

Molecular Cloning and Biological Characterization of cDNAs Encoding Novel Bioactive Peptides, Pilosulin 3 and Pilosulin 4, from a Species of the Australian Ant Genus *Myrmecia*

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Venom of Australian ants of the *Myrmecia pilosula* species complex contains two major allergenic peptides, pilosulin 1 and pilosulin 2. To obtain novel pilosulin-related bioactive peptides, mRNA of another *Myrmecia* species was subjected to RT-PCR in which the forward primer corresponded to a nucleotide sequence in the leader sequences of pilosulin 1 and pilosulin 2. As a result, we isolated cDNA clones encoding novel antimicrobial peptides that we named pilosulin 3 and pilosulin 4 (DDBJ/EMBL/GenBank accession nos. AB128064 and AB128065). The amino acid sequences of the leader sequences of the four pilosulin peptides are more than 84.0% identical. In addition, the nucleotide sequences of the four pilosulin cDNAs show sequence identities more than 89.7% and 73.3% in the 5'- and the 3'-untranslated regions, respectively. The deduced amino acid sequences of the four pilosulin mature peptides have low identities with the other three (13.2-33.3%), however.

In order to obtain sufficient quantities of pilosulins 3 and 4 mature peptides, these peptides were synthesized chemically by the Fmoc method (Fig 1). Synthetic pilosulin 3 and pilosulin 4 peptides caused a significant and dose-dependent histamine release: at a concentration of 5  $\mu$ M, they induced the release of about 50% of the total cellular histamine from rat peritoneal mast cells (Fig 2). To determine whether the histamine-releasing activity of pilosulins 3 and 4 is cytolytic or noncytolytic, LDH (lactate dehydrogenase) release was measured. Pilosulins 3 and 4 were devoid of LDH releasing ability at all concentrations tested. Thus pilosulins 3 and 4 might have a noncytolytic mode of activation. Besides pilosulins 3 and 4 displayed antibacterial activity against some bacteria species, that were not limited to Gram-negative nor to Gram-positive bacteria (Fig 3). Both pilosulins 3 and 4 showed no activity against yeast species, *Candida albicans* and *Saccharomyces cerevisiae*.

Reference:

1) Inagaki H, Masaaki Akagi, Hirokami T. Imai, Robert W. Taylor and Tai Kubo Molecular Cloning and Biological Characterization of Novel Antimicrobial Peptides, Pilosulin 3 and Pilosulin 4, from a Species of the Australian Ant Genus *Myrmecia*.(2004) *Arch. Biochem. Biophys.* 428: 170-178.

