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Heterologous Expression of Human β-Defensin-1 in Bacteriocin-Producing *Lactococcus lactis*

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Abstract *Lactococcus lactis* A164 is a nisin Z-producing strain isolated from kimchi. Its antimicrobial spectrum has been found to be active against most Gram-positive bacteria tested, yet inactive against Gram-negative bacteria [3]. Accordingly, to overcome this drawback, the current study attempted to express human β-defensin-1 (hBD-1), which kills both Gram-positive and Gram-negative bacteria in *L. lactis* A164. When the hBD-1 cDNA was introduced using a nisin Z-controlled expression cassette, the *L. lactis* A164 transformants grew very poorly, due to the bactericidal effect of the expressed hBD-1 against the transformants. Therefore, a gene fusion system was designed to reduce the toxicity of the expressed heterologous protein against the host cells. As such, the hBD-1 gene was fused to the DsbC-Tag of pET-40b(+), then introduced to *L. lactis* A164. The transformants expressed an intracellular 35.6-kDa DsbC-hBD-1 fusion protein that exhibited slight activity against the host cells, yet not enough to strongly inhibit the cell growth. To obtain the recombinant hBD-1, the DsbC-hBD-1 fusion protein was purified by nickel-affinity column chromatography, and the DsbC-Tag removed by cleaving with enterokinase. The cleaved mature hBD-1 exhibited strong bactericidal activity against *E. coli* JM109, indicating that the recombinant *L. lactis* A164 produced a biologically active hBD-1. In addition, the recombinant *L. lactis* A164 was also found to produce the same level of nisin Z as the wild-type.

Key words: Expression, human β-defensin-1, bacteriocin, *Lactococcus lactis*

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Defensins are small cationic antimicrobial peptides produced by vertebrate and invertebrate animals [13] that contribute to the host innate immune defense by killing invading microorganisms. Their microbicidal spectra are active against Gram-positive bacteria, Gram-negative bacteria, mycobacteria, fungi, and certain enveloped viruses [9, 10], making them potential candidates for development as novel antibiotics.

In humans, there are two families of defensins, α and β, which differ in their disulfide bond pairing, genomic organization, and tissue distribution [8]. Human β-defensin-1 (hBD-1) is a short cationic peptide of 36 amino acid residues and has six cysteines residues that form three intramolecular disulfide bonds [2]. hBD-1 is constitutively expressed in the epithelium of the urinary tract, trachea, lungs, gastric tract, gingival tissues, and urogenital tissues [5, 9, 25, 29, 33], while hBD-2, -3, and -4 are induced in the skin, tonsils, testis, and other epithelia during inflammation [10, 12]. The gene encoding hBD-1 is located in the chromosomal region 8p23, which is clustered with known α- and β-defensins genes [11, 15, 21].

Many lactic acid bacteria (LAB) and other Gram-positive bacteria produce small, antimicrobial, and proteinaceous compounds named bacteriocins that typically kill or inhibit the growth of closely related bacteria [16, 17, 19]. Nisin produced by *Lactococcus lactis* strains is a well-characterized bacteriocin with two natural variants, nisins A and Z [6, 20, 23], which have a similar structure and antimicrobial spectrum, but differ in a single amino acid residue at position 27: histidine in nisin A and asparagine in nisin Z. *L. lactis* A164 is a nisin Z producer that has been isolated from naturally fermented kimchi [3]. Like many bacteriocins from

LAB, its antimicrobial spectrum is only against Gram-positive bacteria, and not against Gram-negative bacteria, which would appear to be the main drawback to bacteriocins produced by LAB [24]. To overcome this problem, McCormick *et al.* [22] and van Belkum *et al.* [30] recently successfully expressed colicin V, a bacteriocin produced by *Escherichia coli* that is active against Gram-negative bacteria, in colicin V-resistant LAB by replacing the colicin V leader peptide with a signal peptide from the signal sequence of a LAB bacteriocin. The colicin V-producing LAB were shown to effectively inhibit the growth of *E. coli* DH5α. However, the application of colicin V as a biopreservative and therapeutic will likely be restricted, as it is not a generally regarded to be safe (GRAS) bacteria. Conversely, since human defensins are produced by humans, their application as potential antimicrobial peptides is more feasible.

