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Characterization of the Pediocin Operon of *Pediococcus acidilactici* K10 and Expression of His-Tagged Recombinant Pediocin PA-1 in *Escherichia coli*

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Abstract The relationship between plasmid (~9.5 kb) and pediocin PA-1 in *P. acidilactici* K10 was confirmed by plasmid curing. The pediocin operon of *P. acidilactici* K10 was amplified by PCR (polymerase chain reaction), and the nucleotide sequence was analyzed. The sequence of the pediocin operon of *P. acidilactici* K10 was similar to those of *P. acidilactici* strains producing pediocin PA-1/AcH. For the expression of pediocin PA-1 in *E. coli*, a pQEPED (pQE-30 Xa::mature *pedA*) was constructed. His-tagged recombinant pediocin PA-1 (~6.5 kDa) was translated by cell-free *in vitro* transcription and translation using pQEPED as a DNA template. Theresult of slot blotting assay showed that transcription of recombinant *pedA* in *E. coli* M15 was induced by the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) at the final concentration of 1 mM. Although the recombinant pediocin PA-1 inhibited the growth of *E. coli*, it was expressed in the host strain and purified by nickel-nitrilotriacetic acid (Ni-NTA) metal-affinity chromatography under denaturing condition. This is the first report for the production and one-step purification of biologically active recombinant pediocin PA-1 in *E. coli*.

Key words: Pediocin PA-1, pediocin operon, *Pediococcus acidilactici*, expression in *E. coli*

Bacteriocins are proteinaceous antimicrobial compounds produced by both Gram-positive and -negative bacteria, and they are active chiefly against closely related bacteria [31]. The discovery of bacteriocin dates back to 1925, when *E. coli* V was shown to produce an antimicrobial compound, colicin, active against *E. coli* Φ [5]. The bacteriocins produced by Gram-positive bacteria were defined as proteinaceous compounds that kill only closely related species [7, 31].

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The first report of a bacteriocin produced by lactic acid bacteria (LAB) was made in 1928 [29]. The substance was found to be a polypeptide [33] and subsequently named nisin [6, 17]. Since then, interest on bacteriocin has continuously grown, and now bacteriocins have been reported to be produced by all genera of LAB [9, 10]. During the last decade, bacteriocins have attracted considerable interest, and numerous antimicrobial peptides have been characterized [24]. Bacteriocins produced by LAB are subdivided into three distinct classes, based on the genetic and biochemical properties: class I-the lantibiotics; class II-the heat-stable unmodified bacteriocins; and class III-the larger heat-labile bacteriocins [23, 24].

Pediocin PA-1 is a bacteriocin produced by *P. acidilactici*, and its genetic [16] and biochemical [2, 8, 18, 27] characteristics have been revealed by several research groups. The nucleotide sequence analysis indicated the presence of four clustered genes (*pedA*, *pedB*, *pedC*, and *pedD*) in pediocin producers and showed the presence of a single putative promoter directly upstream of *pedA*. Promoter sequences could not be detected upstream of three other genes, indicating that the four genes are organized in an operon-like structure [16]. This operon structure was further confirmed by Motlagh *et al.* [22]. However, the function of each gene product has not yet been clearly elucidated, except for PedA: Venema *et al.* [32] elucidated the roles of gene products via functional analysis of the pediocin operon: PedA, prepediocin PA-1; PedB, pediocin PA-1 immunity protein; PedC, pediocin PA-1 transport; PedD, processing prepediocin PA-1 and transporting pediocin PA-1.

To date, several researches have engaged in the isolation of bacteriocinogenic LAB from kimchi [12, 13, 14, 19]. However, to the best of our knowledge, there are no indepth reports on the isolation, identification, and physico-chemical/genetic characteristics of pediocin-producing LAB from kimchi. In most cases, pediocin producers have been

isolated from fermented meat [4, 22]. In our earlier study, we first isolated pediocin PA-1-producing *P. acidilactici* K10 from kimchi and characterized its antimicrobial spectrum and amino acid sequence [11]. The pediocin had a broad range of antimicrobial activity, including many foodborne pathogens and spoilage bacteria. Therefore, pediocin together with organic acids and antimicrobial ingredients, such as garlic, will ensure microbiological safety of kimchi. In this study, we confirmed resident plasmids and the nucleotide sequence of the pediocin operon in *P. acidilactici* K10, and tried to express His-tagged recombinant pediocin PA-1 in *E. coli*, and do one-step purification using Ni-NTA affinity column.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, and Culture Conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *P. acidilactici* K10 and *Lactobacillus plantarum* NCDO 955 were grown in Lactobacilli MRS medium (Merck, Darmstadt, Germany) at 37°C without agitation. *E. coli* M15 (pREP4) (Qiagen, Hilden, Germany) was grown in LB medium with 25 μg/ml kanamycin at 37°C in a shaking incubator. *E. coli* M15 transformant (pREP4 and pQEPED) was selected on LB agar plate, containing 25 μg/ml kanamycin and 100 μg/ml ampicillin, at 37°C and cultured in the same medium for further studies.