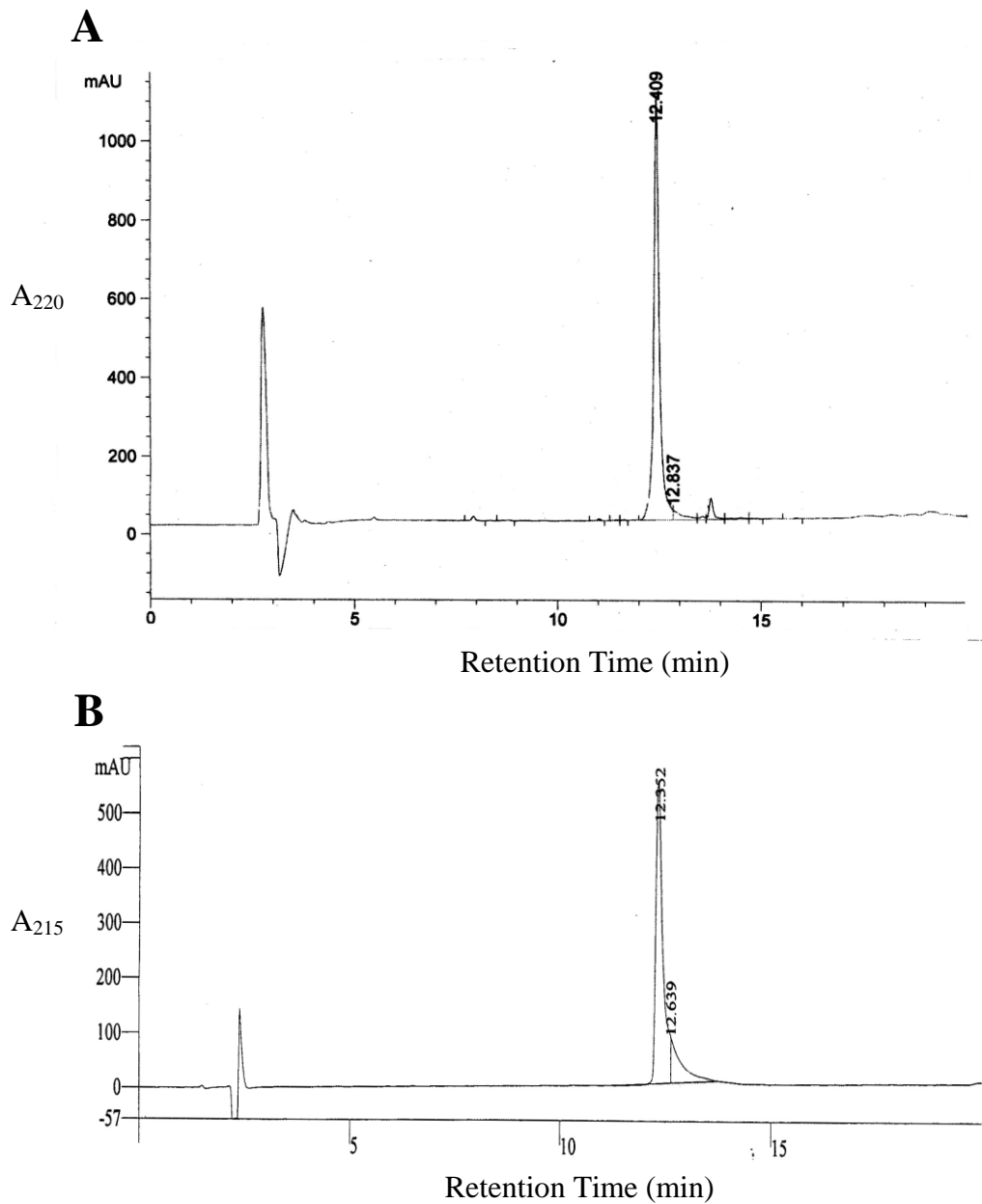


Fig. 1. Reversed -phase HPLC separation of synthetic pilosulin 3 (A) and pilosulin 4 (B) peptide.

(A) Pilosulin 3

Sequence: **IIGLVSKGTCVLVKTVCCKVLKQ-NH<sub>2</sub>**

(disulfide bridge between Cys10-Cys17)

Molecular Weight: 2455.2

Column: VyDAC C-18

Gradient: Linear 5.0%-65.0% CH<sub>3</sub>CN/H<sub>2</sub>O/0.1% TFA

(B) Pilosulin 4

Sequence: **FDITKLNKKLTKATCKVISKGASMCKVLFDKQ**

(disulfide bridge between Cys16-Cys26)

