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ORIGINAL ARTICLE

Microarray Analyses of Shrimp Immune Responses

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Abstract Shrimp aquaculture is one of the major food-producing industries in the world. However, it is being impacted by several problems including diseases, antibiotic use, and environmental factors. The extent of the effects of these problems in the immune system of the shrimp at the molecular level is just beginning to be understood. Here, we review the gene expression profile of shrimp in response to some of these problems using the high-throughput microarray analysis, including white spot syndrome virus, yellow head virus, *Vibrio* spp., peptidoglycan, oxytetracycline, oxolinic acid, salinity, and temperature.

Keywords Expressed sequence tag · Virus · Bacteria · Immunostimulant · Antibiotics

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Introduction

During the past two decades, several high-throughput methods have been established to understand how environmental conditions affect gene expression. These include expressed sequence tag (EST) analyses, suppression subtractive hybridization (SSH), differential hybridization, serial analysis of gene expression, microarray, and two-dimensional electrophoresis (2-DE). In the field of shrimp immunology, EST analyses have been used for several species, including *Litopenaeus vannamei*, *Litopenaeus stylirostris*, *Penaeus monodon*, *Marsupenaeus japonicas*, and *Fenneropenaeus chinensis* (Rojtinnakorn et al. 2002; Shen et al. 2004; de Lorgeril et al. 2005; Tassanakajon et al. 2006; Leu et al. 2007; Preechaphol et al. 2007; Pongsomboon et al. 2008a). The ESTs are derived from cDNA library and SSH clones constructed from various tissues, such as whole postlarvae, hepatopancreas, hemocytes, and lymphoid organ. Shrimp ESTs provide a first observation to describing the host differential expression at the transcriptional level at different experimental conditions. Because the shrimp whole genome has not yet been resolved, large collections of shrimp ESTs are valuable not only for gene identification, but also for probes for cDNA microarrays.

Microarrays have been used to investigate gene expression by white spot syndrome virus (WSSV), which causes enormous economic damage to the shrimp aquaculture industry worldwide. Global viral gene expression profiles have been examined for three white spot syndrome virus strains (WSSV-Taiwan, WSSV-Thailand, and WSSV-China) of experimentally infected *P. monodon* and crayfish (*Cambarus clarkia*) (Wang et al. 2003; Tsai et al. 2004; Marks et al. 2005; Lan et al. 2006). Furthermore, using viral DNA microarray database obtained from different experimental treatments, several specific WSSV properties

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have been characterized including latency-related genes, immediate-early genes, putative promoter motifs, and the most highly expressed viral gene (Khadijah et al. 2003; Liu et al. 2005; Marks et al. 2006; Wang et al. 2007).

In aquaculture, microarrays have been used to investigate host physiological modulations, including development (Darias et al. 2008; Gahr et al. 2008), dietetics (Kirchner et al. 2007; Leaver et al. 2008), environmental sciences (Chou et al. 2008; Steinberg et al. 2008; Ruggeri et al. 2008), and genetic divergence among populations (Larsen et al. 2007; Jeukens et al. 2009). In recent years, there has been an increasing attention on pathogen–host interactions, particularly on the host immune response against invaders. Microarray analyses have provided important insights into the regulation of biodefense mechanisms of aquaculture animals in response to different stimuli (Matsuyama et al. 2007a, b; Darawiroj et al. 2008; Djordjevic et al. 2008; Schiøtz et al. 2008; Peatman et al. 2008). Recently, several shrimp microarrays have also been constructed from studies of different organs and different shrimp species. Dhar et al. (2003) constructed a low-density microarray where the glass chips contained 100 clones obtained from WSSV-infected shrimp hepatopancreas ESTs. Wang et al. (2006a) constructed an *F. chinensis* cDNA microarray obtained from 3,136 cDNA targets form a cephalothorax cDNA library of normal shrimp and SSH libraries of WSSV-infected shrimp. The generation of the first *L. vannamei* cDNA microarray encompassed 2,469 ESTs from standard cDNA libraries and SSH libraries from three tissues under different stimulus (Robalino et al. 2007). The first generation of *P. monodon* cDNA microarray chips contained 3,853 individual cDNA fragments originating from a normal hemocyte cDNA and six SSH libraries (de la Vega et al. 2007). Pongsomboon et al. (2008b) and Fagutao et al. (2008) used a cDNA microarray composed of 2,036 ESTs collected from healthy and WSSV-infected hemocyte cDNA libraries of *P. monodon* and *Marsupenaeus japonicus*. Zeng and Lu (2008) demonstrated the gene expression of WSSV-infected crayfish (*Procambarus clarkia*) hemocytes using nylon membrane-based *P. clarkia* cDNA microarray. Their arrays showed that shrimp gene expression and immune responses associated with a variety of experimental conditions including immunostimulations, stress conditions (hypoxic, hyperthermic, and hypoosmotic responses), WSSV infection, yellow head virus (YHV) infection, and *Vibrio anguillarum* infection (Dhar et al. 2003; Wang et al. 2006; de la Vega et al. 2007; Robalino et al. 2007; Fagutao et al. 2008; Pongsomboon et al. 2008b; Robalino et al. 2007; Wang et al. 2008; Wongpanya et al. 2007; Zeng and Lu 2008). Large percentages of the differentially expressed genes in these reports (47% to 72%) had no significant similarity to any proteins from other organisms and lack identifiable functional domains. These results suggest that shrimp possesses many immune-related genes of unknown function. In the following sections, we review six categories of shrimp immune responses against different stimuli as revealed by microarray analyses. Three of these categories are about shrimp immune modulations caused by three shrimp major infectious pathogens (WSSV, YHV, and *V. anguillarum*), while the other three are about the host immune regulation in response to immunostimulation, antibiotic treatment and environmental stress.