Plasmid Preparation and Curing

Plasmid DNA from *E. coli* was isolated by using a commercial plasmid preparation kit (Qiagen, Hilden, Germany), and plasmids from *P. acidilactici* K10 were prepared according to the O'Sullivan and Klaenhammer method [25]. To obtain a plasmid cured mutant, *P. acidilactici* K10 was continuously cultured in MRS broth with novobiocin (3 μg/ml) and spread on MRS plate, and the mutant was isolated via plasmid profiling of colonies randomly selected on the MRS plate.

Bacteriocin Assay

Production of bacteriocin was assayed by spotting cells on MRS agar plate and incubating at 37°C for 18 h. The plates were overlaid with soft agar (0.7%) seeded with indicator strain (*Lb. plantarum* NCDO 955), incubated at 37°C for 18 h, and examined for inhibition zone around the colony (deferred antagonism assay) [13, 20, 26]. The bacteriocin activity of the culture supernatant was assayed by the well diffusion method [26]. Eighteen-hour culture was neutralized with NaOH, centrifuged, filtered, and an aliquot of the supernatant was loaded in a well made by a punch in MRS agar plate. The plate was overlaid with soft agar (0.7%) seeded with indicator strain (*Lb. plantarum* NCDO 955), and the next step was the same as the deferred antagonism assay.

PCR Amplification of Pediocin Operon and Nucleotide Sequencing

For PCR amplification of the pediocin operon in *P. acidilactici* K10, a primer set (PED1F and PED1R) was designed

Table 1. Bacterial strains, plasmids, and primers used in this study.

Strain, plasmid, or primer	Characteristics or sequence (5'→3')	Source or reference
Strains		
P. acidilactici K10	Pediocin PA-1 producer	[11]
Lb. plantarum NCDO 955	Pediocin PA-1 sensitive indicator	[1]
E. coli M15	Derived from *E. coli* K12, Nal^s, Str^s, Rif^s, Thi^-, Lac^-, Ara^+, Gal^+, Mtl^-, F^-, $RecA^+$, Uvr^+, Lon^+	Qiagen
Plasmids		
pQE-30 Xa	Ap^r, expression vector	Qiagen
pREP4	Km^r, contain *lacI*	Qiagen
pQEPED	Ap^r, pQE-30 Xa::mature *pedA*	This study
PCR primers		
PED1F	ACT TAA AAG ATA CTG CGT TGA TAG CCA	This study
PED1R	GCG AGG ATT TCA CGG ACA AG	This study
Sequencing primers		
pedBF	ATG AAT AAG ACT AAG TCG GAA C	This study
pedCF	TTG TCT AAG AAA TTT TGG TC	This study
pedCF1	CGG GTC TTT CAG CAT GGC	This study
pedDF	ATG TGG ACT CAA AAA TGG CAC	This study
pedDF1	GGT GAT CCT GAT CCA ACC G	This study
pedDF2	GGA CAA CGT CTC ATG ATC GAC	This study
pedDF3	GTC TCA GTG GCA TAG AAA CC	This study
pedDF4	GCT GCC AGA GTG GCT AAT AAT CG	This study

Ap^r, ampicillin resistance; Km^r, kanamycin resistance.

according to published pediocin operon sequence [16] and synthesized by a commercial biotech company (Bioneer, Chongwon, Korea). PCR was performed by using Pyrobest® DNA polymerase (Takara Bio, Shiga, Japan) by the conventional protocol. The nucleotide sequence of the PCR product (~3.6 kb) was determined with PED1F and internal sequencing primers. Nucleotide sequences of the primers are shown in Table 1.

Construction of Recombinant DNA: pQEPED (pQE-30 Xa::mature *pedA*)

For the expression of His-tagged recombinant pediocin PA-1, pQE-30 Xa (Qiagen, Hilden, Germany) expression vector was used. This vector was digested with *Stu*I and *Hin*dIII (Takara Bio, Shiga, Japan). The digested vector was dephosphorylated by alkaline phosphatase (Takara Bio, Shiga, Japan), purified by QIAquick® gel extraction kit (Qiagen, Hilden, Germany), and used for the cloning of mature *pedA*. Mature *pedA* was generated by PCR amplification. Ped$^+$ plasmid (~9.5 kb) of *P. acidilactici* K10 as a template, primer set (phosphorylated pedSF, AAA TAC TAC GGT AAT GGG GTT AC; pedSR-*Hin*dIII, CCC AAG CTT GGG CTA GCA TTT ATG ATT ACC TTG ATG TCC), and Pyrobest® DNA polymerase were used for PCR amplification with the conventional protocol. The PCR product was digested with *Hin*dIII and purified by QIAquick® gel extraction kit. Digested pQE-30 Xa and mature *pedA* were ligated by T4 DNA ligase (Takara Bio, Shiga, Japan), transformed into *E. coli* M15 (pREP4) (Qiagen, Hilden, Germany), and selected on LB agar plate containing ampicillin (100 μg/ml) and kanamycin (25 μg/ml). The nucleotide sequence of the recombinant DNA was analyzed by Takara Co. (Seoul, Korea). Oligonucleotide (CCC GAA AAG TGC CAC CTG) synthesized by Bioneer Co. (Chongwon, Korea) was used as sequencing primer.

