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Research Article

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Expression of Pediocin PA-1 in *Escherichia coli*

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ABSTRACT

Background: Pediocinis an antimicrobial peptide, which strongly *inhibits Listeria monocytogenes*. Previous reports showed that pediocin remarkable and promising potential in the food preservation industry and pharmaceutical. Although pediocin had been expressed in some *E. coli* strains, the production of pediocin still need more study.
Methods: In this study, we present our results on expression recombinant pediocin as solble protein in *E. coli*. N-terminus of the pediocin gene was incorporated into the *NusA* tag, coordinated with 6xHistidine.
Results: Active recombinant pediocin was successfully obtained and showed its strong antibacterial activity against *L. monocytogenes* ATCC.
Conclusion: The result will be opened a new door to produce recombinant pediocin and apply it.

Key-words: Antibacterial activity, *E. coli* expression, Pediocin, *Listeria monocytogene*, NusA tag

INTRODUCTION

Pediocin PA-1 is a class IIa bacteriocin which produced by *Pediococcus acidilactici* PAC1.0. [1,2]. Pediocin PA-1 has a wide antibacterial spectrum against Gram-positive bacterial sp. such as *Lactobacilli, Leuconostoc, Brochothrix thermosphacta, Probionibacteria, Bacilli, Enterococci, Staphylococci, L. clostridia, L. monocytogenes,* and *L. innocua.* Pediocin was commercialized as a food presevative for several types of foods, particularly, which have to be prevented from *L. monocytogenes* [3]. As nisin, pediocin is widely applied in food presevation, particularly, which is strictly prohibited from *L. monocytogenes*.

Previous studies reported that pediocin showed its effectiveness of preservation, several different kinds of food such as sausage, milk, chicken meat, beef, salmon [4-11]. Pediocin could be used directly as metabolite from Pediococcus, when the microorganism was added into sausage and milk [4,5]. Besides, purified pediocin was added into foods [7-10]. In the order hand, pediocin was also reported as anticancer activity on liver cell line A-549 [12]. Recombinant pediocin had been produced from some different expression systems including *E. coli*. Halami, 2007, reported that recombinant pedicoin was successfully produced in inclusion bodies of E. *coli* BL21 (DE3). The recombinant pediocin was then refolded and purified to obtain its antibacterial activity on the *L. monocytogenes* V7 [13]. Pediocin PA-1 was also expressed in E. coli M15 as fusion protein with His-tagged mouse dihydrofolate reductase (DHFR). The recombinant pediocin showed its antibacterial activity against *L. plantarum* NCDO 955 [14]. In this study, we show our results on producing recombinant pediocin as soluble protein in *E. coli* BL21(DE3).

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MATERIALS AND METHODS

The study was performed in the Laboratory of Molecular

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and Environmental Biotechnology, University of Science, VNU.HCM, Vietnam from 2016 to 2019.

Strains and Plasmid- DH5α *E. coli* (F-, ϕ80*lacZ*ΔM15, *recA*1, *endA*1, *hsdR*17 (rk-, mk+), *phoA*, *supE*44, λ-, *thi*-1, *gyrA*96, *relA*1), BL21(DE3) *E. coli* F^-, *ompT*, *hsdSB* (rB^-, mB^-), *gal*, *dcm* (DE3) and plasmid pET43.1a(+) were purchased from Invitrogen. Indicator bacteria were suported by Laboratory of Molecular and Environmental Biotechnology, University of Science, VNU.HCM, Vietnam.

E. coli strain was grown at 37°C in low salt Luria-Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl). Plamsid carried *E. Coli* was grown on LB medium, which supplemented with ampicillin (100 µg/ml). Indicator strains were grown at 37°C in Tryptic Soy Broth (Tryptone 1.7%, Peptone 0.3%, D-glucose 0.25%, NaCl 0.5%, K_2HPO_4 0.25%).

Construction of recombinant vector pET43.1a-*ped*- The construction of recombinant vector was designed following *et al.* [15] with modification. Pediocin encoding gene was synthesized based on nucleotide sequence of pediocin gene in *P. acidilactici* PAC1.0. and amplified by PCR with set of primers (BamHI-ped: CGCGGATCCGATG ACGACGACAAGAAATATTATGGTAATGGTGTTACCTGTGGT AAACATAGC and XhoI-ped: CCGCTCGAGCGGTTAACATTT ATGATTACCCTGATGA CCACC). The *Bam* HI-*Xho*I-pediocin DNA fragment was then inserted into pET43.1a(+) vector by T4 ligase. The T4 ligation product was transformed into DH5α *E. coli.* The recombinant vector was analyzed by PCR and sequencing.