Molecular Weight: 4085.0

Column: Discovery C-18

Gradient: Linear 20.0%-40.0% CH<sub>3</sub>CN/H<sub>2</sub>O/0.1% TFA

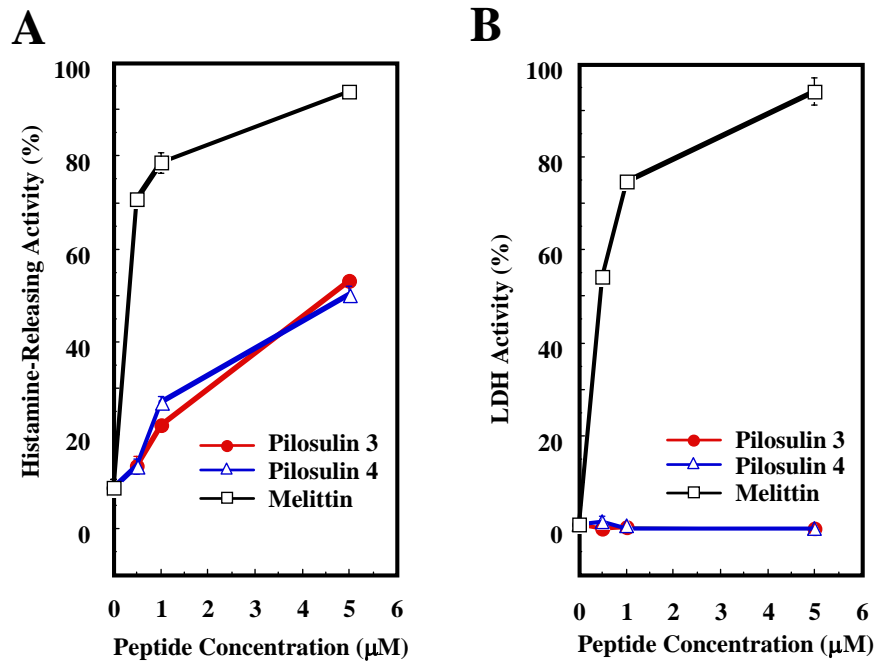


Fig. 2. Histamine (A) and LDH (B) release from rat peritoneal mast cells induced by pilosulin 3, pilosulin 4, and melittin. Ranges of concentration of pilosulin 3 ( ), pilosulin 4 ( ), and melittin ( ) peptides were tested for histamine-releasing or LDH activity from rat peritoneal mast cells.

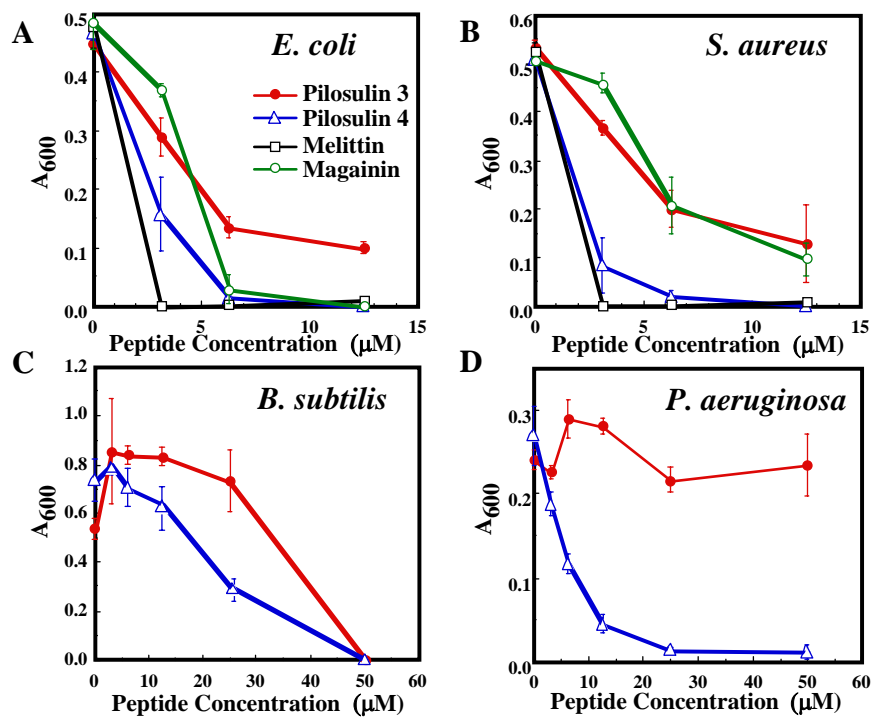


Fig. 3. Antimicrobial activities of pilosulin 3, pilosulin 4, melittin, and magainin. Ranges of concentrations of pilosulin 3 ( ), pilosulin 4 ( ), melittin ( ), and magainin ( ) peptides were tested for antimicrobial abilities against *E. coli* (A), *S. aureus* (B), *B. subtilis* (C), and *P. aeruginosa* (D).