In Vitro Transcription and Translation

For the cell-free *in vitro* transcription and translation, EasyXpress™ protein synthesis mini kit (Qiagen, Hilden, Germany) was used. Briefly, *E. coli* extract was thawed on ice and 22.5 μl of premix (reaction buffer 20 μl+RNase-free water 2.5 μl) was added to *E. coli* extract. Then, 0.5 μg of plasmid DNA (pQEPED) was added to the reaction tube, and the final reaction volume was adjusted to 50 μl with RNase-free water. The reaction mixture was incubated at 37°C for 1 h, and the reaction was terminated by placing on ice, followed by SDS-PAGE and bioassay.

SDS-PAGE and Bioassay

To estimate the molecular mass of expressed protein, tris-tricine SDS-PAGE [30] was performed. Criterion™ 10-20% gradient tris-tricine gel (Bio-Rad, Hercules, CA, U.S.A.) was assembled in Mini-PROTEAN® 3 electrophoresis unit (Bio-Rad, Hercules, CA, U.S.A.), and running buffer (100 mM Tris, 100 mM Tricine, 0.1% SDS, pH 8.3) was poured into the buffer tank and samples were loaded in wells. The unit was run at 150 V for 60 min, and the gel was fixed in fixation solution [40% (v/v) MeOH, 10% (v/v) acetic acid] for 30 min. The fixed gel was stained by Bio-Safe™ Coomassie (Bio-Rad, Hercules, CA, U.S.A.), washed with distilled and deionized H_2O, and protein bands were examined. For confirmation of bacteriocin band, bioassay [21] was performed at the same time. Thus, an identical gel run in the same unit was washed with distilled and deionized H_2O for 5 h, and the gel was loaded on MRS agar plate. Soft agar (0.7%, w/v) seeded with indicator strain (*Lb. plantarum* NCDO 955) was poured on the plate, then the plate was incubated at 37°C for 18 h, and inhibition zone was examined.

Slot Blotting Assay

In order to confirm transcriptional induction of *E. coli* M15 (pREP4 and pQEPED) by the addition of IPTG, slot blotting assay was performed. Overnight culture of *E. coli* M15 (pREP4 and pQEPED) was inoculated in 100 ml of LB broth with ampicillin (100 μg/ml) and kanamycin (25 μg/ml) and incubated up to OD_{600} of 0.5-0.6 with vigorous shaking at 37°C. IPTG was added to the final concentration of 1 mM, and the culture was incubated for 4 h. Subsampling was performed before and after the addition of IPTG, and subsamples were centrifuged and stored at -20°C until used. Total RNA preparation was purified by RNeasy® mini kit (Qiagen, Hilden, Germany). For the slot blotting assay, 10 μg of the purified total RNA was blotted onto Zeta-Probe® membrane (Bio-Rad, Hercules, CA, U.S.A.) by a Bio-Dot® SF microfiltration apparatus (Bio-Rad, Hercules, CA, U.S.A.) according to manufacturer's instruction. Probe (mature *pedA* labeled by digoxigenin, DIG) was hybridized by DIG Easy Hyb (Roche, Mannheim, Germany) and detected by DIG nucleic acid detection kit (Roche, Mannheim, Germany) according to the manufacturer's instruction.

Expression of His-Tagged Recombinant Pediocin PA-1 in *E. coli*

Expression of His-tagged recombinant pediocin PA-1 in *E. coli* was performed according to the manufacturer's instruction (Qiagen, Hilden, Germany). Overnight culture of *E. coli* M15 (pREP4 and pQEPED) was inoculated into 100 ml of LB broth with ampicillin (100 μg/ml) and kanamycin (25 μg/ml) and cultured up to OD_{600} of 0.5-0.6. IPTG was added to the final concentration of 1 mM in the culture and incubated for 4 h. The culture was centrifuged and the cell pellet was stored at -20°C. For the cell lysis, stored cell pellet was thawed on ice for 15 min and resuspended in 4 ml lysis buffer (native condition, 50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0; denaturing condition, 8 M urea, 0.1 M NaH_2PO_4, 0.01 M

Tris-Cl, pH 8.0). The mixture was incubated on ice for 30 min after the addition of lysozyme (1 mg/ml), and sonicated 6×10 s with 10 s pauses in the case of native condition or incubated at room temperature for 1 h with agitation in the case of denaturing condition. The lysate was centrifuged at 13,000 rpm for 20 min, and the supernatant was saved. One ml of the 50% Ni-NTA slurry was added to 4 ml of cleared lysate and mixed gently by shaking at 4°C in the case of native condition or room temperature in the case of denaturing condition for 60 min. The lysate-Ni-NTA mixture was loaded onto a column, and the column was washed twice with 4 ml of wash buffer (native condition, 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0; denaturing condition, 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 6.3). Bound proteins were eluted 4 times with 0.5 ml of elution buffer (native condition, 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0; denaturing condition, 8 M urea, 0.1 M NaH_2PO_4, 0.01 M Tris-Cl, pH 4.5), followed by SDS-PAGE and bioassay as described above.

