

**MOLECULAR CLONING, RECOMBINANT EXPRESSION AND  
FUNCTIONAL CHARACTERIZATION OF A NOVEL ISOFORM OF  
ANTI-LIPOPOLYSACCHARIDE FACTOR FROM THE CRUCIFIX  
CRAB, *CHARYBDIS FERIATUS***

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**ABSTRACT**

Anti-lipopolysaccharide factor (ALF), one of the key effector molecules in innate immune system of crustaceans is a cationic Antimicrobial Peptide (AMP) having broad spectrum antibacterial and antiviral property. In the present study we identified and characterized one isoform of ALF homolog (Cf-ALF2) from the haemocytes of crucifix crab, *Charybdis feriatatus*. The partial cDNA of 294bp encoded for a mature peptide with 98 amino acid constituting a molecular mass of 10.923 kDa with a net charge of +9 and pI of 10.09. Furthermore, different from the previously identified ALF isoforms, Cf-ALF2 has a LPS binding domain with 23 amino acids including two cysteines instead of 22 amino acids and also exhibited less sequence similarity. Secondary structure of Cf-ALF2 predicted using PSIPRED constitutes two alpha helices packed against three beta strands. Multiple alignment performed for Cf-ALF2 with representatives of ALFs from limulids and decapod crustaceans using MEGA 6.0 revealed the existence of conserved regions within the sequence. The recombinant form of Cf-ALF2 (rCf-ALF2) was expressed as a fusion protein in *E. coli*, RosettaGami B DE3 pLysS using the pET-32a+ vector. The purified and refolded rCf-ALF2 protein revealed antimicrobial activity even

at 2.5  $\mu\text{M}$  against Gram-negative bacteria viz., *Pseudomonas aeruginosa*, *Aeromonas hydrophila* and *Edwardsiella tarda* and Gram-positive bacteria viz., *Bacillus cereus* and *Staphylococcus aureus*. Recombinant Cf-ALF2 was found to be non-haemolytic and non-cytotoxic even at a concentration of 8  $\mu\text{M}$  (250 $\mu\text{g}/\text{mL}$ ). These preliminary functional studies strongly suggest that Cf-ALF2 is a potent AMP against bacterial infection and might function as a promising therapeutic candidate in aquaculture and medicine.

**Keywords:** Antimicrobial peptides; Crab; Innate Immunity; Recombinant Expression; Anti-lipopolysaccharide factor.

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