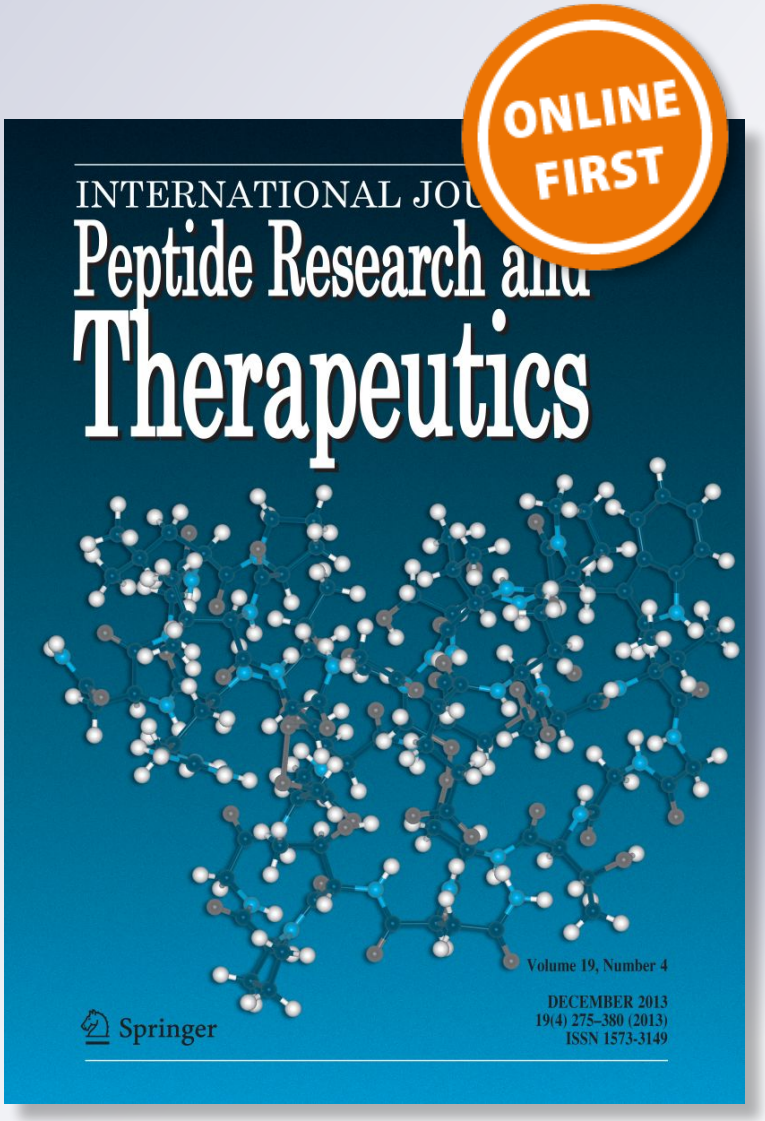
International Journal of Peptide Research and Therapeutics
formerly known as "Letters in Peptide Science"

ISSN 1573-3149

Int J Pept Res Ther
DOI 10.1007/s10989-019-09904-5



Springer

International Journal of Peptide Research and Therapeutics
https://doi.org/10.1007/s10989-019-09904-5

Production of a Recombinant Peptide (Lasioglossin LL III) and Assessment of Antibacterial and Antioxidant Activity

Abbas Tanhaeian[1] · Mohammad B. Habibi Najafi[2] · Paria Rahnama[2] · Marjan Azghandi[3]

Accepted: 26 July 2019

Abstract
The use of antimicrobial peptides (AMPs) as biopreservative to replace chemical preservatives has become of interest among consumers. Current approaches of production and purification of AMPs are time-consuming and costly and may kill the producing host cells. In this study a recombinant peptide was patterned from Lasioglossin LL III which is found naturally in the venom of eusocial bee, this recombinant peptide was produced to inhibit food microbial degradation with the least possible costs. The synthesis of this peptide in the periplasmic space of *Escherichia coli*, has facilitated the production and purification of this additive. The pET based expression system for production of a recombinant form of Lasioglossin LL III using *E. coli* as a host was developed. The antibacterial and antioxidant activities of recombinant peptide were determined. A C-terminal poly histidine tag was used to facilitate purification by standard methods using Ni^{2+} affinity chromatography. The average yield of the recombinant peptide was 0.7 g/L. The findings showed great antimicrobial function of the peptide. The antioxidant activity of the peptide was lower than Butylated hydroxytoluene (BHT) synthesized antioxidant as a common antioxidant but the antioxidant potential of the peptide along with the antimicrobial activity against foodborne pathogens have shown promising benefits of the peptide. These results demonstrated that pET expression system is appropriate for the rapid and simple isolation of recombinant lasioglossin LL III from *E. coli* and the recombinant peptide could be used as a natural preservative source. However, further studies need to be carried out to check the effect of this peptide on more microorganisms.