Bacteriocin Activity and Protein Concentration

The bacteriocin activity, expressed as AU (arbitrary unit) per ml, was defined as the reciprocal of the highest two-fold dilution, showing inhibitory action against the indicator strain [20, 21]. Protein concentrations were measured by using *RC DC*™ protein assay kit (Bio-Rad, Hercules, CA, U.S.A.) according to the manufacturer's instruction.

Nucleotide Sequence Accession Number

The nucleotide sequence of the pediocin operon in *P. acidilactici* K10 has been deposited in the GenBank database under accession number AY705375.

Results and Discussion

Determination of the Locus of the Pediocin Operon in *P. acidilactici* K10

There were two resident plasmids (about 9.5 kb and 36 kb) in *P. acidilactici* K10 (lane 1 in Fig. 1A). The plasmid

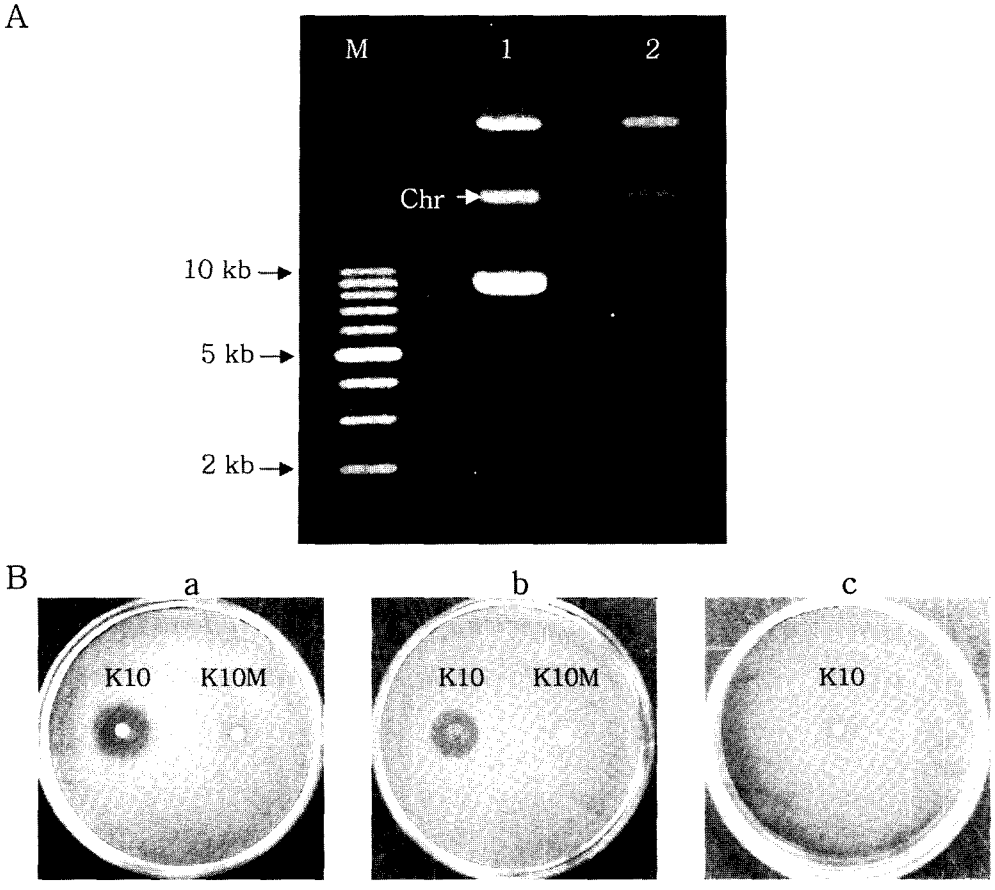

Fig. 1. Plasmid profiles of *P. acidilactici* K10 wild-type and its mutant (K10M) (A) and their phenotypes (B).
Chr, chromosomal DNA fragment. M, supercoiled DNA ladder (Promega, Madison, WI, U.S.A.); lane 1, plasmids of *P. acidilactici* K10 wild-type; lane 2, plasmid of *P. acidilactici* K10M. a, Deferred antagonism assay (indicator strain: *Lb. plantarum* NCDO 955). b, Well diffusion assay for culture supernatant (indicator strain: *Lb. plantarum* NCDO 955). c, Deferred antagonism assay for K10M immunity test against pediocin PA-1 produced by *P. acidilactici* K10 wild-type (indicator strain: *P. acidilactici* K10M).

profile agreed with those of *P. acidilactici* strains producing pediocin PA-1/AcH, isolated from meat sources [4, 22]. In these strains, the smaller plasmid was related to bacteriocin production, and the larger plasmid was related to sucrose fermentation.

