

Nawanith Klongkleaw 2009: Molecular Characterization of Complementary DNAs (cDNAs) and Expression of Anti-lipopolysaccharide Factor Genes in Giant Freshwater Prawn (*Macrobrachium rosenbergii*, de Man). Master of Science (Aquaculture), Major Field: Aquaculture, Department of Aquaculture. Thesis Advisor: Mr. Prapansak Srisapoom, Ph.D. 124 pages.

Full length of complementary DNAs (cDNAs) encoded for anti-lipopolysaccharide factor (ALF) genes of giant freshwater prawn were isolated using Rapid Amplification of cDNA Ends (RACEs) technique and surveying in cDNA libraries of haemocytes and androgenic gland. At least 3 different isoforms (Mr-ALF1, Mr-ALF2 and Mr-ALF3) of ALF were discovered. cDNAs of these isoforms contained nucleotide sequences of 794, 959 and 833 bp respectively. The open reading frames of these cDNAs were 345, 399 and 366 bp (114, 132 and 121 amino acid residues) respectively. Amino acid sequence analysis indicated that these cDNAs bearing the LPS binding motif which actually use to bind cell wall components of pathogens were identified. This motif was observed to situate between two conserved cysteines at the middle part of the protein molecules. The consensus pattern [(W/T/R)CP(G/S)W(T/A)] which similar to those of invertebrates ALFs was also found in Mr-ALF1, Mr-ALF2 and Mr-ALF3. In addition, one glycosylation site was shown in only Mr-ALF2 and Mr-ALF3. Nucleotide and amino acid sequence analysis demonstrated that Mr-ALF1 was closely similar to bristled river shrimp (*Macrobrachium olfersii*), corresponding to phylogenetic tree analysis. Mr-ALF2 was more similar to white shrimp (*Litopenaeus schmitti*) and kuruma prawn (*Marsupenaeus japonicus*), while Mr-ALF3 relatively homologous to american lobster (*Homarus americanus*) and bristled river shrimp (*M. olfersii*). Tissues expression analysis of Mr-ALF1 and Mr-ALF2 gene investigated by RT-PCR indicated that Mr-ALF1 gene was highly expressed in haemocytes, heart, mid gut and hepatopancreas. On the contrary, Mr-ALF2 gene was observed to be expressed lightly in haemocytes, eyestalk, fore gut, gills and heart. Transcriptinal responses of giant freshwater prawn Mr-ALF1 and Mr-ALF2 to *Aeromonas hydrophila* and  $\beta$ -glucan were investigated by quantitative real-time PCR. Mr-ALF1 was showed to be significantly up-regulated only in day 1 after injected with *A. hydrophila*. Interestingly, Mr-ALF2 showed the expression levels higher than that of control ( $P < 0.05$ ) at day 1, 3 and 7 after injection. Transcriptinal levels of Mr-ALF1 and Mr-ALF2 were also determined in prawns that experimentally fed with  $\beta$ -glucan supplemented and normal feed for 14 days. No significant differences of these ALFs between two prawn groups were observed during experimental periods ( $P \geq 0.05$ ).

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Thesis Advisor's signature