Accordingly, the objective of this study was to express human β-defensin-1 into nisin Z-producing *L. lactis* to control the growth of Gram-positive and Gram-negative bacteria by producing nisin Z and hBD-1 simultaneously. As such, this report covers the cloning and expression of the hBD-1 cDNA in nisin Z-producing *L. lactis* A164 using a nisin Z-producing gene cassette and gene fusion-tag system with an *E. coli* expression vector. This is also the first report dealing with the heterologous expression of human defensin in LAB.

MATERIALS AND METHODS

Bacterial Strains, Media, and Growth Conditions

The bacterial strains and plasmids used in this study are listed in Table 1. The *E. coli* was grown in a Luria-Bertani medium (BD Biosciences, Sparks, MD, U.S.A.) at 37°C with shaking at 180 rpm. The nisin Z-producing *L. lactis* A164 and other *L. lactis* were grown in an M17 medium (BD Biosciences) supplemented with 0.5% lactose at 30°C without shaking. The *Weissella paramesenteroides* was grown in a Lactobacilli MRS medium (BD Biosciences) at 30°C without shaking. When appropriate, antibiotics were added to the culture medium. Erythromycin (Sigma Co., St. Louis, MO, U.S.A.) was used at a final concentration of 5 μg/ml for the *L. lactis* strains and 500 μg/ml for the *E. coli*, while ampicillin and kanamycin (Sigma) were used at a final concentration of 100 and 30 μg/ml, respectively, for the *E. coli*. All cultures were maintained as frozen stocks held at -70°C in an appropriate broth containing 20% glycerol.

DNA Manipulations

Plasmid DNA was isolated from the *E. coli* using a QIAprep spin plasmid preparation kit (Qiagen Inc., Valencia, CA, U.S.A.) and from the *L. lactis* as described by Anderson and McKay [1]. The extraction of chromosomal DNA

Table 1. Bacteria strains and plasmids used for expression of hBD-1.

Strain or plasmid	Relevant characteristics	Source or reference
Strain		
E. coli		
DH5α	*F⁻ endA1 hsdR17* ($r_k^- m_k^+$) *glnV44 this-1 recA1 gyrA* (Nal^r) *relA1* *Δ(lacIZYA-argF)*U169 *deoR* φ80d*lacΔ(lacZ)*M15	Clontech
TOP10	*F⁻ endA1 mcrA Δ(mrr-hsd*RMS-*mcr*BC) *recA1* φ80d*lacΔ(lacZ)*M15 *ΔlacX74 deoR araD139 Δ(ara-leu)7697 gal*U *gal*K *rsp*L (*Str^r*) *nupG*	Invitrogen
BL21 (DE3)	*F⁻ ompT hsdDB* ($r_B^- m_B^+$) *gal dcm* (DE3)	Novagen
BL21 (DE3) (pHCD1)	BL21 (DE3) carrying plasmid pHCD1	This study
JM109	Indicator strain of recombinant hBD-1	Promega
L. lactis		
A164	Nisin Z producer, plasmid-free	[3]
A164 (pOri23)	A164 carrying plasmid pOri23	This study
A164 (pOED1)	A164 carrying plasmid pOED1	This study
W. paramesenteroides ATCC 40114	Indicator strain of nisin Z	ATCC
Plasmid		
pGEM-T Easy	Cloning vector (T-overhangs), 3.0 kb, Amp^r *lacZ* f1-*ori*	Promega
pET-40b(+)	Expression vector, 6.2 kb, Kan^r T7*lac* f1-*ori* DsbC-Tag His-Tag S-Tag	Novagen
pGKV210	*Lactococcus-E. coli* shuttle vector, promoterless, Em^r *cat86* pWV01-*ori*	[31]
pOri23	*Lactococcus-E. coli* shuttle and expression vector, Em^r ColE1-*ori*	[26]
pGBD1	hBD-1 cDNA inserted into pGEM-T easy	This study
pLZh1-1	*nisZ-hbd1* cassette inserted into pGEM-T easy	This study
pGLZh1-1	pLZh1-1 inserted into the *Pst*I site of pGKV210	This study
pHCD1	hBD-1 cDNA inserted into the *Kpn*I/*Bam*HI site of pET-40b(+)	This study
pOED1	DsbC-hBD-1 cDNA cassette inserted into the *Bam*HI/*Sal*I site of pOri23	This study