Keywords Lasioglossin LL III · Antimicrobial peptide · Foodborne pathogen · pET system · Antioxidant activity

Introduction

One of the major concerns of consumers, food industries, and food safety organizations are foodborne diseases. Researchers have identified more than 250 foodborne diseases. Most of them are infections, caused by a variety of bacteria, viruses, and parasites. Therefore, the use of food preservatives is inevitable to protect food against microbial degradation and maintain its quality. However, the side effects of most chemical preservatives on the health of consumers have become a health challenge. Finding natural antimicrobial agents that inhibit bacterial and fungal growth for better quality and shelf-life has been of considerable interest in recent years. Unlike synthetic food preservatives, natural agents as alternative food preservatives have had a growing demand. The main categories of artificial preservatives are antimicrobials, antioxidants, and chelating agents. Antimicrobials preservatives include benzoates, sorbates, propionates, nitrates and nitrites. They can cause serious

✉ Mohammad B. Habibi Najafi
habibi@um.ac.ir

Abbas Tanhaeian
ab_tanhaeian@yahoo.com

Paria Rahnama
Rahnama92p@gmail.com

Marjan Azghandi
Azghandi.marjan@gmail.com

[1] Department of Plant Protection, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

[2] Department of Food Science & Technology, Ferdowsi University of Mashhad, Mashhad, Iran

[3] Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran

Published online: 05 August 2019

health hazards such as hyperactivity, allergy and cancer (del Olmo et al. 2017; Tajkarimi et al. 2010). Common antioxidants used include sulfites, synthetic vitamin E, C, butylated hydroxyanisole (BHA), and (BHT) (Tajkarimi et al. 2010). In addition to beneficial effects of these compounds, there are some complications in using them like liver cancer and allergic reactions (Pandey et al. 2014). Since we are aware of the adverse effects of chemical preservatives and common antioxidants, it is essential to find ways to reduce the use of such compounds for health promotion. One of the most important solutions is the use of natural compounds or the production of a synthetic or recombinant peptide with a natural pattern (Hassan et al. 2012). Main natural compounds to protect food products are essential oils derived from plants, enzymes obtained from animal sources, organic acids, naturally occurring polymers and peptide from microbial sources like Nisin from *Lactococcus lactis* (Gutierrez et al. 2008). In this study, achieving the antimicrobial peptide with an appropriate antimicrobial potential in a cost-effective way is considered to continue this research pathway. This peptide could be considered as a candidate for food preservation in the future. Antimicrobial peptides (AMPs) are a diverse class of naturally occurring molecules that are produced as the first line of defense by all multicellular organisms (Toke 2005; Parisien et al. 2008). This kind of biomolecule may have an amphipathic or cationic structure and may manifest a wide spectrum of action against Gram-positive and negative bacteria, fungi, viruses and protozoa (Zhao et al. 2013). In addition to the microbial activity against a vast spectrum of fungal, viral and bacterial species, the AMP has been mentioned as potential antitumor and anti-parasitic substances, as well. The interest of the food industry in the application of AMPs led to manufacture healthy and natural products through inhibition of the growth of certain microorganisms as well as inhibition of contamination risk of food. The major benefit of using antimicrobial peptides is that they can preserve food without changing its quality and they are not harmful (Wang et al. 2016). The ability of AMPs to kill bacteria usually depends upon their ability to interact with bacterial membranes or cell walls. Generally, AMPs exhibit a net positive charge and a high ratio of hydrophobic amino acids, which allow them to bind selectively to negatively-charged bacterial membranes. Binding of AMPs to the bacterial membrane leads to non-enzymatic disruption. Selectivity for specific species is due to differences in the membrane composition of different microbes and cell types (Joo et al. 2016). The natural origin of some antimicrobial peptides is insects. Lasioglossin LL-III peptide is an antimicrobial peptide found in the venom of wild bees (Čeřovský et al. 2009). Isolation of peptide from natural sources is a labor-intensive and time-consuming process and therefore does not provide an efficient method to obtain peptides in large amounts. So, by simulating the natural form and amino acid structure of these peptides, various synthetic and recombinant types of them have been created and used. Among different methods of antimicrobial peptides synthesis, recombination technique is preferred in comparison to chemical and enzymatic methods (Wang et al. 2011). Although chemical synthesis is very efficient, it is a complex and costly process. It is also not an ideal platform for large-scale peptide production. Fortunately, recombinant DNA technology provides an economical means for protein manufacture. In this study, to produce the peptide, *E. coli* strain BL21 was used as the host organism. Designing and cloning and the expression of Lasioglossin LL-III in the form of accumulation in periplasmic space were performed. The function of antimicrobial peptide against foodborne pathogens was then examined. Also, the antioxidant activity of the recombinant peptide was measured.