Immune-Related Gene Expression Following WSSV Infection

White spot syndrome (WSS) causes enormous economic damage to the shrimp aquaculture industry worldwide. The causative pathogen of WSS is white spot syndrome virus (WSSV), which is a large dsDNA virus. It is thus essential to elucidate the interactions between WSSV and host, both the host immune response and the WSSV pathogenesis. Some recent studies have investigated the molecular mechanisms in WSSV-infected shrimp. Leu et al. (2007) performed a comparative EST analysis of differentially expressed genes in normal and WSSV-infected *Penaeus monodon* postlarvae. This study suggested that WSSV infection modulates the expressions of various kinds of genes involved in the immune response such as genes involved in oxidative activity, protein synthesis, energy metabolism, glycolytic pathway, and calcium ion homeostasis. To identify host genes that are involved in WSSV resistance, several research groups used SSH to identify differentially responsive genes in WSSV-resistant *L. vannamei* and *M. japonicus* (He et al. 2005; Pan et al. 2005; Zhao et al. 2007). Most strongly expressed genes in subtractive libraries function in antiviral activity, prophenoloxidase (proPO) system, lysosomal proteolytic system, non-self recognition system, clotting system, apoptosis and antioxidant enzyme. Furthermore, based on the protein expression profiles of WSSV-infected and WSSV-free white shrimp using 2-DE, several differentially expressed proteins were identified. These proteins are involved in mitochondrial pathways, energy production, calcium homeostasis, nucleic acid synthesis, glycolysis/gluconeogenesis pathways, and other cellular processes (Wang et al. 2007).

The microarray technique was also applied to discover differentially expressed host genes in response to WSSV infection (Dhar et al. 2003; Wang et al. 2006, 2008; Robalino et al. 2007; Zeng and Lu 2008). However, the shrimps used in these studies were all WSSV susceptible, while the challenged agent was infectious WSSV. Therefore, the responsive gene profile may not only reflect the

host immune response, but also the WSSV pathogenesis and host physiological stress response. At present, the genes involved in the host immune response and WSSV pathogenesis, as well as the coordinated interaction between these sets of genes remain unclear. Nevertheless, these studies identified differentially expressed global host responses after WSSV infection.