A colony, which simultaneously lost both bacteriocin activity and the smaller plasmid, was isolated after plasmid curing by novobiocin (Fig. 1). This result indicates that the pediocin operon of *P. acidilactici* K10 exists on that specific plasmid, in agreement with the results by others [4, 22]. Interestingly, the Ped$^+$ (pediocin-producing phenotype) plasmid-cured *P. acidilactici* K10 mutant was still resistant to its own pediocin PA-1 (Fig. 1Bc). Similar result was also presented by Gonzalez and Kunka [4], suggesting that there may exist another mechanism for pediocin PA-1 immunity in this strain.

PCR Amplification of the Pediocin Operon and the Nucleotide Sequencing

The pediocin operon was amplified by PCR, when Ped$^+$ plasmid was used as a PCR template (lanes 1 and 2 in Fig. 2B). However, the operon was not amplified when plasmid purified from Ped$^+$ plasmid-cured *P. acidilactici* K10 mutant was used (lane 3 in Fig. 2B). This result confirms that the pediocin operon exists in the smaller plasmid. The bands of lanes 3 and 4 in Fig. 2B are nonspecific amplicons. Next, the nucleotide sequence of amplified pediocin operon (~3.6 kb) was determined by using designed sequencing

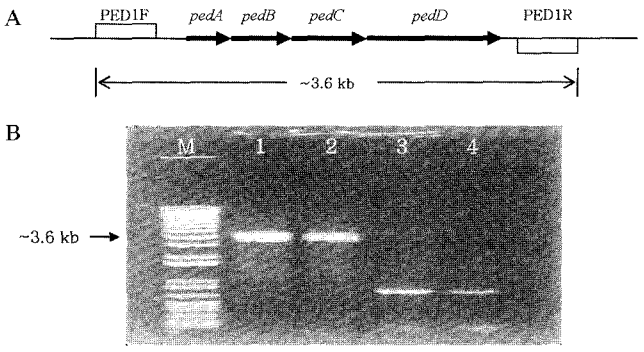

Fig. 2. Primer design (A) and PCR (B) for the amplification of the pediocin operon.

A: The structure of the pediocin operon (*pedA*, pediocin PA-1 structural gene; *pedB*, immunity gene; *pedCD*, ABC transporter and accessory gene) and loci of primer set designed by the sequence data published [16]. B: PCR using *P. acidilactici* K10 plasmids (lane 1), gel extracted Ped$^+$ plasmid (lane 2), and *P. acidilactici* K10M plasmid (lane 3) as a PCR template; lane 4, negative control (no template); M, 1 kb plus ladder (Invitrogen, Carlsbad, CA, U.S.A.).

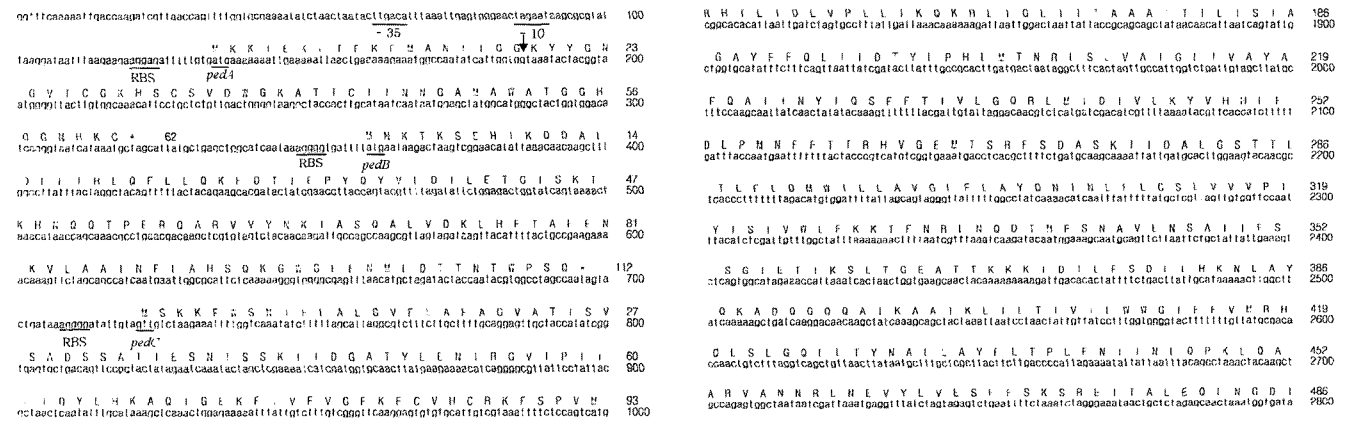

Fig. 3. Nucleotide sequence and predicted amino acid sequences of the PCR fragment containing the pediocin operon in *P. acidilactici* K10 Ped$^+$ plasmid.

