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Short crystallization paper

Crystallization and preliminary X-ray crystallographic analysis of the pediocin immunity protein (PedB) from *Pediococcus pentosaceus* at 1.35 Å resolution

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Abstract

PedB, a bacterial immunity protein conferring immunity to a newly identified pediocin (pediocin PP-1), was crystallized by the hanging-drop vapor diffusion method at 296 K. A 1.35 Å data set has been collected from a single crystal at 100 K using synchrotron-radiation source. The PedB crystals belong to the hexagonal space group $P6_2$ or $P6_4$, with unit cell parameters $a=b=62.2$, $c=39.9$ Å. Analysis of the packing density shows that the asymmetric unit probably contains one molecule with a solvent content of 33.8%.

Keywords: PedB; Bacteriocin; Pediocin; Immunity protein

Bacteriocins are ribosomally-synthesized antimicrobial peptides produced by many gram-positive bacteria like lactic acid bacteria. Most bacteriocins are generally synthesized as premature forms and can be divided into several classes depending on their post-translational processing and the mode of action [1–3]. Type I bacteriocins, of which a nisin is most well known, undergo post-translational modifications such as formation of dehydrated residues and lanthionine bridges [4]. In contrast, type IIa bacteriocins are matured by simple cleavage of a leader peptide, and characterized by a conserved YGNGVXC motif in the N-terminus [5–7]. These bacteriocins contain 37–48 residues and show potent activity against related Gram-positive bacteria, e.g., *Listeria monocytogenes* [8,9].

Pediocin secreted by *Pediococcus* spp. is a representative of the type IIa bacteriocins. Among pediocins, pediocin PA-1 of *Pediococcus acidilactici* PAC1.0 is the most extensively studied and its operon, pediocin PA-1 operon, is also well characterized [10–12]. Recently, we identified another pediocin (pediocin PP-1) operon from *Pediococcus pentosaceus* CBT-8, which is isolated from the Korean traditional fermentative food, kimchi. This operon encompasses four genes: *pedA*, *pedB*, *pedC*, and *pedD*. Since the deduced amino acid sequences of these genes show remarkable degree of homology (>98%) to those of corresponding genes in the pediocin PA-1 operon, the role of each protein in the pediocin PP-1 operon can be safely assumed based on the sequence homology: *pedA* encodes the 62-amino-acid precursor of pediocin PP-1 and *pedD* is implicated in maturation and translocation of pediocin PP-1, while the exact function of *pedC* gene product is still unknown. In general, type IIa bacteriocins are coexpressed with cognate immunity proteins (pediocin-like immunity proteins) composed of 88–115 amino acids, in order to protect the organism from the antimicrobial activity of its own bacteriocin [13–17]. In the pediocin PP-1 operon, the *pedB* gene product (PedB) corresponds to the immunity protein.

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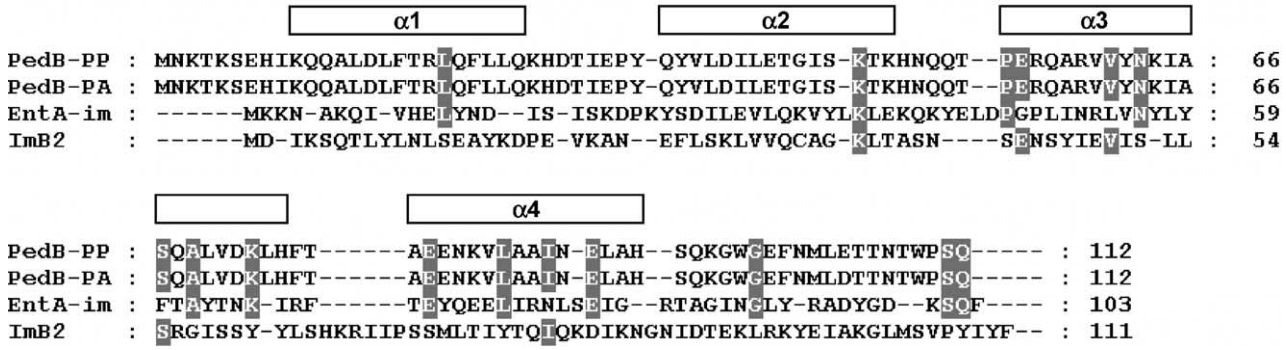

Fig. 1. Sequence alignment of PedB from *P. pentosaceus* (PedB-PP) with PedB from *P. acidilactici* (PedB-PA), EntA-im, and ImB2. Secondary structure of PedB predicted by Jpred server is presented above the alignment, and compared with the observed secondary structures of EntA-im and ImB2. The color scheme of white on dark grey indicates the consensus residue derived from the occurrence of >70% of a single residue at a given position.