Expression of NusA-his-pediocin fusion protein- The expression of recombinant pediocin was performed followed by Moon *et al.* [15] with modification. Recombinant plasmid pET43.1a-*ped* was transformed into BL21 (DE3) *E. coli.* TheBL21(DE3) *E. coli* transformed strain were induced by IPTG 0.8 mM when the optical density reaches 0.6-0.8 units (OD_{600}= 0.6–0.8) then harvested by centrifugation at 5000 rpm for 7 minutes after 2 hours further grown. The cell pellet after harvested was re-suspended in the binding buffer containing Na_2HPO_4 50mM, NaCl 300 mM, Imidazole 10mM pH 7.4 then sonicated using a homogenizer to disrupt the cells. To separate the precipitate and soluble fractions, the cell lysates then obtained by centrifugation at 13000 rpm for 15 minutes. To determine the presence and location of fused-pediocin, 3 fractions: total, precipitate and soluble of was checked for the expression by SDS-PAGE and confirmed indirectly by Western blot with anti-his antibody (Invitrogen).

Pediocin purification by affinity chromatography- Thepurification of recombinant pediocin was followed by Moon *et al.* [15] with modification. Soluble fraction from *E. coli* lysate was filtered by 0.2 mm low-protein-binding membrane and 10 ml of sample was applied to nickel-NTA agarose resin, which was first equilibrated by 5 CV binding buffer, followed by 15 CV buffer A containing Na_2HPO_4 50 mM, NaCl 300 mM, pH 7.4 to wash the column. The NusA-his-pedioc infused pediocin was eluted by buffer B (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM Imidazole pH 7.4). Eluted protein was analyzed by SDS-PAGE and Bradford assay.

Antimicrobial assay on Tricine SDS-PAGE gel- Antibacterial assay on tricine SDS-PAGE gene was applied as described by Bhunia *et al.* [16]. Two SDS-PAGE gels were run under the same condition, one was used for silver stained and the other was fixed with a solution containing 10% acetic acid and 20% isopropanol for 30 minutes, wash carefully with deionized water overnight. The gel was placed into a sterile petri dish and overlaid with 5 ml soft TSB-agar medium containing indicator bacteria, which was prepared the same as in the agar diffusion test. The test plate was incubated at 37°C until the inhibition zone was observed.

RESULTS

Pediocin expression in *E. coli*- To express pediocin in the cytoplasm of the *E. coli* cells, we introduce *pediocin* encoding gene into pET43.1a vector (Fig. 1).

After cloning *E. coli Bl21 Dec3* strain was utilized to express pediocin. Since pET43.1a was designed to express the soluble heterologous protein in *E. coli,* the recombinant pediocin was obtained as a soluble protein in the cytoplasm (Fig. 2). Besides, N-terminus of the pediocin encoding gene (*ped*) was fused with 6x histidine, sequenced by *NusA* tag, thereby the NusA-his-Pediocin fusion protein was detected by Wetern Blot with anti-his antibody. After the expression process, recombinant protein was obtained and introduced into SDS-PAGE analysis. The results showed that pediodin was expressed as NusA-his-pediocin fused protein with the molecular mass of more than 66 k Da, confirmed by the Western blot with anti-his-antibody (Fig. 2).