from the *L. lactis* was performed according to the method of Choi *et al.* [3]. The restriction enzymes and other DNA modifying enzymes from various sources were used according to the manufacturer's recommendations, and the PCR fragments were purified with a QiaQuick purification kit (Qiagen). The transformation of the *E. coli* was performed using cells made competent with $CaCl_2$, while the electrotransformation of the *L. lactis* was performed using cells grown in the presence of glycine to weaken the cell wall [14]. All DNA sequences were verified using a *Tag* Dye-Deoxy terminator cycle sequencing kit (Perkin-Elmer Co., Wellesley, MA, U.S.A.) and ABI PRISM 377 automatic DNA sequencer (Perkin-Elmer).

Cloning of hBD-1 cDNA Using a Nisin Z-Producing Gene Cassette

The plasmid vectors and oligonucleotide primers used in this study are listed in Tables 1 and 2, respectively. A PCR was carried out to obtain the mature hBD-1 cDNA, *nisZ* promoter, and leader sequence [3]. The PCR reaction to obtain the mature hBD-1 cDNA was performed with human lung Marathon-Ready cDNA (BD Biosciences Clontech, Palo Alto, CA, U.S.A.) as the template, HBD1-5 and HBD1-3 as the primers (listed in Table 2), and *Vent* polymerase (New England Biolabs Inc., Beverly, MA, U.S.A.). The proximal 170 bp to the *nisZ* promoter and leader sequence was amplified from the *L. lactis* A164 chromosomal DNA using the Nis-Pro5 and Nis-Pro3 primers (Table 2). The PCR products of the hBD-1 cDNA were fused downstream of the *nisZ* promoter and leader sequence by ligation, and the fusion products then used as the template DNA for the amplification of the *nisZ-hbd1* cassette. After conducting a fusion PCR using the HBD1-5 and Nis-Pro3 primers and *Taq* polymerase (New England Biolabs), the amplified *nisZ-hbd1* cassettes were cloned into a pGEM-T Easy Vector (Promega Co., Madison, WI, U.S.A.) to obtain pLZh1-1. Plasmid pLZh1-1 was then digested with *Pst*I and cloned into the *Pst*I-digested lactococcal plasmid vector pGKV210 [31] to obtain pGLZh1-1, which was used to transform *L. lactis* A164. The finally constructed vectors were verified by plasmid extraction and DNA sequencing.

Cloning of hBD-1 cDNA Using a DsbC-Tag Fusion System

The mature hBD-1 gene was obtained by PCR amplification with the HBD1-PET5 and HBD1-3 primers (Table 2), then digested with *Kpn*I and *Bam*HI and cloned into *Kpn*I/*Bam*HI-digested pET-40b(+) (Novagen Inc., Madison, WI, U.S.A.) to produce pHCD1. Thereafter, the plasmid pHCD1 was propagated in *E. coli* BL21 (DE3), checked for the correct sequence, and used as the template DNA for the PCR amplification of the pET-40b(+) fusion-tag part (DsbC-Tag) and *hBD-1* gene using the PET-TAG5 and HBD1-3SAL primers (Table 2). The PCR product was then digested with *Bam*HI and *Sal*I and cloned into the corresponding site of the lactococcal expression vector pOri23 [26]. The resulting plasmid pOED1 was used to transform *L. lactis* A164.