Robalino et al. (2007) established tissue-specific transcriptional profiles of four tissues (hemocytes, hepatopancreas, gills, and muscle) of WSSV-infected *L. vannamei* by using a microarray platform. A cluster analysis indicated that the host gene response patterns of hemocytes and gills were highly similar, while those of two other tissues were different. The similar gene response patterns of hemocytes and gills is thought to be due to the fact that hemocytes and gills are derived from ectodermal and mesodermal tissues, which are major targets of WSSV, while tissues of endodermal origin are more resistant to WSSV infection (Chang et al. 1996; Wang et al. 1999). This apparently prompted the authors to choose the hepatopancreas as the best organ for examining the immune response after WSSV infection. The hepatopancreas was collected from control and experimental groups 40 h after injecting a lethal dose of WSSV, and total RNA was extracted. Although the authors did not mention the health status of the WSSV-infected shrimp, the WSSV viral gene expression profile showed that several WSSV structural protein genes such as *wsv 421*, *wsv311*, *wsv465*, and *wsv077* corresponding to *vp28*, *vp26*, *vp136B*, and *vp36A* and very late gene, *wsv230* have already been detected. This suggests that the shrimp were at the late stage of WSSV replication, but not at the moribund stage (Tsai et al. 2004; Wang et al. 2007). A total of 64 differentially expressed host genes were identified while 38% are novel genes without known function. WSSV infection up-regulated the genes for anti-lipopolysaccharide factor, cathepsin-like cysteine protease and lysozyme, and anti-apoptotic-related gene (platelet-derived growth factor). Microarray analyses revealed several immune-related genes that were up-regulated in response to WSSV infection in crayfish and Chinese shrimp (Dhar et al. 2003; Zeng and Lu 2008; Wang et al. 2006, 2008). These include the genes for lipopolysaccharide-β-1,3 glucan-binding protein, chaperone-related genes (chaperonin, heat shock protein 70, heat shock protein 90, calreticulin precursor, and endoplasmin precursor), and three kinds of serine protease inhibitor, which can function as defense components.

These microarray results, together with the results of other shrimp gene studies, indicated not only the importance of innate immunity for defense against WSSV, but also the novel antiviral functions of known and unknown proteins in innate immunity. For example, shrimp anti-lipopolysaccharide factor is a well-known antimicrobial peptide against bacteria and fungi, such as *Vibrio* species and *Fusarium oxysporum* (Somboonwiwat et al. 2005; de la Vega et al. 2008). Liu et al. (2006b) found that crayfish anti-lipopolysaccharide factor from crayfish (*Pacifastacus leniusculus*) can interfere with WSSV replication in vitro and in vivo. Therefore, anti-lipopolysaccharide factor may also have an antiviral potential against WSSV in penaeid shrimp.

Microarrays analyses have shown that, while WSSV triggered the expressions of some host genes, it repressed other host genes. Some of these genes function in anti-oxidative stress system (glutathione-*S*-transferase and thioredoxin-related genes), JAK/STAT pathway (signal transducer and activator of transcription), energy metabolism (arginine kinase), and glycolysis pathway (phosphopyruvate hydratase) (Wang et al. 2006, 2008; Robalino et al. 2007; Zeng and Lu 2008). Insights into anti-oxidative stress system showed that WSSV can modulate these host defense mechanisms. Free radicals, also called reactive oxygen species (ROS), are involved in immune responses against bacteria, fungi, and viruses in crustaceans (Munoz et al. 2000). In order to prevent cell harm caused by ROS accumulation, the anti-oxidative system must associate well with the oxidative system during the defense process (Mohankumar and Ramasamy 2006). The anti-oxidative stress system has been shown to be down-regulated after WSSV infection, leading to a significant increase of oxidative stress in infected shrimp (Mohankumar and Ramasamy 2006; Mathew et al. 2007).

The JAK/STAT signaling pathway has been shown to be involved in immune- and stress-induced responses in various organisms (Loo and Gale 2007; Pastor-Pareja et al. 2008). In the lymphoid organ of WSSV-infected shrimp, the expression of STAT was decreased, but its activation (i.e., phosphorylation) was increased (Chen et al. 2008b). This suggests that WSSV uses STAT to enhance its immediate early gene expression in infected shrimps (Liu et al. 2007b). Together, these results indicated that WSSV can benefit from the host JAK/STAT immune response. This is a good example of how WSSV can exploit the host immune response. In addition, this may also be the reason why WSSV replication can be triggered in shrimp under environmental stress (reviewed below).