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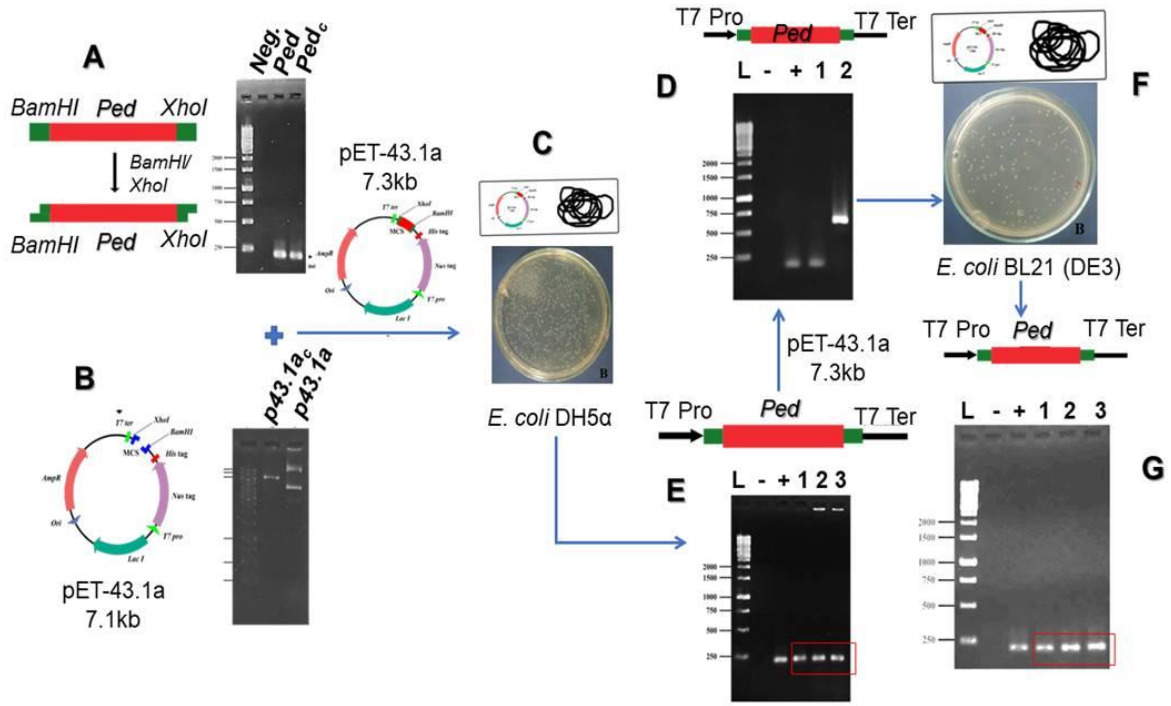

Fig. 1: Construction of peidocin expression vector in *E. coli*

A: Obtaining pediocin gene with BamHI/XhoI cohesive ends

B: Obatining pET43.1a with BamHI/XhoI cohesive ends

C: Introduction of recombinant vector into DH5α *E. coli*

D-G: Confirmation of pediocin expression vector and recombinant vector carried BL21 Dec. PCR