Western Blotting

Total protein extracts were prepared from exponential cultures of the *L. lactis* A164 harboring pOED1. The cells were harvested by centrifugation for 15 min at 8,000 ×*g*, washed twice in a 10 mM sodium phosphate buffer (pH 7.4), and resuspended in the same buffer. The cell suspension was then treated with 20 mg/ml lysozyme (Sigma) for 1 h at 37°C to remove the cell wall, followed by complete disruption using a Bead Homogenizer (bead diam., 0.2 mm; B. Braun Biotech, Germany). Next, the supernatant was separated by centrifugation for 20 min, 12,000 ×*g* at 4°C, and the protein concentrations measured according to the method of Lowry. The total protein extracts were then applied to an His-Bind Column (Novagen), and the hBD-1 fusion protein eluted according to the manufacturer's recommendations. The eluted fractions were subjected to sodium dodecyl sulfate-polyacrylamide gel elctrophoresis (SDS-PAGE) and subsequently electroblotted onto a polyvinylidene difluoride (PVDF) membrane ((Bio-Rad

Table 2. Oligonucleotide primers used for expression of hBD-1.

Primer	Sequence (5′→3′)[a]	Application
HBD1-5*	GATCATTACAATTGCAGCAGTGGAG	Amplification of hBD-1 cDNA for pLZh1-1
HBD1-3	CGGGATCC**TCA**CTTGCAGCACTTGGCCTTCCCTCTG	Amplification of hBD-1 cDNA for pLZh1-1
Nis-Pro5	CGGAATTCGAAACATTAACAAATCTAAAACAGTCTTAA	Amplification of *nisZ* promoter and leader sequence for pLZh1-1
Nis-Pro3*	GCGTGGTGATGCACCTGAATCTTTCTT	Amplification of *nisZ* promoter and leader sequence for pLZh1-1
HBD1-PET5	GGGGTACCGGTCGGGATCTGTAC*GACGATGACGATAAG*G ATCATTACAATTGCGTCAGCAGTGGAGG	Amplification of hBD-1 cDNA for pHCD1
PET-TAG5	CGGGATCC**ATG**AAGAAAGGTTTTATGTTGTTTACTTTG	Amplification of hBD-1 cDNA for pOED1
HBD1-3SAL	ACGCGTCGAC**TCA**CTTGCAGCACTTGGCCTTCCCTCTG	Amplification of hBD-1 cDNA for pOED1

[a]Restriction sites are underlined, translation start and stop codons are represented in boldface, and enterokinase cleavage site is represented in italic. Asterisks represented 5'-phosphorylated primers.

Laboratories, Hercules, CA, U.S.A.) using a Mini-Trans-Blot electrophoretic transfer cell (Bio-Rad). The membranes were then incubated with Penta-His antibodies (Qiagen) as the primary antibody and goat antimouse IgG antibodies coupled with alkaline phosphatase (Sigma) as the secondary antibody. The immunological detection of the hBD-1 fusion protein was carried out using a 5-bromo-4-chloro-3-indol-1-phosphate-p-toluidine/nitroblue tetrazolium (BCIP/NBT) substrate system (Sigma).

Comparison of Cell Growth

The recombinant *L. lactis* A164 strains carrying pOED1 or pOri23 were statically incubated in an M17 broth at 30°C, and the cell growth measured by monitoring the optical density at 600 nm at 2-h intervals.

