

ANTI-LIPOPOLYSACCHARIDE FACTOR (ALF) IN SHRIMP SPECIES: FROM CHARACTERIZATION TO BIOLOGICAL ACTIVITIES AND GENE EXPRESSION ANALYSES

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Introduction

Antimicrobial peptides (AMPs) are effector molecules that play important role in innate immune system. In all kingdoms, from bacteria to human, a variety of AMPs has been identified and characterized. Anti-lipopolysaccharide factor (ALF) is an AMP previously found in haemocytes of horseshoe crabs, *Limulus polyphemus* and *Tachypleus tridentatus*. This molecule is a small basic protein located in large granule haemocytes of horseshoe crab. It binds and neutralized the toxic lipid A moiety of LPS leading to inhibition of the endotoxin-mediated activation of the coagulation cascade. It exhibits strong antibacterial activity on the growth of Gram-negative bacteria. Studies in shrimp concern the characterisation of the antimicrobial activities and properties of the recombinant ALF and the analysis of its gene expression in response to infections.

Materials and methods

Recently, ALF cDNA clones were identified from the haemocytes of the black tiger shrimp, *Penaeus monodon* by Expressed sequence tag (EST) analysis (Supungul et al. 2002). A full-length cDNA clone containing the sequence of the ALF $Pm3$, the most abundant isoform of ALF found in *P. monodon*, was selected for expression via *Pichia pastoris* system. The region encoding for ALF mature peptide was amplified and cut with SnaBI and NotI in order to ligated to the pPIC9K plasmid. The SacI-linearized pALFPmK plasmid was then transformed to *P. pastoris KM71* to generate the recombinant ALFPm3 (rPmALF). ALFK9 *Pichia* transformant was selected by G418-sulfate resistace screening.

Results and Discussion

Large-scale production in fermentor provided around 200-300mg/L of recombinant ALF that was purified to homogeneity by weak cation exchange chromatography on a Sep-Pak[®] Accell[™] Plus CM cartridges and by reversed-phase HPLC on a SuperPac Sephasil

C₈ column. Then, the spectrum of antimicrobial activity of *Penmon-ALF3* was established *in vitro* against a range of microorganisms (Table I) as well as its *in vivo* effect on larvae.

Table I. Antimicrobial activity of the recombinant *Penaeus monodon* ALF

Microorganisms	Minimum inhibitory concentration (MIC) (μM)
Gram (+) bacteria	
<i>Bacillus megaterium</i>	0.08-0.12
<i>Micrococcus luteus</i>	3.07-4.61
<i>Staphylococcus aureus</i>	>35
Gram (-) bacteria	
<i>Enterobacter cloacae</i>	3.07-4.61
<i>Erwinia carotovora</i>	1.37-2.05
<i>Escherichia coli 363</i>	0.08-0.12
<i>Klebsiella pneumoniae</i>	2.05-3.07
<i>Salmonella thyphimurium</i>	6.91-10.37
<i>Vibrio alginolyticus</i>	0.27-0.40
<i>Vibrio anguillarum</i>	0.91-1.37
<i>Vibrio harveyi</i>	0.91-1.37
<i>Vibrio penaeicida</i>	>23.3
Filamentous fungi	
<i>Fusarium oxysporum</i>	2.07-3.05

MIC are expressed as the interval *a-b*, where *a* is the highest concentration tested at which the growth of the microorganism is not inhibited and *b* the lowest concentration that causes the 100% growth inhibition.

The spatio-temporal expression of *Penmon-ALF3* encoding gene has been studied in shrimps in response to microbial challenge to approach the function of this effector, and compared to penaeidin that is known to be constitutively expressed in granular cells. Due to its properties, these molecules are predominant candidates for potential therapeutic agents for prophylaxis and therapy of viral and bacterial infectious diseases.

Reference

Supungul P., Klinbunga S., Pichyangkura R., Jitrapakdee S., Hirono I., Aoki T., Tassanakajon A., 2002. Identification of immune-related genes in hemocytes of black tiger shrimp (*Penaeus monodon*). Marine Biotechnology 4: 487-494.

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