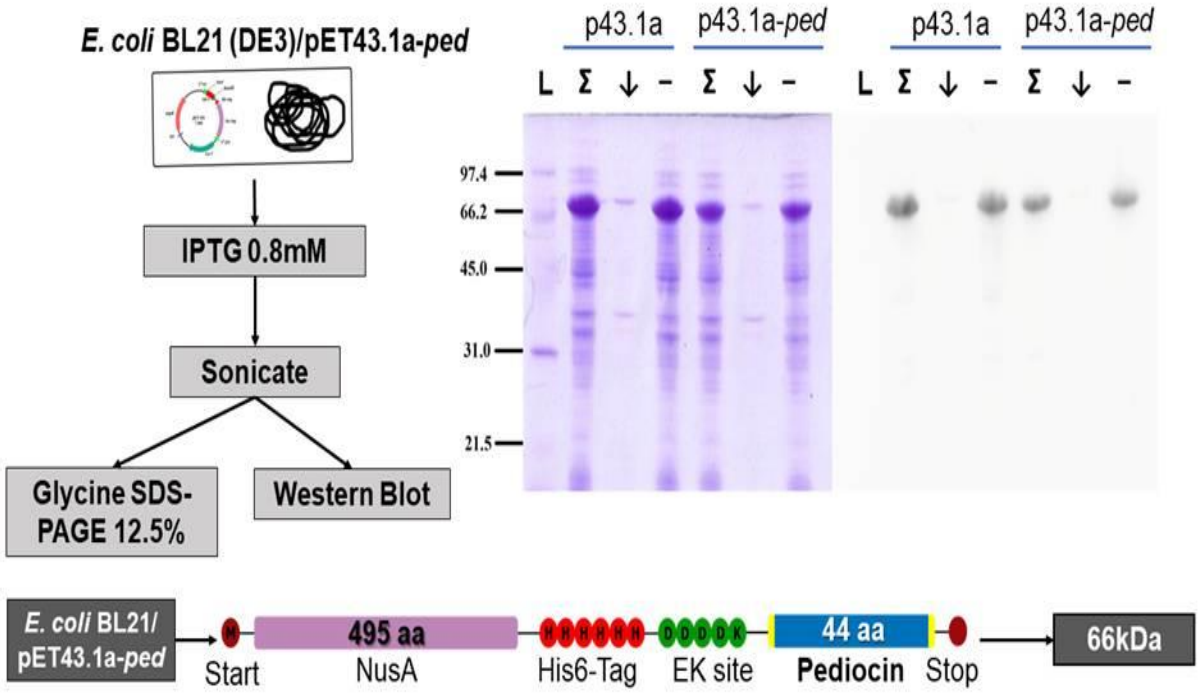

Fig. 2: Expression of recombinant pediocin in *E. coli*

L: protein ladder; p43.1a: extracted protein from *E. coli/ pET43.1a*; p43.1a-ped: extracted protein from *E. coli* with carrying pediocin expressing vector */ pET43.1a*-ped; **∑**: Total protein from the *E. coli* cells; **↓**: Peletted protein from the *E. coli* cells. **–:** Soluble protein from the *E. coli* cells

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Collection of active recombinant pediocin- In order to collect active pediocin, NusA-his-pediocin was obtained from *E. coli* and was purified by nickel-NTA column. The results in Fig. 3 showed that we have successfully obtained NusA-His-pediocin after elution by 20% buffer B (50 mM NaH_2PO_4, 300 mM NaCl, 500 mM Imidazole pH 7.4). The eluted protein was in 46.92±3.12% purity and in 77.15±11.79% collected yield. Although we could collect NusA-his-pediocin in the fraction which was eluted by 30% buffer B with 95.3% of purity, the recovery was quite low (18.44±5.53 %).

Table 1: Purify of NusA-his-pediocin protein

NusA-his-pediocin	Total protein in supernatant	Eluted protein (20% B)	Eluted protein (20% B)
Purity (%)	24.43±3.57	46.92±3.12	95.3%
Yield (mg)	2.793±0.869	2.155±0.549	0.515±0.034
Recovery (%)	100	77.15±11.79	18.44±5.53

The eluted NusA-his-Pediocin protein was then traeted by enterokinase enzyme in order to collect pediocin. The collected pediocin was applied to check its antibacterial activity by using *L. monocytogene* MT as indicator bacteria (Fig. 4). The results demonstrated that after treated by enterokinase we could release pediocin from the fusion NusA-his-pediocin protein and the free pediocin was about 4.6 kDa with the antibacterial activity of *L. monocytogene* MT.

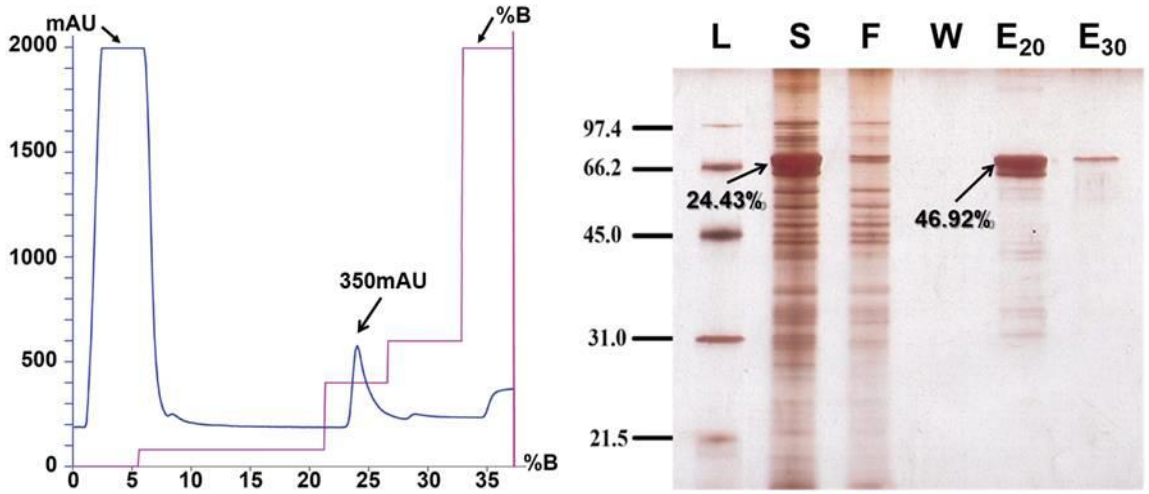

Fig. 3: Purification of NusA-his-pediocin
L: Protein ladder; **S:** Supernatant fraction from *E. coli* cell extract; **F:** Follow through fraction; **W:** Wash fraction; **E_{20}:** elution fraction with 20% buffer B; **E_{30}:** elution fraction with 30% buffer B

Analysis of the antimicrobial spectrum of recombinant pediocin: Beside *L. monocytogenes,* antimicrobial spectrum of recombinant pediocin was also analyzed.
The results showed that recombinant pediocin has antibacterial activity against Gram positive bacteria such as *L. monocytogene, L. inocua, Enterococcus faecalis*. Besides, the recombinant peptide pediocin also inhibited Gram negative bacteria such as *Shigella boydii, Vibrio parahaemolyticus* (Table 2).