The predicted amino acid sequences of the open reading frames (*pedA*, *pedB*, *pedC*, and *pedD*) are shown above the nucleotide sequence. The putative -35 and -10 promoter regions as well as the putative ribosome binding sites (RBS) are underlined. The vertical arrow in the amino acid sequence of PedA indicates the mature processing site. The horizontal arrows indicate a potential transcription terminator sequence.

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ATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTCACCTCGAGAAA   60

TCATAAAAAATTTATTTGCTTTGTGAGCGGATAACAATTATAATAGATTCAATTGTGAGC   120
               -35   Operator I            -10         Operator II
                                                 M  R  G  S  H     5
GGATAACAATTTCACACAGAATTCATTAAAGAGGAGAAATTAACTATGAGAGGATCGCAT   180
                            RBS
H  H  H  H  H  G  S  G  S  G  S  G  I  E  G  R↓ K  Y  Y  G     25
CACCATCACCATCACGGATCTGGCTCTGGATCTGGTATCGAGGGAAGGAAATACTACGGT   240
   6× His                                         mature pedA →
N  G  V  T  C  G  K  H  S  C  S  V  D  W  G  K  A  T  T  C     45
AATGGGGTTACTTGTGGCAAACATTCCTGCTCTGTTGACTGGGGTAAGGCTACCACTTGC   300

I  I  N  N  G  A  M  A  W  A  T  G  G  H  Q  G  N  H  K  C     65
ATAATCAATAATGGAGCTATGGCATGGGCTACTGGTGGACATCAAGGTAATCATAAATGC   360

*
TAGCCCAAGCTTAATTAGCTGAGCTTGGACTCCTGTTGATAGATCCAGTAATGACCTCAG   420
      HindIII

AACTCCATCTGGATTTGTTCAGAACGCTCGGTTGCCGCCGGGCGTTT   467
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Fig. 4. Nucleotide sequence and predicted amino acid sequence of the ligation junctions in pQEPED.

The predicted amino acid sequence is shown above the nucleotide sequence. The putative -35 and -10 promoter regions, *lac* operator, RBS, 6x histidine residues, and *Hin*dIII site are underlined. The vertical arrow in the amino acid sequence indicates the Factor Xa Protease cleavage site, and IEGR residues in the recognition site of Factor Xa Protease.

primers, as described in Materials and Methods (Fig. 3). The nucleotide sequence of the pediocin operon of *P. acidilactici* K10 was similar to those of *P. acidilactici* strains producing pediocin PA-1/AcH [16, 22]. The pediocin operon consisted of four genes (*pedABCD*).

Construction of pQEPED (pQE-30 Xa::mature *pedA*)

For the expression of His-tagged recombinant pediocin PA-1 in *E. coli*, a pQEPED was constructed, as described in the Materials and Methods. The sequence analysis of the junction regions confirmed the correct ligation, as shown in Fig. 4.

In Vitro Transcription and Translation Using pQEPED

Before *in vivo* expression, *in vitro* transcription and translation was performed by using pQEPED. As shown in Fig. 5A, the expressed protein band (~32 kDa) was visualized in lane 2 of SDS-PAGE gel by Bio-Safe™ Coomassie (Bio-Rad, Hercules, CA, U.S.A.) staining when positive control (DNA that encodes the 32 kDa elongation factor EF-Ts) was used as a template. However, the expressed protein band was not visualized when pQEPED was used as a template (lanes 4 and 5 in Fig. 5A). Nevertheless, an inhibition zone corresponding to 6,500 Da was visible in activity staining of the gel. The size of the inhibition zone of IPTG-treated sample (lane 5 in Fig. 5B) was larger than that of nontreated sample (lane 4 in Fig. 5B). Formation of the inhibition zone in the sample without IPTG-treatment might be due to leakage of transcription by a little repressor present in the cell-free extract. The above result indicates that His-tagged recombinant pediocin PA-1 (~6,500 Da) was correctly translated and remained as a biologically active peptide.

Slot Blotting for Confirming Transcriptional Induction

To confirm transcriptional induction of recombinant mature *pedA* by IPTG (transcription inducer), slot blotting assay was performed. As shown in Fig. 6C, recombinant mature *pedA* in *E. coli* M15 (pREP4 and pQEPED) was rapidly transcribed after the addition of IPTG. On the other hand,