PedB is a small positively charged protein with 112 amino acids and exists as a monomer in solution (data not shown). Although a number of pediocin-like immunity proteins have been known and characterized, so far, only two structures have been reported: immunity proteins conferring immunity to enterocin A (EntA-im) [18] and carnobacteriocin B2 (ImB2) [19]. Interestingly, EntA-im and ImB2 commonly adopt an antiparallel 4-helix bundle, strongly suggesting that this is a conserved scaffold in pediocin-like immunity proteins. In fact, despite low sequence identity between PedB and EntA-im/ImB2 (<15%), PedB was predicted to consist of four helices by secondary structure prediction using the Jpred server [20] (Fig. 1).

It is known that the immunity proteins usually contain high specificity against their cognate bacteriocins [21]. However, in contrast to the type IIa bacteriocins that exhibit high sequence conservation in their N-terminus, the degree of sequence homology between the pediocin-like immunity proteins is various (5–85%) and their mode of action still remains elusive [21,22]. The only thing known is that the C-terminal half, especially the possible flexible C-terminal end, of immunity proteins is an important determinant for the specific recognition of their cognate bacteriocins [23,24]. Thus, the three-dimensional structure of PedB will provide insights into how the immunity proteins inactivate their cognate bacteriocins and how the recognition between the immunity proteins and cognate bacteriocins is achieved in a highly specific manner. Here, we report the crystallization and preliminary X-ray analysis of the PedB at 1.35 Å resolution as a first step towards the structure determination.

The gene sequence encoding the PedB was amplified by polymerase chain reaction (PCR) using *P. pentosaceus* genomic DNA as a template. The PCR product was digested with *Bam*HI and *Sal*I and inserted into the pGEX4T-1 (Amersham Biosciences), generating pGEX-PedB and the plasmid was transformed into *E. coli* strain BL21 (DE3). Cells were grown up to OD_{600} of approximately 0.5 in Luria–Bertani media containing 0.1 mg/ml ampicillin (Duchefa) at 37 °C and expression was induced by 1 mM isopropyl-β-D-thiogalactoside (Duchefa) at 22 °C. After overnight induction, cells were harvested and resuspended in 1× PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.3). The cells were disrupted by sonication. After the cell debris was discarded by centrifugation at 20,000×*g* for 30 min, the supernatant was loaded to a column containing 3 ml of glutathione agarose (Amersham Biosciences). The column was washed with 20 ml of 1× PBS buffer and GST-PedB was eluted

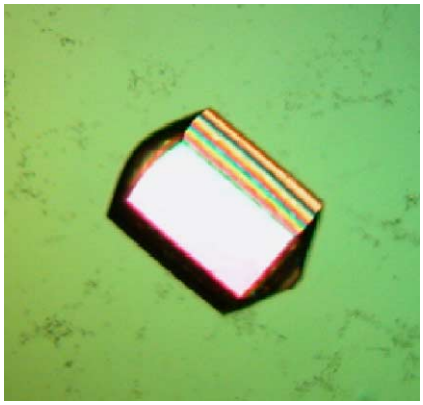

Fig. 2. A crystal of recombinant PedB from *P. pentosaceus*. Crystal dimensions are about 0.2 × 0.2 × 0.3 mm.

Table 1
Data collection and processing statistics

Source	Pohang Light Source, beamline 6B
Data collection temperature	100 K
Wavelength (Å)	0.91841
Space group	$P6_2$ or $P6_4$
Unit–cell parameter (Å)	$a=b=62.2$, $c=9.9$
Resolution range (Å)	30.0–1.35
Unique reflections	18,942
Redundancy	14.2
Completeness (>0 σ, %)	97.4 (95.2)
R_{sym} (%)[a]	7.3 (47.9)
Average $I/\sigma(I)$	53.3 (2.6)

Values in parentheses refer to the highest resolution shell, 1.351.40 Å.
[a] $R_{sym}=\sum|I_{obs}-I_{avg}|/\sum I_{obs}$.