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Table 2: Antimicrobial spectrum of recombinant pediocin

S. No.	Indicator	Gram	Antibacterial activity
1	*Aeromonas caviae* B168	–	–
2	*Aeromonas dhakensis* B77	–	–
3	*Aeromonas hydrophila* B56	–	–
4	*Aeromonas hydrophila* B66	–	–
5	*Aeromonas veronii* B141	–	–
6	*Bacillus cereus*	+	–
7	*Bacillus subtilis* DHCT	+	–
8	*Clostridium botulinum* E	+	–
9	*Clostridium botulinum* D	+	–
10	*Clostridium perfringen* 1	+	–
11	*Enterococcus faecalis*	+	+
12	*Escherichia coli* 1/6	–	–
13	*Escherichia coli* DHCT	–	–
15	*Edwardsiella ictaluri* LMG-Gly09M	–	–
16	*Edwardsiella tarda* ATCC 15947	–	–
17	*Enterobacter cloacae* DHCT	–	–
18	Enterotoxigenic *Escherichia coli* (ETEC)	–	–
19	*Listeria inocua*	+	+
20	*Listeria monocytogen* 364	+	+
21	*Listeria monocytogene*	+	+
22	*Listeria monocytogene* MT	+	+
23	*Pseudomonas aeruginosa*	–	–
24	*Pseudomonas aeruginosa* DHCT	–	–
25	*Staphylococcus aureus* 1	+	–
26	*Staphylococcus aureus* 2	+	–
27	*Staphylococcus aureus* B12	+	–
28	*Staphylococcus aureus* DHCT	+	–
29	*Salmonella dublin*	–	–
30	*Salmonella enteritidis*	–	–
31	*Salmonella sonei*	–	–
32	*Salmonella typhi*	–	–

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33	*Salmonella typhimurium*	–	–
34	*Shigella boydii*	–	+
35	*Shigella dysenteria*	–	-
36	*Shigella flexneri*	–	-
37	*Vibrioparahaemolyticus*	–	+
38	*Vibrio parahaemolyticus* L2	–	-

DISCUSSION

Pediocin PA-1 is well known as an antimicrobial peptide with strong antibacterial activity against quite wide, broad of Gram positive pathogens such as *L. monocytogenes*, *Staphylococcus aureus*, *Enterococcus faecalis.* Pediocin thereby was reported as food preservatives to inhibit the growth of *L. monocytogenes* in some kind of meat and meat-related products such as Frankfurters, breast meat as well as fish product [17,18]. In this study, we successfully obtained recombinant pediocin from *E. coli* by using pET43a.1 vector. The recomniant pediocin was about 4.6kDa and showed its trong activity against not only on several gram positive bacteria as *Enterococcus faecalis, L. innocua, L. monocytogen 364, L. monocytogene, L. monocytogene MT*. Interestingly, the recombinant pediocin in this study also inhited two Gram negative bacteria *Shigella boydii, Vibrio parahaemolyticus,* which had not been reported before on bactibase database. *The results suggested a deffirent mechanism of pediocin activity.*The antibacterial activity of recombinant pediocin in this study is similar to pediocin from in *Pediococcus pentosaceus K23-2* Shin *et al.* [19] and Papagianni *et al.* [20].

CONCLUSIONS

Pediocin is an antibacterial peptide which owns a strong potential on application for food and pharmaceutical industry. In this study, pediocin was introduced and expressed in *E. coli* by using pET43.a. The recombinant pediocin was successfully clevated from NusA-his-pediocin fusion protein and showed its strong antibacterial activity. The results in this study enable a new door for further study on pediocin production and application.

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CONTRIBUTION OF AUTHORS

Research concept- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Research design- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Supervision- Dr. Dang Thi Phuong Thao

Materials- Dr. Dang Thi Phuong Thao

Data collection-Nguyen Hieu Nghia, Nguyen Thi Cam Nhung

Data analysis and Interpretation- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Literature search- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Writing article- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Critical review- Dr. Dang Thi Phuong Thao

Article editing- Dr. Dang Thi Phuong Thao, Nguyen Hieu Nghia

Final approval- Dr. Dang Thi Phuong Thao

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