Assay for hBD-1 Activity

To obtain the mature hBD-1, the fusion part of the purified DsbC-hBD-1 fusion protein was removed using a Recombinant Enterokinase kit (Novagen) according to the manufacturer's instructions. Different concentrations of hBD-1 were created by dilution with a 10 mM sodium phosphate buffer (pH 7.4). A colony count assay [27, 32] was used to determine the bactericidal activity of the expressed hBD-1 or DsbC-hBD-1 fusion protein. *E. coli* JM109 was freshly prepared by growing cells in an appropriate broth to 10^8 CFU/ml, then diluted in a 10 mM sodium phosphate buffer to obtain a final cell concentration of 10^5 CFU/ml. The cells were harvested and washed twice in the same buffer. Various concentrations of the recombinant hBD-1 or DsbC-hBD-1 fusion protein were added to 0.1 ml of the bacterial suspension or 10 mM sodium phosphate buffer (control) in a total volume of 0.2 ml of the 10 mM sodium phosphate buffer, and the mixtures incubated for 2 h at 37°C. Following incubation, the mixture was serially diluted in the same buffer, and the population of *E. coli* JM109 determined by plating 0.1 ml of each dilution in duplicate on the appropriate agar plates. The resulting colonies were counted after incubation at 37°C for 24 h and the bactericidal activity calculated in comparison with the control. When appropriate, the bactericidal activity was calculated as follows: loss of cell viability (%)=[1-(cell survival after incubation with hBD-1)/(cell survival in buffer alone)]×100.

Assay for Nisin Z Activity

The wild-type and recombinant *L. lactis* A164 strains were statically grown in an M17 broth at 30°C until the growth reached the stationary phase. The bacteriocin activity was then assayed by the critical-dilution method using a microtiter plate [4]. In brief, the cell-free stationary phase cultures of the wild-type and recombinant *L. lactis* A164 strains were serially two-fold-diluted in 125 µl volumes of an MRS broth in a 96-well microtiter plate. Each well was inoculated with 50 µl of a 100-fold-diluted overnight culture of the indicator organism, *Weissella paramesenteroides* ATCC 40114. After incubating at 30°C for 18 h, the nisin Z activity was calculated and expressed as arbitrary units (AU) per milliliter. *L. lactis* A164 (pOri23) was used as the defensin-negative control.

RESULTS AND DISCUSSION

Cloning and Expression of hBD-1 cDNA in *L. lactis* A164 Using a Nisin Z-Producing Gene Cassette

To attempt the expression of hBD-1 cDNA in *L. lactis* A164, the hBD-1 cDNA was fused in the frame behind the *nisZ* promoter and leader sequence to obtain a *nisZ-hbd1* cassette, which was cloned into pGKV210, a promoterless and high-copy-number *E. coli-Lactococcus* shuttle vector. The resulting plasmid pGLZh1-1 was then introduced into *L. lactis* A164. Despite multiple attempts, no *L. lactis* A164 transformants harboring pGLZh1-1 were obtained. The colonies that appeared on the M17 erythromycin agar plate grew poorly, and only pinpoint colonies were observed (results not shown), which did not grow further even in an M17 erythromycin broth. Since pGLZh1-1 carries a chloramphenicol-resistant gene downstream of its multi-cloning site [31], transformants were also selected from the M17 agar plate that contained different concentrations of chloramphenicol. However, no transformants were recovered, as their growth stopped when pinpoint colonies appeared on the M17 erythromycin agar plate, probably due to the toxicity of the expressed hBD-1 against the host cells. de Vuyst and Vandamme [7] analyzed the transcription and translation levels of nisin A-producing *L. lactis* NIZO 22186 at different growth phases. In the early growth phase, they detected a large number of nisin A transcripts, with only a few transcripts translated into nisin A precursors. The translation of the nisin A transcripts then began as the cells reached the exponential phase, and most transcripts were translated in the late exponential phase. In this study, after the transformation of *L. lactis* A164 with pGLZh1-1, hBD-1 mRNAs were accumulated, yet they were not translated until the early exponential phase. As such, it would appear that the translation of the hBD-1 mRNAs occurred in the early exponential phase, then the synthesized hBD-1 subsequently interfered with the growth of the transformants, resulting in the pinpoint colonies on the M17 erythromycin agar plate. Therefore, the current results indicate that a cloning strategy using a nisin Z-producing gene cassette is inadequate to express hBD-1 in *L. lactis* A164.

