Identification of genes associated with Vp_PirAB-like toxin resistance in Litopenaeus vannamei

［課程博士・論文博士共通］

博士学位論文内容要旨

Abstract

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論文題目 Title	Identification of genes associated with Vp_PirAB-like toxin resistance in *Litopenaeus vannamei*		

Whiteleg shrimp, *Litopenaeus vannamei* accounts for about 75% of global farmed shrimp production. They are vulnerable to viral and bacterial diseases that cause mass mortality in shrimp farms. In the last decade, shrimp farmers have suffered from an infection disease, named acute hepatopancreatic necrosis disease (AHPND). AHPND has spread to several countries in Asia and South America, resulting in a great reduction of production. AHPND is caused by a pathogenic *Vibrio parahaemolyticus* carrying a plasmid encoding Vp_PirAB-like toxin which are identified as the virulence factor. Recently, the plasmid encoding Vp_PirAB-like toxin was found in other three AHPND-causing *Vibrio* species, but not *V. parahaemolyticus*.

Vp_PirAB-like composes of two genes, Vp_PirA-like (336 bp) and Vp_PirB-like (1,313 bp), which are co-transcribed and translated to two proteins Vp_PirA-like and Vp_PirB-like. The structural topology of Vp_PirA- and B-like toxins show similarity to *Bacillus thuringiensis* delta-endotoxin or Cry toxins, insecticidal proteins. In the previous study revealed that the virulence of Vp_PirAB-like toxins, unlike the virulence of normal bacterial exotoxins, they are heat stabile and formalin-resistant. Therefore, the formalin-killed cells of *V. parahaemolyticus* AHPND-causing strain D6 (FKC-VpD6) was used to select the Vp_PirAB-like toxin-resistant *L. vannamei* by oral administration. After two weeks of feeding, only 2.5% shrimp survived, then they were collected as toxin-resistance shrimp. It is interesting to figure out how can these shrimps be resistant to toxin virulence, which may be the advantage to control an AHPND infection in shrimp farming.

Previous studies have reported that the high expressions of some immune-related genes induce protection against AHPND. On the other hand, Vp_PirAB-like toxin and Cry toxins may use similar mechanisms for insertion into host cells and share sequence similarity of receptors. The studies of Cry toxins suggested that mutations of Cry toxin-receptors are the most common mechanism for insect resistance by altering the binding between toxin and receptor. To identify genes that are associated with Vp_PirAB-like toxin resistance, I am focusing on 1) immune-related genes that show specific response to Vp_PirAB-like toxin and 2) genetic variation of toxin-receptor candidates.

At first, stomach and hepatopancreas tissues of resistant shrimps (sur-FKC) were subjected to RNA sequencing. The differentially expressed genes (DEGs) between sur-FKC, AHPND-infected shrimp (Vp-inf), and non-treated shrimp (control) were identified. From a total of 79,591 genes, 194 and 224 DEGs were identified in the stomach and hepatopancreas transcriptomes, respectively. The expressions of ten selected DEGs were validated by qPCR. Only one gene, DN21485 (a gene homologous to *L. vannamei* anti-lipopolysaccharide factor AV-R isoform (*Lv*ALF AV-R)) was expressed significantly more strongly in the hepatopancreas of sur-FKC than in the other groups.

Moreover, searching of Cry toxin receptor homologs was performed to identify the gene candidates of Vp_PirAB-like toxin receptors. Five Cry toxin receptors (cadherin, alkaline phosphatase, aminopeptidase N, and ATP-binding cassette transporter subfamily C) were used as query genes for homology search against *L. vannamei* control/Vp-inf transcriptome. A total of thirteen Vp_PirAB-like toxin-receptor candidates were identified.

From the results of DEG analysis, *Lv*ALF AV-R was further investigated to clarify the association of *Lv*ALF AV-R and shrimp resistant to Vp_PirAB-like toxin. In the hepatopancreas, expression of *Lv*ALF AV-R mRNA was not affected by VpD6-immersion or dietary of FKC-VpN7 (*V. parahaemolyticus* non-AHPND causing strain) but was highly induced during FKC-VpD6 feeding. Moreover, significantly higher expression of *Lv*ALF AV-R was also observed in shrimp that survived in three other trials of FKC-VpD6 selection. Histological analysis of four resistant shrimps showed that three of them exhibited normal characteristic of hepatopancreas cells without signs of AHPND.

To investigate the function of *Lv*ALF AV-R that reacts to toxin, the recombinant protein, rALF AV-R was produced for *in vitro* and *in vivo* studies. rALF AV-R bound to LPS, PGN, Gram-negative bacteria, and some Gram-positive bacteria in ELISAs. But rALF AV-R did not interact with native Vp_PirAB-like toxin in an ELISA or a Far-Western blot analysis. For *L. vannamei* orally fed rALF AV-R for 3 days, the survival rate following challenge with VpD6-immersion was not significantly different from that of shrimp fed two control diets (rGFP and PBS).

Genetic variations of Vp_PirAB-like toxin receptor candidates between resistance and other groups were analyzed. Homologous genes of pre-identified receptor candidates from control/Vp-inf were identified in sur-FKC transcriptome. Between control/Vp-inf and sur-FKC, 4 genes (cadherin-like 1, APN-like 1, ABCC2-like 1 and 2) which showed high identity but not 100%, were selected for further analysis. Validation by sequencing showed that only 4326 C/A SNP in cadherin-like 1 gene was detected in pooled cDNAs of resistant shrimp according RNA-seq data, whereas no variation was detected in the other three genes. SNP 4326 C/A was genotyped in the cDNA of resistance group (3 trials) and control/Vp-inf group. The frequencies of genotype AA, AC, and CC in resistance group were 64%, 36%, and 0%, respectively, while those of control/Vp-inf group were 17%, 0%, and 83%, respectively. In addition, SNP 4326 C/A was genotyped in genomic DNA of susceptible and resistance groups. Association analysis indicated that the genotype frequency distributions between two groups was significantly different ($P < 0.05$). In the susceptible group, the AA, AC and CC genotypes were 45%, 34%, and 21%, respectively. The frequency distributions in the resistance group were 64%, 36%, and 0%, respectively. Interestingly, the genotype CC was not observed in resistant group.

Lastly, to determine the stability of resistance phenotype, I had re-challenged the survival shrimp with dietary FKC-VpD6. The result showed that all shrimp died in 3 days after re-challenging. These results indicate that the resistant characteristic of these collected shrimp is not permanently expressed.

In conclusion, *Lv*ALF AV-R plays an indirect role in shrimp resistant to Vp_PirAB-like toxin by promoting other shrimp molecules to directly inhibit virulence of toxin. On the other hand, the SNP 4326 C/A of cadherin-like 1 gene might be used as a genetic marker to select Vp_PirAB-like toxin-resistant shrimp, although this SNP is not directly related to the resistance mechanism.