Materials and Methods

Bacterial Strains, Vectors and Other Regents

E. coli DH5α (f-gyr A96 Nalr, recA1 relA1 Thi-1 hsdR17 r − k m + k, Stratagene, USA) and BL21 (DE3) pLysS (f-ompthsdB, rB- mB-, dcm gal, DE3, pLYsScmr, Stratagene, USA) strains were used as the host amplifying and the expression strain respectively. Moreover, pET22b(+) vector (5493 bp, Novagene, USA) and PGH vector were used as expression vector and cloning vector. The T7 promoter sequence and the T7 terminator sequence consist of these oligonucleotides 5′-TAATACGACTCACTATAGGG-3′ and 5′-GCTAGTTATTGCTCAGCGG-3′, respectively and were obtained from Macrogen Co. (Korea). All chemicals, commercial kits, and restriction enzymes were purchased from Sigma Chemical co. (USA), Roche co. (Germany) and Thermo Fisher Scientific co. (USA), respectively.

Gene Synthesis and Vector Construction

The amino acid sequence of the peptide, lasioglossin LL III, was selected from *Antimicrobial Peptide Database with APD ID: AP01467.* The peptide was codon optimized for proper expression in *E.coli* and chemically synthesized by Genscript® (USA). A poly-histidine tag consists of six histidine residues was added to C-terminal for peptide purification. PGH vector was digested by *Nco*I and *Sal*I restriction enzymes. The synthesized gene was inserted into the plasmid and then transformed into DH5α-competent *E. coli*. The transformed colonies containing the recombinant plasmid were cultured and were used for plasmid extraction. After plasmid extraction from the bacterium and PCR amplification, the products were purified by gel extraction kit then double digested by *Nco*I and *Sal*I restriction enzymes. The

purified product was inserted into a pET22b(+) vector (Tanhaiean et al. 2018b).

Cloning of Lasioglossin III Gene into pET22b(+), Peptide Expression and Purification

After double enzyme digestion, the ligation of the coding sequence of Lasioglossin LLIII in a vector of expression pET22b(+) was performed using *T4* enzyme with a 3: 1 ratio vector to fragment. pET22b(+) recombinant expression vector was confirmed by restriction mapping and sequencing. The recombinant expression vector was transformed into the expression host *E. coli* BL21 (DE3) pLysS and was then cultured on an agar plate containing Ampicillin (100 µg/mL). The positive colonies were inoculated into 2 mL Luria–Bertani broth (LB) medium containing Ampicillin (100 µg/mL) in shaker incubator at 37 °C with 200 rpm overnight, according to the standard protocol (Sambrook et al. 1989; Richard et al. 2004). On the next day, 500 µL of the cultured materials was inoculated in 50 mL LB containing Ampicillin (100 µg/mL) and then incubated at 37 °C with 200 rpm until OD 600 nm reached to 1.5. The expression of lasioglossin III was induced with Isopropyl-β-D-thiogalactopyranoside (IPTG) and 1 mM of IPTG was added. The culture was grown for 6 h post induction at 37 °C 200 rpm (Studier 1990). Periplasmic protein was harvested at different intervals (2, 4 and 6 h) after IPTG induction (Tanhaeian et al. 2018a) and was loaded on 17.5% SDS-PAGE. The Ni–NTA agarose column was used to purify the expressed protein according to the manufacturer's instruction (Thermo, USA). The quality of the purified recombinant peptide was analyzed on a 17.5% SDS-PAGE gel electrophoresis and the total protein was quantified by Bradford method.

Bacterial Strains for Antimicrobial Test

Bacterial species which were used in this study were considered food pathogens namely *Staphylococcus aureus* ATCC25923, *Salmonella typhimurium* ATCC14028, *Enterococcus faecalis* ATCC29212, *Listeria monocytogenes* ATCC19111 and *Escherichia coli* ATCC25922. All the strains were grown in *Muller-Hinton broth* at 37 °C.

