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PenBase, the shrimp antimicrobial peptide penaeidin database: Sequence-based classification and recommended nomenclature

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Abstract

Antimicrobial peptides play a major role in innate immunity. The penaeidins, initially characterized from the shrimp *Litopenaeus vannamei*, are a family of antimicrobial peptides that appear to be expressed in all penaeid shrimps. As of recent, a large number of penaeid nucleotide sequences have been identified from a variety of penaeid shrimp species and these sequences currently reside in several databases under unique identifiers with no nomenclatural continuity. To facilitate research in this field and avoid potential confusion due to a diverse number of nomenclatural designations, we have made a systematic effort to collect, analyse, and classify all the penaeidin sequences available in every database. We have identified a common penaeidin signature and subsequently established a classification based on amino acid sequences. In order to clarify the naming

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process, we have introduced a 'penaeidin nomenclature' that can be applied to all extant and future penaeidins. A specialized database, PenBase, which is freely available at http://www.penbase.immunaqua.com, has been developed for the penaeidin family of antimicrobial peptides, to provide comprehensive information about their properties, diversity and nomenclature.

Keywords: Penaeidin; Penaeid shrimp; Antimicrobial peptide; Crustacea; Sequence database; Nomenclature

1. Introduction

Antimicrobial peptides (AMP) play a major role in innate immunity, conserved in evolution, and present in all phyla of the living kingdom. They are mostly cationic and amphipathic molecules although they present a great diversity in terms of structural features as well as biological properties and functions. More than 850 antimicrobial peptides have now been discovered in plants, vertebrates, and invertebrates (Antimicrobial sequences database, AMSDb http://www.bbcm.units.it/~tossi/amsdb.html [1]) and very recently, two databases dedicated to antimicrobial peptides, the Antimicrobial peptide database (APD) [2] and ANTIMIC [3], were created. To date, in penaeid shrimps, two kinds of antimicrobial peptides have been fully characterized, namely the penaeidins from hemocytes [4] and anionic hemocyanin-derived peptides isolated from shrimp plasma [5]. In addition, recent studies utilizing a genomic approach led to the characterization of other antimicrobial effectors in shrimp, i.e. anti-LPS factor (ALF) and crustin [6–8].

Penaeidins were first characterized from *Litopenaeus vannamei* using a biochemical approach and molecular cloning techniques. Three peptides (initially named penaeidin 1, 2, and 3) were isolated in their active and mature forms (5.48–6.62 kDa) from the hemocytes of shrimp [4]. However, subsequent phylogenetic analysis indicated that penaeidin 1 and -2 could be classified in the same class [9]. Penaeidins are composed of an N-terminal proline-rich domain, followed by a C-terminal domain containing six cysteine residues organized in two doublets. This overall structure is quite unique among the AMP families [4]. The antimicrobial activity spectrum of penaeidin 2 and 3 from *L. vannamei* has been established through the production and analysis of recombinant peptides [10]. More recently, a new subgroup of penaeidins, named penaeidin 4 by their authors, has been identified in *L. vannamei* using a genomic approach [9], and a synthetic chemical peptide was produced to investigate and characterize the functional properties and spectrum of activity [11]. Penaeidins possess antibacterial activity predominantly directed against Gram-positive bacteria and antifungal activity against filamentous fungi. Very recently, the solution structure of the recombinant penaeidin 3 from *L. vannamei* and of the synthetic penaeidin 4 from *L. setiferus* have been determined, revealing the overall organization of the two domains and the arrangement of the disulfide bonds [12,13].

Recent studies have revealed the presence of penaeidin mRNAs in different penaeid shrimp species [6,8,14,15]. Moreover, in both *L. vannamei* and *L. setiferus*, the penaeidin subgroups (penaeidin 2, 3, and 4) were shown to be expressed in a single individual [6]. Expressed sequence tag (EST) analysis from hemocyte cDNA libraries has shown that penaeidin transcripts are very abundant. In fact, penaeidins appear to represent 10.7 and 20% of all the sequences isolated from hemocytes of *L. vannamei* and *L. setiferus*, respectively [6].

Most groups working on shrimp from different parts of the world have now isolated numerous penaeidin sequences mostly by genomic approaches, and this family appears to be ubiquitous among penaeid shrimp species. At the moment more than 200 sequences can be found in the EMB/GenBank/DDBJ databases, some of which have yet to be published. The current nomenclature for penaeidins is very confusing, with several different names given to the same gene product and distinct names (i.e. penaeidin number) given to variants of the same molecule. Therefore, we suggest that the 'penaeidin field' adopts a common nomenclature based on amino acid sequence similarity. Herein, we introduce a new database, PenBase, to provide comprehensive information about penaeidin properties, diversity and nomenclature. PenBase has been implemented according to the IMGT Scientific chart rules that are based on the IMGT-ONTOLOGY concepts [16].