Cloning and Expression of hBD-1 cDNA in *L. lactis* A164 Using a DsbC-Tag Fusion System

To reduce the toxic effect of the recombinant hBD-1 protein against the host cells, the hBD-1 cDNA was fused in the frame behind the pET-40b(+) fusion-tag part (DsbC-

Tag, 236 amino acid residues), which increases solubility and ensures proper folding of the expressed proteins by enhancing the periplasmic localization and disulfide bond-isomerizing activity [18]. The fused gene of DsbC-Tag and hBD-1 was cloned into pOri23, a lactococcal constitutive expression vector, to produce pOED1. *L. lactis* A164 transformants carrying pOED1 were successfully recovered, as the transformants grew well on the M17 erythromycin agar plates, suggesting that the DsbC-hBD-1 fusion protein was not too toxic to inactivate the host cells. The plasmid pOED1 was recovered from the appropriate transformants and identified by restriction digest and agarose gel electrophoresis (results not shown).

The expression of the DsbC-hBD-1 fusion protein was determined by Western blot analysis using a Penta-His antibody. As shown in Fig. 1, a similar 35.6-kDa protein corresponding to the size of the DsbC-hBD-1 fusion protein was detected from the cell extracts of *L. lactis* A164 harboring pOED1, whereas no band was detected from the cell extracts of *L. lactis* A164 carrying pOri23. Therefore, *L. lactis* A164 carrying pOED1 was found to express an intracellular 35.6-kDa DsbC-hBD-1 fusion protein.

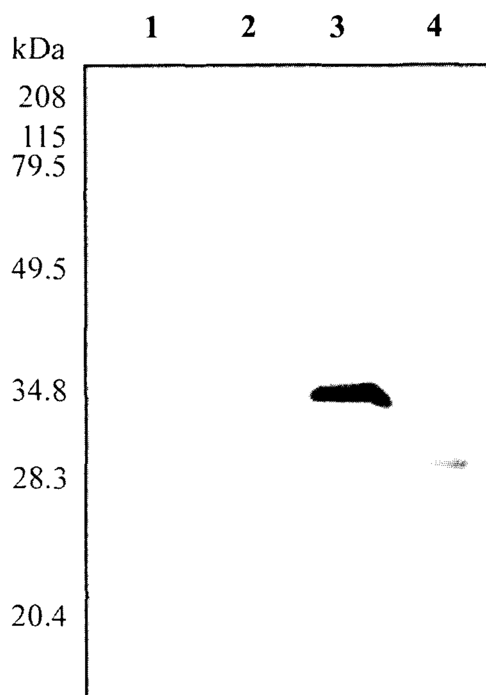

Fig. 1. Detection of DsbC-hBD-1 fusion protein (35.6 kDa) in cytoplasmic fractions of overnight cultures of recombinant *L. lactis* A164.
The total cell proteins from the cell extracts were separated by SDS-PAGE and detected by Western blotting with Penta-His antibodies. Lane 1, Pre-stained SDS-PAGE Marker (Bio-Rad); lane 2, total cell proteins from *L. lactis* A164 (pOri23); lane 3, total cell proteins from *L. lactis* A164 (pOED1); lane 4, Dsb-hBD-1 fusion protein digested with enterokinase. The positions of the molecular weight markers (in kilodaltons) are indicated on the left.