Bacterial Susceptibility Assay

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) assays were measured in broth microdilution. Bacterial cells were grown overnight in *Muller-Hinton broth* and diluted to 10^5 CFU/mL in culture medium. A volume of 200 µL of bacterial suspension was inoculated in micro-titer plates. Each plate also included a positive growth control and a negative control. 100 µL peptide was added to the first well and after being completely mixed, 100 µL of it was transferred to the next well and this serial dilution method was repeated for the remaining wells. The plates were then incubated at 37 °C under aerobic conditions for 18 h. MIC was defined as the lowest peptide concentration present in the clear well at OD 620 nm by ELISA reader. For MBC, 50 µL of the well contents was spread on agar and was grown at 37 °C for 24 h. MBC was defined as the lowest peptide concentration resulting in no bacterial growth in the media. Each assay was performed at least three times for all bacteria (Melvin et al. 2009; Wiegand et al. 2008; Zhang et al. 2018).

Cytotoxicity Assay

Cytotoxicity of Lasioglossin LL III peptide in normal cells was checked using *human embryonic kidney cell* line cells (HEK293-T ATCC® CRL-1573™). Approximately, 3×10^3 cells in 100 µL of DMEM [10% v/v FBS] were seeded per well in 96 well plates. The cells were incubated overnight at 37 °C in a humidified incubator of 5% CO_2. The cells were then treated with the peptide. The cells were incubated for 72 h at 37 °C under the same conditions. Furthermore, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay was performed (van de Loosdrecht et al. 1991) to check the inhibitory effect of the peptide in normal cells. The absorbance of the colored solution was determined with an ELISA microplate reader at a wavelength of 570 nm (Pascariu et al. 2011; Uhlig et al. 2014).

Antioxidant Activity

The antioxidant activity of recombinant peptide was measured by reducing power assay and DPPH radical scavenging activity. BHT was used as a standard antioxidant compound.

Determination of Antioxidant Property by DPPH

The ability of the recombinant peptide to scavenge DPPH free radical was assessed. 2 mL of the peptide (5 mg/mL) was mixed with 1 mL of 0.2 mmol/L DPPH solution and was allowed to react in the dark for 30 min. The absorbance was measured at 517 nm against blank. The ability of the peptide to reduce DPPH was quantified using the following equation (Bai et al. 2017).

$$\text{DPPH radical scavenging ability } \% = \left[1 - A_s/A_b\right] \times 100,$$

where, A_s is the absorbance of the sample, and A_b is the absorbance of blank.

Reducing Power Assay

The reaction was carried out in a mixture containing 1 mL of the sample (0.7 mg/mL), 1 mL of double-distilled water and

1 mL of K3Fe (CN)6 (1%, w/v) by incubating at 50 °C for 20 min. Trichloroacetic acid (2.5 mL, 10%, w/v) was added to the reaction mixture and was then centrifuged at 5000×*g* for 5 min. The upper layer (2 mL) was mixed with 2 mL of double-distilled water and 1 mL of fresh $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power (Li et al. 2013).

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) with post hoc Tukey's multiple comparisons test using statistical software, GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). In all cases, a value of $p < 0.05$ was considered significant.

Results

Vector Construction and Expression of Recombinant Lasioglossin LL III

We confirmed the introduction of pET-22(+) recombinant plasmid into the strain of *E. coli* by performing colony PCR process. The PCR results confirmed the presence of DNA fragment into pET-22(+) vector. The length of DNA band was 426 bp (Fig. 1a, b). To confirm the accuracy of subcloning, the structure of pET-22(+) recombinant plasmid containing the gene was digested by *sal*1 and *Nco*1 enzymes. The result of double enzyme digestion showed the fragment had 126 bp length (Fig. 1c). The sequencing results also showed that Lasioglossin LL III sequence was correctly subcloned into pET22b(+) vector without changing the frame.

The coding sequence of Lasioglossin LL III was successfully cloned into the pET expression vector. The results of the recombinant vector construction are illustrated in (Fig. 2). Sequencing without any mutation showed validation of vector construction. The results of purification of the recombinant peptide on SDS-PAGE gel revealed that purification was done desirably and no protein contamination in the purified expression peptide was detected. A single band with an approximate molecular mass of 2.58 kDa was observed after nickel affinity column purification and SDS-PAGE analysis (Fig. 3). Ni–NTA chromatography system is a rapid and convenient purification technique. Proteins fused with His-tag at either end (N- or C-terminus) in native or denatured state bind tightly with high affinity on immobilized nickel ions (Malik et al. 2016). Protein concentration was measured using Bradford method and based on the standard curve. The results showed that the mean concentration of peptide was about 0.7 g/L.