Immune-Related Gene Expression Following YHV Infection

Yellow head virus (YHV), the pathogen of yellow head disease syndrome, is an enveloped, rod-shaped virus containing positive-sense stranded RNA genome (Rattanarojpong et al. 2007). Like WSSV, YHV also cause serious mortalities and economical loss in farmed penaeid shrimp. In 2002, YHV was classified as a species under a new family called

Roniviridae (genus *Okavirus*) within the order Nidovirales (Sittidilokratna et al. 2002; Walker et al. 2005). Although YHV have been considered as a highly virulent shrimp pathogen, the global host gene/protein responses involved in the host–YHV interaction are still limited and poorly described. So far, there is one transcriptomic study and two proteomic studies to clarify the differentially expressed host responses following YHV infection using microarray and 2-DE approaches, respectively (Rattanarojpong et al. 2007; Bourchookarn et al. 2008; Pongsomboon et al. 2008b). Because of the limitation of host protein abundance, few host protein spots (18 to 33 spots) with significant alterations after YHV infection can be identified and sequenced. On the other hand, fluorescent cDNA probes prepared form the higher abundance of mRNA can increase the sensitivity of detection using microarray platform.

Pongsomboon et al. (2008b) investigated responsive genes in *P. mondon* hemocytes from YHV-infected and control shrimp at different times (0.25, 6, 24, and 48 hour post infection (hpi). To exclude elements that did not have at least a twofold change in at least one time course after injection, 105 YHV responsive genes were filtered from 2,028 analyzed gene probes spotted on microarray chip and revealed five differentially expressed transcription patterns (cluster I–V). Here, over ten responsive genes were shrimp immune-related genes, such as crustin-like peptide, penaeidin, hemocyte kazal-type proteinase inhibitor, transgulaminase, anti-lipopolysaccharide factor, WAP domain-contained protein, C-type lectin, prophenoloxidase, and cathepsin L-like cysteine peptidase. In cluster I, the most responsive host genes at 48 hpi were ribosomal proteins and unknown proteins. There were two immune-related genes, ferritin and carboxylesterase, which are involved in detoxification in this cluster. In addition, immune-related genes function in proPO cascade, clotting system, antimicrobial peptides, and pathogen recognition were significantly down-regulated at 24 and 48 hpi and grouped into cluster IV. The down-regulation during the late infection stages may have been caused by YHV pathogenesis due to YHV-infected shrimp. Based on this reason, it has been hypothesized that genes up-regulated at the late infection stage may be involved in YHV pathogenesis or host physiological stress response.

Interestingly, five genes grouped into cluster V showed relatively consistent high expression throughout the YHV infection, although the expression patterns in cluster V were slightly decreasing compared with the earlier stage. Just one of these genes was a known protein, cathepsin L-like cysteine peptidase, which is involved in apoptosis, and others were hypothetical proteins and unknown proteins. In this study, again, 50 of 105 YHV responsive genes (around 47%) were also without any homolog with known gene/protein.

Immune-Related Gene Expression Following *Vibrio* Infection

In addition to viral diseases, bacterial diseases such as vibriosis are also a concern of the shrimp aquaculture industry. Vibriosis causes high mortalities and serious economic damage in both shrimp hatcheries and grow-out ponds. The major causative agents of vibriosis are *Vibrio* spp., including *Vibrio campbellii*, *V. anguillarum*, *Vibrio harveyi*, *Vibrio alginolyticus*, and *Vibrio parahaemolyticus* (Lightner 1988; Saulnier et al. 2000; Somboonwiwat et al. 2006).

In 2006, George and colleagues found that after treatment with *V. harveyi*, *P. monodon* showed higher resistance to WSSV infection. However, a prior non-lethal WSSV infection can increase the susceptibility to *V. campbellii* infection in *L. vannamei* due to rapid *Vibrio* multiplication (Phuoc et al. 2008). In this dual infection, the *V. campbellii* load was significantly higher, but no WSSV load, rather than single bacterial infection. These studies suggest that shrimp have similar immune responses and coordinate their responses to these two pathogens (Phuoc et al. 2008; Wang et al. 2008).

Wang et al. (2006) used a 3,136-gene microarray to compare the transcriptomes of Chinese shrimp (*F. chinensis*) challenged with WSSV and heat-killed *V. anguillarum* at 6 and 12 hpi. Interestingly, 155 genes, of which 77% had unknown functions, were differentially expressed at 6 hpi in response to the inactivated *Vibrio* but not to WSSV, which shows that that the responsive gene profile was caused by the host immune response rather than pathogenesis. The known genes included proteases, protease inhibitors, chaperones, nucleic acid-binding proteins, and transporters. Lysosome-related genes (beta-hexosaminidase, CUB-serine protease, and saposin-related protein) were up-regulated two- to tenfold by inactivated *Vibrio* but only at 6 hpi. A similar lysosome-related immune response was observed in *V. harveyi*-infected *P. monodon* by using differential display PCR (Somboonwiwat et al. 2006). This suggests that different immune modulation responses can be triggered by different pathogens. Otherwise, compared with inactivated *Vibrio* challenged, infectious WSSV may modulate the expression of host cell genes to enhance or inhibit some host responses.

