2-16. Purification and cDNA Cloning of Insect Defensin from Lepidopteran Lavae, *Galleria mellonella*

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Here we report an antifungal peptide isolation from *G. mellonella* larvae. The peptide shows a high degree of sequence homology to an insect defensin, named heliomicin, first reported in Lepidoptera. The peptide was purified by a three-step procedure consisting of acid extraction, gel permeation chromatography and reversed-phase HPLC. First the N-terminal amino acid sequence of the purified peptide was determined by automated Edman degradation. The peptide possesses a molecular mass of 4720.57 Da and consists of 43 amino acid residues. And then the complete sequence of the peptide was disclosed by cDNA cloning performed via a combination of reverse transcriptase (RT)-PCR and 5'-RACE PCR. RT-PCR was performed with degenerated primer prepared based on N-terminal sequence. The cDNA consisted of 642 nucleotide and contained an open reading frame of 216 nucleotide corresponding to a protein of 72 residues. The first 29 amino acid in the precursor are highly hydrophobic and might represent a putative signal sequence. Northen blot analysis revealed that the peptide was specifically produced in the midgut and the fat body of *G. mellonella*. Also, it shows that mRNAs for insect defensin in *G. mellonella* is up-regulated in response to bacterial challenge. The antifungal activity of the peptide was assessed toward several yeasts and filamentous fungi through a micro dilution assay.