Antimicrobial Activity

The antimicrobial activity of Lasioglossin-LL III was tested against Gram negative bacteria (*E. coli* and *S. typhi-murium*) and Gram positive bacteria (*S. aureus*, *E. faecalis* and *L. monocytogenes*). The results which are determined via MIC and MBC assays are shown in (Table 1). MIC and MBC of the peptide against the tested bacteria were 3.85–8.625 µg/L and 7.703–15.406 µg/L, respectively. Lasioglossin-LL III

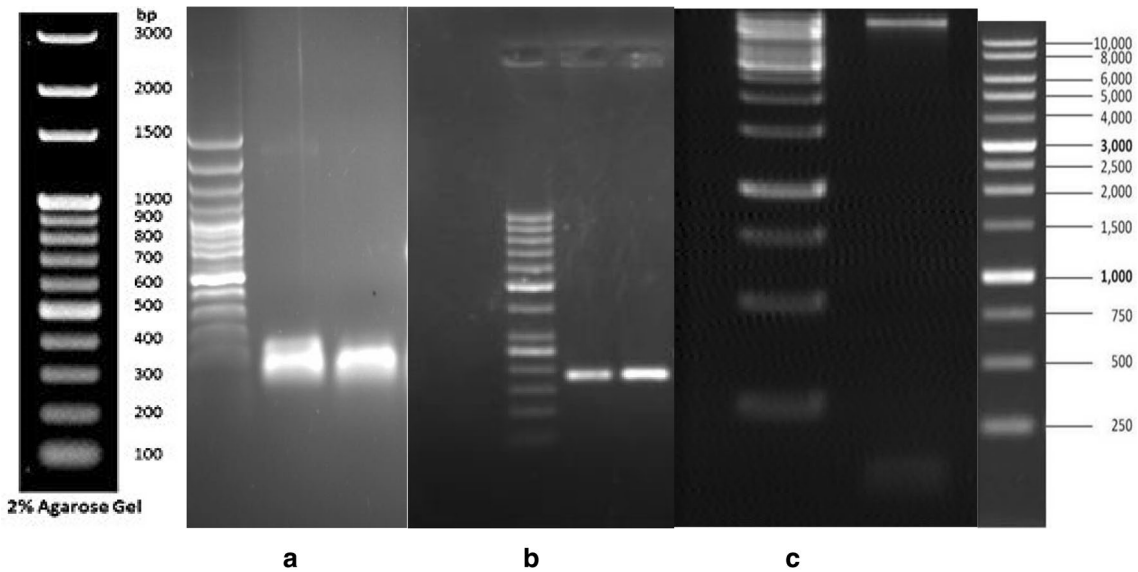


Fig. 1 **a** Result of colony PCR with the specific primer of Lasioglossin LL III sequence (126 bp) in duplicate, **b** result of colony PCR with T7 primer (vector primer) in duplicate, **c** result of double enzyme digestion