Furthermore, 188 genes, of which 67% had unknown functions, were differentially expressed in response to both WSSV and inactivated *Vibrio*. Just 1 of 62 known genes in this group was down-regulated at 6 and 12 h after inactivated *Vibrio* challenge. On the other hand, although 62 known genes were all up-regulated at 6 h post WSSV infection, significant down-regulation or suppression was investigated at 12 h post WSSV infection. This may be caused by WSSV pathogenesis. Therefore, it is important to

consider the relationship between the host immune responses and the pathogenesis of invaders. Even so, the up-regulated genes at 6 hpi in this group appear to be involved in the shrimp defense mechanism against both pathogens, whereas WSSV can suppress these responses at 12 h post infection. Another interesting observation was that hemocyanin expression was significantly suppressed in WSSV-treated shrimp, whereas it was up-regulated in inactivated *Vibrio*-treated shrimp. This clearly shows that hemocyanin, an antiviral, antibacterial, and antifungal protein (Zhang et al. 2004; Pan et al. 2005; Lei et al. 2008), is also involved in the defense against inactivated *Vibrio*. Wang et al. (2006a) also observed repression of hemocyanin in WSSV-infected Chinese shrimp (at 6 hpi and moribund satiations) by cDNA microarray. Using SSH, Pan et al. (2005) found that the antiviral activity of WSSV-resistant shrimp may be due to strong expression of hemocyanin in the hepatopancreas. Therefore, WSSV may suppress the expression of hemocyanin in order to complete its viral replication in WSSV-susceptible shrimp.

Immune-Related Gene Expression Following Immunostimulation

Immunostimulants, agents which can increase the resistance of the host by enhancing the nonspecific immune response, are comprised of several main groups: live bacteria, killed bacterial cells, β-1,3 glucans, peptidoglycans (PG), lipopolysaccharides, and selected plant extracts (Sakai 1999; Smith et al. 2003; Montero-Rocha et al. 2006). Several studies have reported positive consequences of immunostimulation on shrimp disease resistant capacities, e.g., increasing phenoloxidase activity, antibacterial activity, phagocytic activity, clearance efficiency against pathogens, and antioxidant activity (Song et al. 1997; Huang and Song 1999; Takahashi et al. 2000; Campa-Córdova et al. 2002; Rattanachai et al. 2005; Montero-Rocha et al. 2006; Okumura 2007; Bacano Maningas et al. 2008; Fagutao et al. 2008; Tseng et al. 2008).

The first study about the gene expression profile of shrimp following immunostimulation using microarray approach was reported in 2008 (Fagutao et al. 2008). The immunostimulant and shrimp organ used in this study were PG and hemocytes of kuruma shrimp (*M. japonicus*), respectively. The microarray analysis showed that PG administration up-regulated the expression of immune-related genes. These differentially expressed immune-related genes function in antibacterial responses, clotting system, prophenoloxidase (proPO) cascade and wound healing. The significant shrimp immune system triggered by PG immunostimulation is the proPO cascade of humoral responses, and six responsive genes are involved in this cascade as detected by microarray including prophenoloxidase and Kazal-type proteinase inhibitor. Other up-regulated genes have roles in innate immunity, including single WAP domain-containing protein, crustin, lysozyme, transglutaminase and alpha 2-macroglobulin. Other studies found that PG had similar effects on shrimp immune-related genes (Rattanachai et al. 2004; 2005; Lin et al. 2007). Based on these studies, the humoral response is an important innate immune mechanism in shrimp, which is triggered immediately after non-self component stimulation. Interestingly, the strong expression of genes related to the proPO cascade was observed at 1 day, but not at 7 or 14 days, post-PG administration. The genes involved in other immune responses also showed similar phenomenon. The short-lived primary immune response of shrimp provides further evidence that specific memory in shrimp immunity is non-existent. As in the shrimp transcriptomic assays, approximately 48% differentially expressed genes were unknown genes.