To isolate the recombinant mature hBD-1, the DsbC-hBD-1 fusion protein was purified from total cell extracts of *L. lactis* A164 carrying the plasmid pOED1 using nickel-affinity column chromatography. The DsbC-Tag was removed by digestion with enterokinase to obtain the cleaved mature hBD-1. When an n-terminal amino acid sequence analysis of the mature hBD-1 was performed, the sequences were found to be identical to those of the natural hBD-1 peptide (results not shown).

The bactericidal activities of the cleaved hBD-1 against the Gram-negative *E. coli* JM109 are shown in Fig. 2A. A strong bactericidal activity was observed for the cleaved mature hBD-1: at concentration of 2 μg/ml, 99% of the *E. coli* JM109 cells were inactivated. The DsbC-hBD-1 fusion protein also exhibited bactericidal activity against *E. coli* JM109, yet its activity was less than that of the cleaved hBD-1 at a given concentration (Fig. 2B).

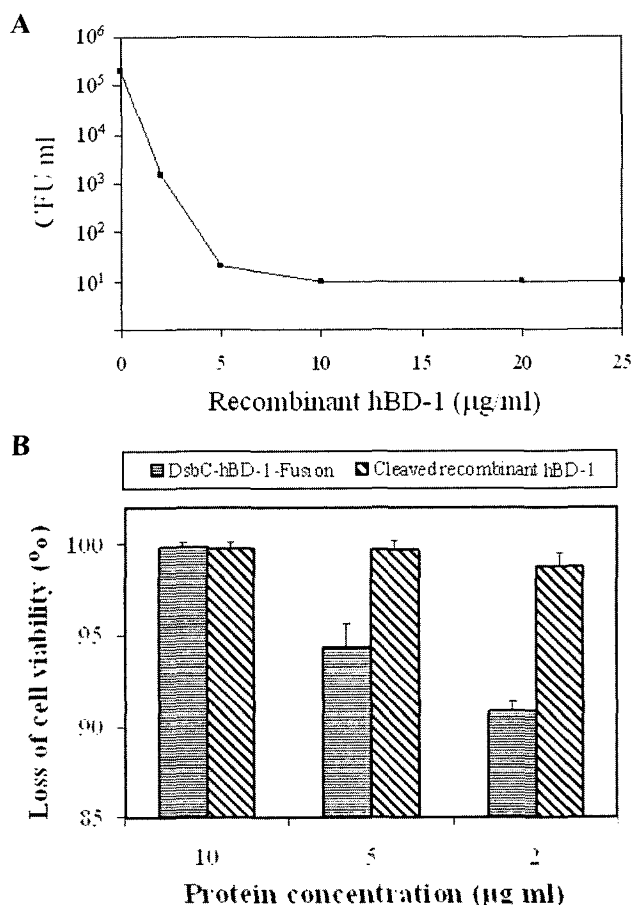

Fig. 2. Bactericidal activity of recombinant hBD-1 (A) and comparison of bactericidal activities of DsbC-hBD-1 fusion protein and cleaved mature hBD-1 (B).
The *E. coli* JM109 cells were incubated for 2 h at 37°C with the cleaved mature hBD-1 or DsbC-hBD-1 fusion protein, as described in Materials and Methods.

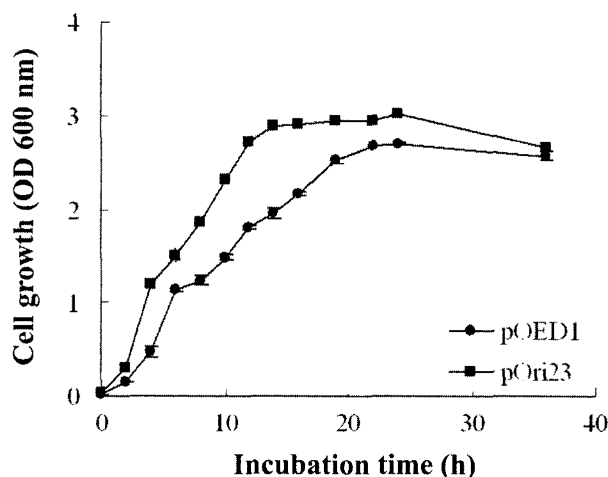

Fig. 3. Comparison of cell growth patterns of *L. lactis* A164 (pOED1) and (pOri23) in M17 broth at 30°C.