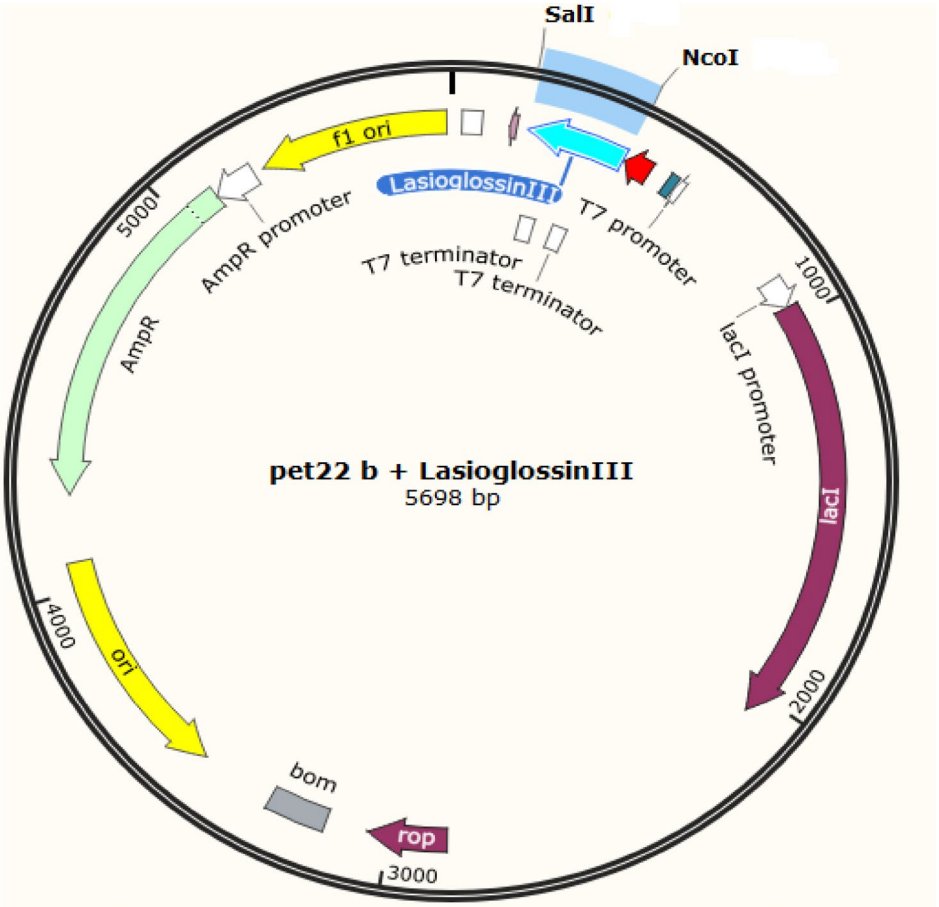


Fig. 2 The schematic representation of cloning of Lasioglossin LL III in pET22b(+) expression vector

showed the lowest MIC and MBC for *Staphylococcus aureus* (3.851 µg/L and 7.703 µg/L), respectively. However, the difference between the mean values of the MIC against *S. aureus* and *S. typhimurium* was not statistically significant ($p > 0.05$).

Measurement of Cytotoxicity

The evaluation of Lasioglossin LL III toxicity toward HEK293 showed that the initial concentration of non-cytotoxicity was 1652 µg/mL. At this concentration, that was the highest possible level of the peptide, no significant toxicity was observed ($p > 0.05$). Cytotoxic concentration of Lasioglossin was 429, 383, 292, 214.5 and 191.5 fold higher than MIC dose and 214.5, 191.5, 146, 107 and 191.5 fold higher than MBC dose of it against *S. aureus*, *S. typhimurium*, *E. faecalis*, *L. monocytogenes* and *E. coli*, respectively. There were significant differences between them ($p < 0.05$).

Determination of Antioxidant Activity

Antioxidant activities have been performed with different reaction mechanisms including DPPH free radical scavenging activity and reducing power efficiency. DPPH is a stable free radical and accepts an electron or a hydrogen radical to become a stable diamagnetic molecule (Rao et al. 2011). In the reducing power assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The presence of reducers reduces Fe^{3+}/ferricyanide complex to ferrous form. Therefore, measuring the formation of Perl's Prussian blue at 700 nm can monitor Fe^{2+} concentration (Vhangani and Van Wyk 2013). The results are illustrated in (Table 2). The mean scavenging ability was found to be 37.18% for DPPH which was lower than scavenging activity of BHT. This difference was statically significant ($p < 0.05$). The inhibitory activity of peptide may be due to its electron transfer ability in order to react with free radicals and convert them to stable compounds, terminate free radical chain reactions. Availability of tyrosine at C-terminal is important to radical scavenging by some peptides (Peng et al. 2010). The reducing power of Lasioglossin LL III showed the mean value of 0.33 which is significantly lower than synthetic antioxidant ($p < 0.05$). Peptide reducing activity can be due to the presence of hydrogen ions in the peptide sequence (Peng et al. 2010).

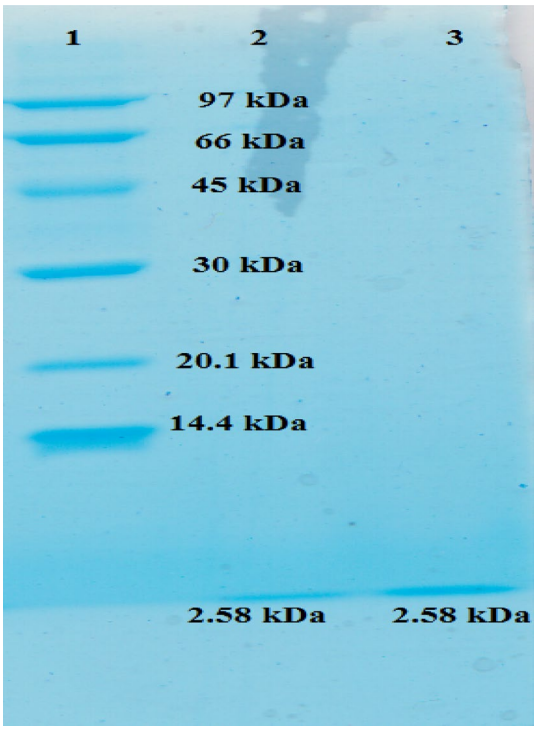


Fig. 3 Purified peptide was extracted by Ni–NTA affinity chromatography with ~2.58 kDa molecular mass. Lane 1: protein size marker, Lane 2 and 3: purified peptide

