ANTI-LIPOPOLYSACCHARIDE FACTOR (ALF): A BROAD SPECTRUM ANTIMICROBIAL PEPTIDE ESSENTIAL FOR SHRIMP IMMUNITY AGAINST BACTERIAL AND FUNGAL INFECTIONS

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Antimicrobial peptides are an essential component of the innate immune system of most organisms. Expressed sequence tag analysis from various shrimp (*Litopenaeus vannamei*) tissues revealed transcripts corresponding to two distinct sequences (ALFLv1 and ALFLv2) with strong sequence similarity to anti-lipopolysaccharide factor (ALF), an antimicrobial peptide originally isolated from the horse shoe crab *Limulus polyhemus*. Full-length cloning revealed a 528 bp transcript with a predicted open reading frame coding for 120 amino acids in ALFLv1, and a 623 bp transcript with a predicted open reading frame coding for 93 amino acids in ALFLv2. Transcript abundance in the various cDNA libraries, suggests that ALFLv1 is expressed at significantly higher levels than ALFLv2, thus ALFLv1 was selected for further analyses. A reverse genetic approach was implemented to study the *in vivo* role of ALFLv1 in protecting shrimp from bacterial, fungal and viral infections. Injection of double-stranded RNA (dsRNA) corresponding to ALFLv1 gene into the shrimp resulted in a significant reduction of ALFLv1 mRNA transcript abundance as determined by qRT-PCR. Following knockdown, shrimp were challenged with low pathogenic doses of *Vibrio penaeicida*, *Fusarium oxysporum* or white spot syndrome virus (WSSV) and the resulting mortality curves were compared with those obtained from injecting either saline, non-specific dsRNA (catfish gene) or a non-immune shrimp gene followed by experimental infection. A significant increase of mortality in the ALFLv1 knockdown shrimp was observed in the *V. penaeicida* and *F. oxysporum* infections when compared to controls, showing that this gene has a role in protecting shrimp from both bacterial and fungal infections. In contrast, ALFLv1 dsRNA activated the sequence-independent innate anti-viral immune response giving increased protection from WSSV infection. These results have demonstrated the usefulness of reverse genetics for the functional characterization of genes *in vivo*.

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