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Short Communication

Molecular characterization and phylogenetic analysis of two antimicrobial peptides: Anti-lipopolysaccharide factor and crustin from the brown mud crab, *Scylla serrata*

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ABSTRACT

AMPs are evolutional weapons, widely used by animals and plants in their innate immune system to fend off invading microbes. The present study reports characterization of a new ALF isoform (Sc-ALF; **HQ638024**) and the first crustin (Sc-crustin; **HQ638025**) from the mud crab, *Scylla serrata*. The full-length cDNA of Sc-ALF consisted of 477 bp with an ORF of 123 amino acids and a putative signal peptide of 26 amino acids. Sc-ALF had a predicted molecular weight (MW) of 11.17 kDa and theoretical isoelectric point (*pI*) of 9.95. Two highly conserved cysteine residues and putative LPS binding domain were observed in Sc-ALF. Comparison of amino acid sequences with neighbor-joining tree indicated that Sc-ALF shared maximum similarity with ALF of *S. paramamosain*. Peptide model of Sc-ALF created using SWISS-MODEL server was found to consist of two α-helices crowded against a four-strand β-sheet. The full-length cDNA of Sc-crustin consisted of 433 base pairs with an ORF of 111 amino acids and a putative signal peptide of 21 amino acids. Comparison of amino acid sequences with a neighbor-joining tree revealed that Sc-crustin shared high identity with other known crustins characterized from *S. paramamosain*, *P. trituberculatus*, *H. araneus*, *C. maenas* and *F. chinensis*. A whey-acidic-protein domain could be detected at the C-terminus with the characteristic four disulfide core. Sc-crustin had a predicted MW of 10.24 kDa and a *pI* of 8.76. Peptide model of Sc-crustin created using SWISS-MODEL server indicated a random coiled structure that is with two possible β-sheets but no helices.

1. Introduction

The economic role of marine and freshwater crustaceans as a food source in the export market demands the need to augment fishery resources through the adoption of intensive culture practices. This has led to physiological stress on cultured organisms, often increasing the incidence of diseases [1]. Despite the development of safe and potent antibiotics, bacterial diseases remain a worldwide health crisis due to the emergence of multiple drug resistant pathogens [2]. The use of antimicrobial peptides (AMPs) as a therapeutic tool has been among the most promising avenues investigated to date for addressing antibiotic resistance [3]. AMPs are found in a wide range of prokaryotic and eukaryotic organisms from plants to human beings [4–7]. In crabs, several AMPs have been isolated and characterized, viz. the 6.5 kDa AMP and a cysteine-rich 11.5 kDa AMP (carcinin) from the shore crab, *Carcinus maenas* [8,9], callinectin from the blue crab, *Callinectes sapidus* [10], scygonadin, an anionic antimicrobial peptide from the mud crab, *Scylla serrata* [11], anti-lipopolysaccharide factor (ALF) and crustin from the mud crab, *Scylla paramamosain* [12,13] and arasin and hyasin from the spider crab, *Hyas araneus* [14]. Hemocytes have been proved to be the site of production and storage of AMPs in invertebrates such as horseshoe crabs, mussels and decapod crustaceans [9,15–18].

The brown mud crab, *Scylla serrata*, is a decapod crustacean of the brachyuran family, Portunidae. They are tolerant to wide range of environmental parameters and there has been a huge interest in the aquaculture of this species due to its high demand/price, high flesh content and rapid growth rates in captivity. Mud crabs are a highly delicious seafood commodity and are therefore an important candidate species for aquaculture. *S. serrata* is a well known commercial species in India, Philippines and Vietnam. Like many other decapod crustaceans [8,19–21], the mud crab also possesses broad-spectrum antibacterial activity in its hemolymph that constitutes part of its nonspecific defenses. However, there are hardly any published works on major AMP families (ALF and crustins) present in this species. The present study aims at the

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molecular characterization and phylogenetic analysis of two major families of AMPs, viz. anti-lipopolysaccharide factor and crustin from *S. serrata*.

2. Materials and methods

Healthy adults of *S. serrata* (~300 g body weight) were collected from the backwater stream along Vypeen, Kochi, India. Hemolymph was collected from the base of abdominal appendages using specially designed capillary tubes (RNase-free) rinsed using pre-cooled anticoagulant solution (RNase-free 10% sodium citrate, pH 7.0). Total RNA was extracted from the hemocytes using TRI reagent (Sigma) following manufacture's protocol. RNA was quantified by spectrophotometry at 260 and 280 nm. Only RNAs with absorbance ratios (A_{260}:A_{280}) greater than 1.8 were used for the present work. First strand cDNA was generated in a 20 μl reaction volume containing 5 μg total RNA, 1*x* RT buffer, 2 mM dNTP, 2 μM oligo d(T_{20}), 20 U of RNase inhibitor and 100 U of M-MLV reverse transcriptase (Ferementas, Inc.). The reaction was conducted at 42 °C for 1 h followed by an inactivation step at 85 °C for 15 min. PCR amplification of 1 μl of cDNA was performed in a 25 μl reaction volume containing 1*x* standard Taq buffer (10 mM Tris–HCl, 50 mM KCl, pH 8.3), 3.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM each primer and 1 U Taq DNA polymerase (Fermentas Inc.). PCR primers were designed using GeneTool software based on consensus sequence. Amplifications were performed using the primers (1) Sc-ALF-F (5′-ggacagaagaaacattgaggacgacgca-3′), Sc-ALF-R (5′-ggaaatcaaaaacatc-cattacaggtca-3′) and (2) Sc-Crus-F (5′-gagagcagaattagacactgt-3′), Sc-Crus-R (5′-atatagtataacataaccatacttc-3′). The thermal profile used