Comparison of the Cell Growth of *L. lactis* A164 Carrying pOED1 and pOri23

To investigate the toxicity of the DsbC-hBD-1 fusion protein against the host cells, the cell growth of *L. lactis* A164 carrying pOED1 and pOri23 was compared (Fig. 3). *L. lactis* A164 carrying the empty vector, pOri23, reached the stationary phase after 12 h incubation at 30°C in the M17 broth, whereas *L. lactis* A164 carrying the hBD-1 expression vector pOED1 required more time to reach the stationary phase. This delayed growth of *L. lactis* A164 expressing the DsbC-hBD-1 fusion protein may have been due to the partial antibacterial activity of the fusion protein against the host cells.

Production of Nisin Z from *L. lactis* A164 Carrying Recombinant Plasmid pOED1

To evaluate whether *L. lactis* A164 harboring the hBD-1 expression vector pOED1 produced nisin Z, the activity of nisin Z was determined in the culture supernatants of the wild-type *L. lactis* A164 and *L. lactis* A164 harboring pOED1 or pOri23 (empty vector) against *Weissella paramesenteroides* ATCC 40114 as the indicator strain. The activity in *L. lactis* A164 carrying pOED1 or the empty vector pOri23 was found to be the same as that in the wild-type (4,096 AU/ml), indicating that *L. lactis* A164 carrying pOED1 produced both nisin Z and hBD-1 simultaneously. In addition, the pOED1 appeared to be stable in *L. lactis* A164, since the viable cell count with and without erythromycin selection after growth in the M17 broth without erythromycin was not different (results not shown).

There have already been a few reports on the expression of antimicrobial peptides in *E. coli*, but no reports on Gram-positive or LAB, reflecting the difficulty of expressing bactericidal peptides in bacteria [12]. When the human α-defensin-1 gene was previously cloned and expressed in *E. coli* using a glutathione-*S*-transferase (GST) fusion-tag, no biological activity was detected [28]. Only hBD-3 has successfully expressed in *E. coli*, in which case its biological and biochemical properties were indistinguishable from those of naturally occurring hBD-3 [12]. In this study, the bactericidal activity of the recombinant hBD-1 peptide produced by the recombinant *L. lactis* A164 against *E. coli* was similar to that of a recombinant hBD-1 expressed by a baculovirus protein expression system (about 2 μg/ml) [29]. Consistent with a previous finding that NaCl inhibits the antibacterial activity of a synthetic hBD-1 [9], the activity of the recombinant hBD-1 produced by the recombinant *L. lactis* A164 was also reduced in the presence of 100 mM NaCl (data not shown). Consequently, all these features indicate that *L. lactis* A164 (pOED1) produced a biologically active hBD-1 that was indistinguishable from naturally occurring hBD-1.

In the present study, the heterologous production of hBD-1 in nisin Z-producing *L. lactis* A164 was achieved. Although the expressed DsbC-hBD-1 fusion protein exhibited partial antibacterial activity against the host cells, the fusion-tag system was found to be useful for expressing antimicrobial peptides in LAB. As potential therapeutics, the antimicrobial peptides produced by humans possess several desirable properties, including broad antimicrobial spectra and a swift killing effect against conventional antibiotic-resistant microorganisms such as methycillin-resistant *Staphylococcus aureus* (MRSA). As such, the use of hBD-1 either alone or in synergy with nisin Z can be expected to control not only Gram-positive pathogens, such as *Listeria monocytogenes*, but also bacteriocin- and antibiotic-resistant microorganisms such as *E. coli* O157:H7 and MRSA, respectively [28].

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