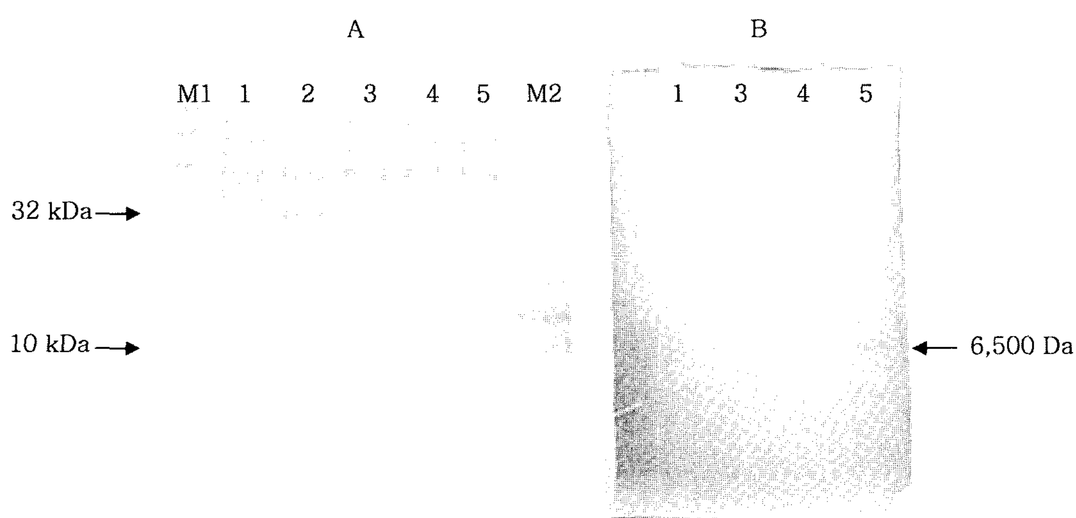

Fig. 5. *In vitro* transcription and translation using pQEPED.

A, Bio-Safe™ Coomassie staining after SDS-PAGE (tris-tricine gel); B, activity staining after SDS-PAGE (indicator: *Lb. plantarum* NCDO 955). M1, precision plus protein standards (Bio-Rad, Hercules, CA, U.S.A.); M2, polypeptide standards (Bio-Rad, Hercules, CA, U.S.A.); 1, negative control (no DNA); 2, positive control (DNA that encodes the 32 kDa elongation factor EF-Ts with a C-terminal 6xHis tag); 3, pQE-30 Xa; 4, pQEPED (no IPTG); 5, pQEPED (IPTG).

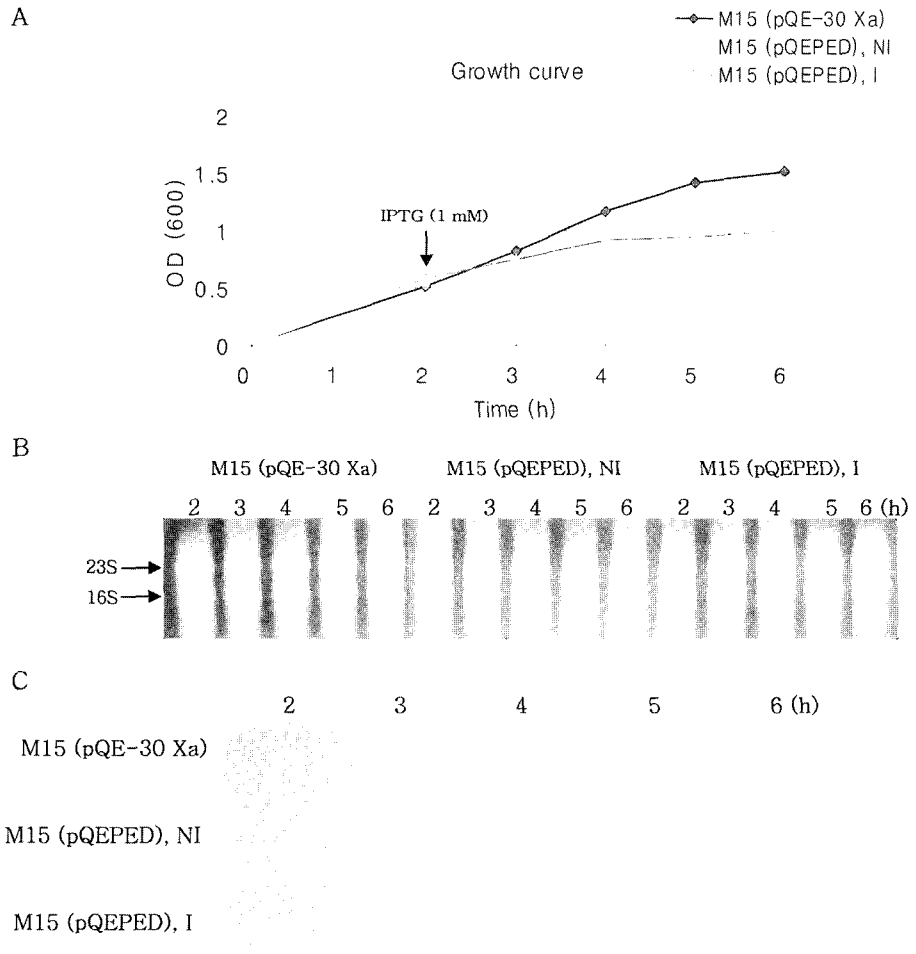

Fig. 6. Slot blotting for confirming transcriptional induction of recombinant mature *pedA* after the addition of IPTG in *E. coli* M15 (pREP4 and pQEPED).
A, Growth curve; B, electrophoresis of total RNA (2 μg loading); C, slot blotting (10 μg loading). M15, *E. coli* M15 (pREP4); NI, IPTG not added; I, IPTG (final concentration: 1 mM) added.