Table 1 minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of lasioglossin LL III

Bacterial strain	MBC (µg/mL)	MIC (µg/mL)
Gram negative bacteria		
E. coli	8.625 ± 0.09^{c}	8.625 ± 0.41^{a}
S. typhi-murium	8.625 ± 0.15^{c}	4.3125 ± 0.225^{d}
Gram positive bacteria		
S. aureus	7.703 ± 0.57^{d}	3.851 ± 0.31^{d}
E. faecalis	11.31 ± 0.22^{b}	5.655 ± 0.24^{c}
L. monocytogenes	15.406 ± 0.33^{a}	7.703 ± 0.43^{b}

The different letters in each column shows significant differences

Table 2 Comparison of antioxidative activities of Lasioglossin LL III with BHT

Sample	DPPH scavenging effect (%)	Reducing power
Lasioglossin LL III	37.18 ± 6.8^{b}	0.33 ± 0.01^{b}
BHT	82.37 ± 2.26^{a}	0.587 ± 0.04^{a}

Different letters within a column show significant differences

Discussion

Nowadays the harmful effects of chemical preservatives such as carcinogenic effects and toxic residues on human health have been proven. Because of the excess use of chemical preservatives, the bacteria have developed resistance; therefore, there is a pressing need to find the new natural food preservatives. To prevent food-spoilage, food products can be preserved by microbes and their antimicrobial products as bio preservatives, which improve the shelf-life of food and enhance food safety (Okuda et al. 2009; Wang et al. 2016). The development of expression systems of the vectors in *E. coli* has made it an appropriate host for producing large quantities of recombinant proteins. The pET expression system is one of the most widely used systems for recombinant protein production in *E. coli*; pET vectors contain N-terminal pelB secretion signal, which directs synthesized polypeptides to *E. coli* periplasm (Yoon et al. 2010). Therefore, this work aimed at increasing the expression of the peptide, and the gene was inserted into pET22b(+) expression vector and since this plasmid contains a pelB signal peptide, it facilitates the release of target proteins to periplasmic space, which provides a very favorable environment for the formation of di-sulfide bonds and the correct protein folding (Sockolosky et al. 2013; Tanhaiean et al. 2018b). The results indicated a successful expression of Lasioglossin LL III peptide in pET22b(+) vector.

The secretion of recombinant protein into periplasm has several advantages over its cytoplasmic production including simplified downstream processing, enhanced biological activity, conducting in vivo activity assays due to greater access of the targeted protein to the substrate, higher product stability and solubility, N-terminal authenticity of the expressed protein, simpler and cost-effective purification due to a lower protein content compared to cytoplasm, and finally providing oxidative environment required for correct protein folding (Murby et al. 1996; Choi et al. 2004; Mergulhao et al. 2005).

As mentioned before, one of the major problems in food industries is the contamination of food by pathogens, such as *Salmonella* spp., *Shigella* spp., *Micrococcus* spp., *Enterococcus faecalis*, *Bacillus licheniformis*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Vibrio parahemolyticus*, *Escherchia coli* 0157:H7, and *Clostridium botulinum*, etc. Food preservation by the use of chemical reagents is one of the widely used and traditional methods; however, using chemical reagents as food additives and preservatives is very sensitive due to its health concerns (Mursalat et al. 2013). For example, sodium nitrite widely used in meat and fish products and the certain kind of cheeses as a

preservative is a chemical compound that is a precursor of N-nitroso carcinogenic compounds (Maekawa et al. 1982). According to Toaima et al. (2015), MIC and MBC values of sodium nitrite in different pH values against *S. aureus* and *E. coli* were (pH 7: 2500 and 3500; 750 and 750 ppm), (pH 6: 750 and 1500; 500 and 500 ppm) and (pH 5.5: 500 and 500; 200 and 350 ppm) respectively, while these values were much higher than those of the lasioglossin recombinant peptide in this study (Toaima et al. 2015). Many researchers pointed out that antimicrobial peptides demonstrated activity against several food-borne pathogens (Hintz et al. 2015; Kraszewska et al. 2016; Wang et al. 2016). The mechanism of action of AMPs has not yet been fully explained. But there is intracellular and extracellular intervention. The interaction of peptides with phospholipid membranes of microorganisms is mainly due to their biochemical properties. Based on the amino acid sequence, innate features, such as amphipathic charge, structure and hydrophobicity, they have been determined through a local thinning layer by the expansion of the outer membrane (Scocchi et al. 2016).