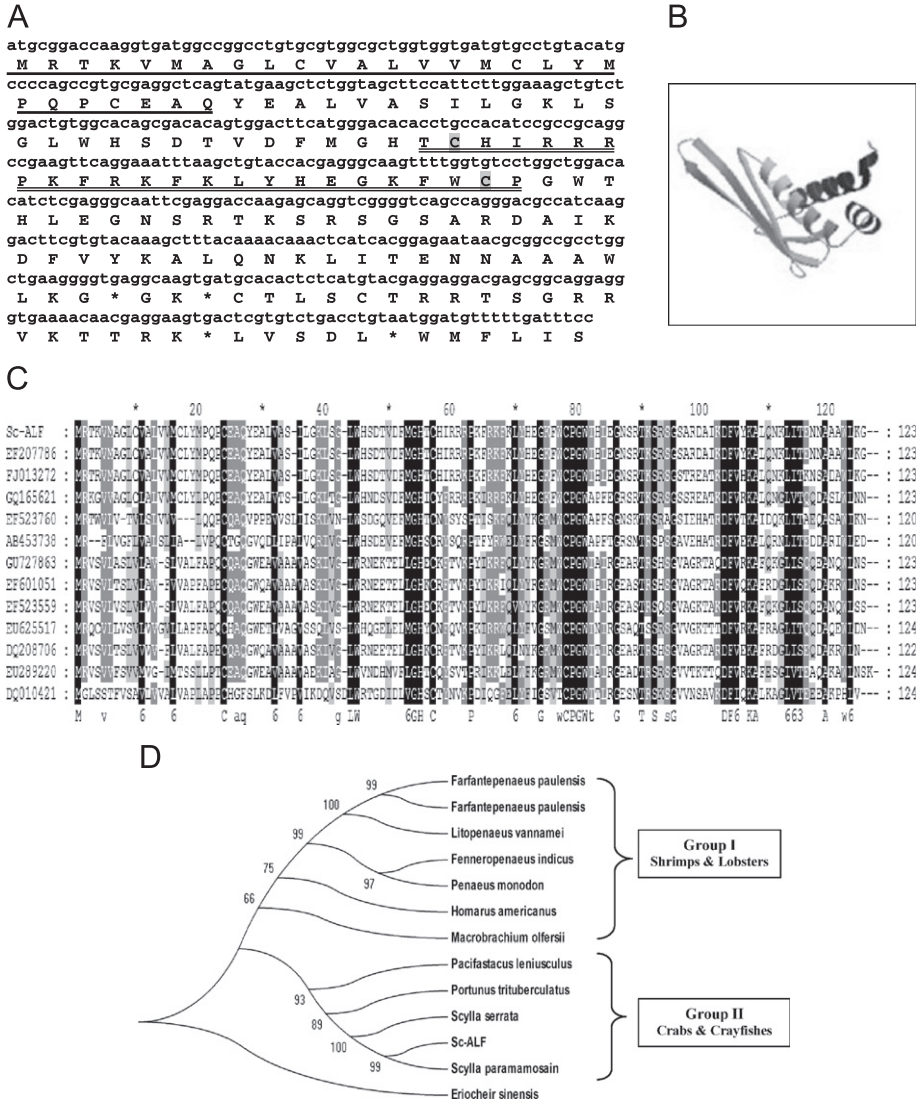

Fig. 1. (A) Nucleotide and amino acid sequences of Sc-ALF from the haemocyte of the mud crab, *Scylla serrata* (HQ638024). The underlined amino acid residues indicate a putative signal sequence. LPS binding domain characteristic of the ALF family is double underlined and the two conserved cysteine residues important for one disulfide bond (loop) formation are highlighted in gray. An asterisk is the stop codon. (B) Structural model of Sc-ALF (HQ638024) of *Scylla serrata* created using SWISS-MODEL server. (C) Multiple alignment of nucleotide sequence of the Sc-ALF (HQ638024) with other ALFs (*Scylla paramamosain* EF207786, *Scylla serrata* FJ013272, *Portunus trituberculatus* GQ165621, *Pacifastacus leniusculus* EF523760, *Marsupenaeus japonicas* AB453738, *Fenneropenaeus indicus* GU727863, *Farfantepenaeus paulensis* EF601051, *Penaeus monodon* EF523559, *Homarus americanus* EU625517, *Litopenaeus vannamei* DQ208706, *Macrobrachium olfersii* EU289220, *Litopenaeus stylirostris* DQ010421) obtained using GeneDoc programme Version 2.7.0. Black and gray indicate conserved sequences. (D) A bootstrapped neighbor-joining tree obtained using MEGA version 4.0 illustrating relationships between the deduced amino acid sequence of the Sc- ALF (HQ638024) with other ALFs of decapod crustaceans (*Scylla paramamosain* EF207786, *Scylla serrata* FJ013272, *Portunus trituberculatus* GQ165621, *Pacifastacus leniusculus* EF523760, *Fenneropenaeus indicus* GU727863, *Farfantepenaeus paulensis* EF601051, *Penaeus monodon* EF523559, *Homarus americanus* EU625517, *Litopenaeus vannamei* DQ208706, *Macrobrachium olfersii* EU289220, *Farfantepenaeus paulensis* EF601052. *Eriocheir sinensis* (GU014699) was used as an outgroup. Values at the node indicate the percentage of times that the particular node occurred in 1000 trees generated by bootstrapping the original deduced protein sequences.