E. coli M15 (pREP4 and pQE-30 Xa) with IPTG and *E. coli* M15 (pREP4 and pQEPED) with no IPTG, used as negative controls, did not indicate any transcriptional induction. This indicates that pQEPED is tightly controlled by IPTG due to two *lac* operators and repressors produced by pREP4 in *E. coli* M15. However, as shown in Fig. 6A, production of His-tagged recombinant pediocin PA-1 after the addition of IPTG inhibited the growth of *E. coli* M15 (pREP4 and pQEPED), whereas *E. coli* M15 (pREP4 and pQE-30 Xa) with IPTG and *E. coli* M15 (pREP4 and pQEPED) without IPTG were not inhibited.

Expression of His-Tagged Recombinant Pediocin PA-1 in *E. coli*

The slot blotting assay proved that His-tagged recombinant pediocin PA-1 was toxic to *E. coli* host cell, indicating that biologically active recombinant pediocin PA-1 was expressed in the host. In general, small-sized foreign peptides in *E. coli* are easily cleaved by various proteases [15]. Nevertheless, His-tagged recombinant pediocin PA-1 was expressed in *E. coli* and maintained as a biologically active peptide. The recombinant pediocin PA-1 in *E. coli* cell lysate prepared by native condition did not bind to Ni-NTA affinity resin in general condition (data not shown), most likely due to masking of histidine residues by the tertiary structure of His-tagged recombinant pediocin PA-1. However, this problem was overcome by denaturing purification, as described in the Materials and Methods, and His-tagged recombinant pediocin PA-1 (~6,500 Da) was purified by 20-fold purity in one step (Fig. 7 and Table 2).

Because pediocin PA-1 is a representative class IIa bacteriocin, having strong antilisterial activity and a broad-range antimicrobial spectrum, this bacteriocin is a main strategic target for developing biopreservative. In this respect, overexpression of pediocin PA-1 and its simple

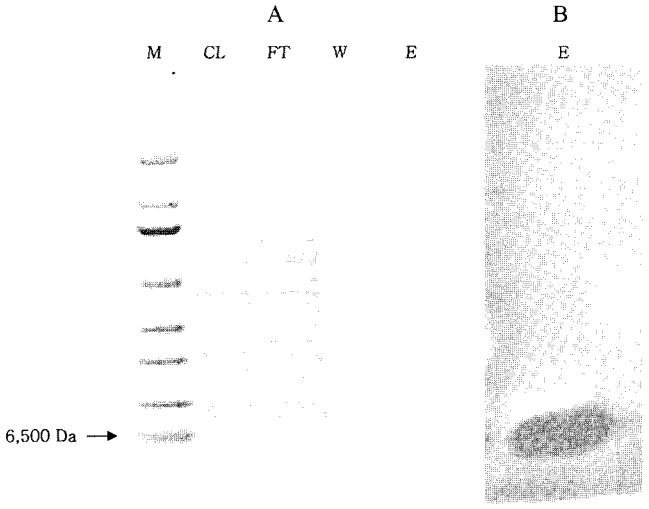

Fig. 7. Tris-tricine SDS-PAGE after purification of His-tagged recombinant pediocin PA-1.
A, Bio-Safe™ Coomassie staining; B, activity staining (indicator: *Lb. plantarum* NCDO 955). M, broad-range marker (Bio-Rad, Hercules, CA, U.S.A.); CL, cleared lysate; FT, flow-through; W, wash; E, eluate.

purification method can be valuable. To meet this demand, tagging target peptide and affinity chromatography can be a solution. In this study, we expressed His-tagged recombinant pediocin PA-1 in *E. coli* and purified the recombinant pediocin PA-1 by Ni-NTA affinity chromatography. Recently, piscicolin 126 and divercin V41, belonging to class IIa bacteriocin, were expressed in *E. coli* with fusion protein form [3, 28]. These fusion proteins were cleaved by cyanogens bromide and enterokinase, respectively, and biologically active recombinant piscicolin 126 and divercin V41 were purified by reversed-phase HPLC and metal affinity chromatography, respectively. These methods for the expression of class IIa bacteriocins could be useful, however, cleavage and further purification steps appear to be disadvantageous. In this respect, our direct expression of biologically active bacteriocin in *E. coli* can be a powerful method if it can be maintained stably in the host.

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Table 2. Purification of recombinant pediocin PA-1 from *E. coli* cleared lysate.

Fraction	Volume (ml)	Protein conc. (mg/ml)	Activity (AU/ml)	Total activity (AU)	Specific activity (AU/mg)	Fold in specific activity	Yield (%)	Total protein (mg)
Cells	100							
Cleared lysate	3.8	2.9	3,000	11,400	1,034	1		11.02
Eluate	2	0.24	5,000	10,000	20,833	20.1	4.4	0.48

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