The toxicity of antimicrobial peptides against eukaryotic cells has limited their application as antibacterial agents in food and drug. Therefore, antimicrobial peptides, in addition to strong antimicrobial properties, should be assessed for non-cytotoxicity in food and drug application (Lee et al. 2013). So in this study antimicrobial activity and cytotoxicity of the recombinant peptide was evaluated.

Cerovský et al. (2009) have reported that non recombinant lasioglossins (I, II and III) have strong antimicrobial activity against both gram-positive and -negative bacteria in concentration from 0.7 to 18.7 mM without detectable hemolytic activity (Čeřovský et al. 2009). Mishra et al. (2013) discussed the antimicrobial effect of Lasioglossin LL III, that was chemically synthesized against *E. coli* ATCC8739 and *S. aureus* ATCC6538, was higher than the recombinant peptide (Mishra et al. 2013). The result obtained by Ebbensgaard et al. (2015) revealed an increased antimicrobial effect of cap11 and cap18 peptides against a different strain of *E. coli* compared to gram positive bacteria. These results were in contrast with our results in which the inhibitory effect of Lasioglossin LL III against *E. coli* was lower than gram positive bacteria. *Antibacterial activity of* Melittin peptide, that was isolated from the venom of a bee, was twofold lower than Lasioglossin LL III against *E. coli* (Ebbensgaard et al. 2015). Ilic et al. (2013) exhibited a bactericidal effect of ADP3 peptide against *E. coli* which was similar to Lasioglossin LL III. While, the inhibitory effect of this peptide against *S. aureus* and ADP2 peptide against *S. typhi-murium* was lower than Lasioglossin LL III (Ilić et al. 2013). Findings of Cerovský et al. (2009) was in accordance with our results, in which they demonstrated that Lasioglossin LL III had toxic effect against PC-12 cancer cell line and this peptide showed high potency against leukemia cells. However, this peptide was two to three-times less potent against cells derived from solid tumors (PC12, HeLa S3 and SW480) (Čeřovský et al. 2009). In our study, the lowest toxicity concentration of recombinant peptide was 1652 µg/mL which was far higher than the results obtained by Cerovsky (19 µM). One interesting finding that shows the importance of our study was obtained by Toaima et al. (2015). They demonstrated that nisin, a natural antimicrobial peptide that exhibits antibacterial activity against a wide range of gram-positive and gram-negative bacteria, and diversely used as a preservative in the food industry, has higher MIC and MBC values than those of recombinant lasioglossin peptide (de Arauz et al. 2009; Toaima et al. 2015). The results of Li et al. (2018) showed MIC of nisin against *E. coli* LMG15862 was about 40 µg/mL that was approximately five times higher than our results about lasioglossin peptide (Li et al. 2018).

In this research, the recombinant peptide containing Histidine showed antioxidant activity. Saidi et al. (2014) reported that the presence of Tyrosine, Methionine, Histidine and Lysine contributes to the antioxidant activity of peptides (Saidi et al. 2014). Particularly, Histidine and peptides containing Histidine exhibit strong radical scavenging activity because of the presence of imidazole ring (Wiriyaphan et al. 2015).

The antioxidative properties of peptides were highly influenced by molecular mass and molecular structure properties. Most of the reported peptides exhibiting antioxidative activity were those with low molecular weights (Rajapakse et al. 2005; Pihlanto 2006); this complied our results.

Conclusion

Most food products require protection against microbial spoilage during storage. Since there is a growing demand for food that is free of synthetic chemicals as preservatives; it is necessary to examine and identify alternative and safe approaches for controlling foodborne pathogens. In this context, antimicrobial peptides as the natural compounds are gaining a great interest from research and industry, due to their potential to provide quality and safety benefits, with a reduced impact on human health. In this study, we have reported a simple system for production of a patterned recombinant peptide from the natural peptide in the venom of eusocial bee directed to *E. coli* periplasm via a pET-based expression vector. The results of this study showed that this peptide has a considerable antibacterial activity toward some foodborne pathogens with low cytotoxicity against HEK293 normal cell line which can be used as a preservative agent in food products, and they also have potential to be further modified into therapeutics in the treatment of certain infections. The production of a recombinant peptide

in pET22b(+) expression system can result in the production of a high-quality recombinant peptide in a cost-effective manner.

Acknowledgements We hereby acknowledge Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad for providing us with all facilities and financial supports required to perform the practical work.

Funding This study was funded by the deputy of Research and Technology, Ferdowsi University of Mashhad, Iran (Grant No: 2/45804).

Compliance with Ethical Standards

Conflict of interest The authors declare there is no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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