Immune-Related Gene Expression Following Antibiotic Treatment

Antibiotics, biologically or synthetically produced substances that display antagonistic activity against microorganisms, are used in shrimp culture for both therapeutic and prophylactic purpose (Tendencia and Peña 2001). Two of the most common antibiotics used in shrimp are oxytetracycline and oxolinic acid. Oxytetracycline (OTC) is a broad-spectrum antibiotic active against a wide variety of bacterial species including Gram-negative and Gram-positive aerobic and anaerobic bacteria and is perhaps one of the most widely used therapeutics in aquaculture because of its effectiveness, relative safety, low rate of accumulation in edible tissue, and short tissue elimination time (Bray et al. 2006). OTC is also one of the only four antimicrobial agents approved by the US Food and Drug Administration for use in food fish and only one such drug approved for use in invertebrates (Nolan et al. 2007). It is used in the treatment of various bacterial diseases of aquatic animals and in farm-raised shrimp such as vibriosis and necrotizing hepatopancreatitis infections (Reed et al. 2004). On the other hand, oxolinic acid (OA), a quinolone, is an antibiotic effective against a variety of Gram-negative bacteria and works primarily by inhibiting the synthesis of bacterial DNA (Pianotti et al. 1968). It is mainly used in shrimp farms in Asia primarily as a treatment against vibriosis (Gräslund and Bengtssona 2001; Tendencia and Peña 2001; Uno 2004).

Shrimp lymphoid organ is reported to be a primary site of bacterial accumulation and bacteriostasis (Burgents et al. 2005; van de Braak et al. 2002) and one of the organs that

plays a role in the elimination of viral particles and other infectious agents (Hasson et al. 1999; Duangsuwan et al. 2008). We previously showed that the expressions of genes in the shrimp lymphoid organ including those that are involved in immune response were altered after the administration of antibiotics (Fagutao et al. 2008). However, the effects OA and OTC are slightly different from each other, i.e., there were far more differentially expressed genes in the lymphoid organ of OA-treated samples, particularly down-regulated genes, than in the lymphoid organ of OTC-treated samples.

Immune-related genes that were significantly down-regulated by OA and OTC were penaeidin, proPO, clotting protein, profiling, and whey acidic protein. Penaedins, first isolated in pacific white shrimp, *Litopenaeus vannamei*, display antimicrobial activity against fungi and bacteria with a predominant activity against Gram-positive bacteria (Destoumieux et al. 1997) and have been proposed to be linked the survivability of shrimp (de Lorgeril et al. 2008). ProPO, which is an essential component in melanin synthesis, was shown to have an antibacterial role in shrimp (Amparyup et al. 2009; Liu et al. 2007a). Importantly, both the penaeidins and ProPO are mainly synthesized in the hemocytes (Destoumieux et al. 2000; Hose et al. 1987). Shrimp lymphoid organ was found to contain many hemocytes (van de Braak et al. 2002), and lymphoid organ cells also share similar characteristics to semi-granular and, in particular, large granular hemocytes with phenoloxidase activity (Anggraeni and Owens 2000). Clotting protein is strongly expressed in the lymphoid organ in shrimp and its absence renders shrimp susceptible to viral and bacterial pathogens (Maningas et al. 2008). The lymphoid organ was reported to be one of the major tissues producing this gene (Yeh et al. 2007). Profilin is a protein important for regulating actin polymerization essential for many cellular processes, and in shrimp, it was found to be up-regulated after *Vibrio* challenge (Somboonwiwat et al. 2006). On the other hand, genes having whey acidic protein (WAP) domains were shown to possess antimicrobial properties (Jia et al. 2008). It is expressed at a higher level in virus-resistant shrimp and is highly up-regulated during early phase of WSSV infection (Chen et al. 2008a, b).

Immune-Related Gene Expression Following Environmental Stress

WSSV pathogenesis is believed to be closely related to the host stress-induced response (via the JAK/STAT pathway). Therefore, stress should decrease the ability of shrimp to defend themselves against pathogens. Furthermore, viral and bacterial pathogenesis in shrimp reflects the correlation of environmental stresses and immunity. For examples, *L. vannamei* had lower resistance to *V. alginolyticus* under nitrite stress and higher WSSV resistance under high temperature (Tseng and Chen 2004; Granja et al. 2006), *P. monodon* showed higher susceptibility to *Photobacterium damselae* subsp. *damselae* following a change in salinity (Wang et al. 2006), and Taura syndrome virus-infected shrimp showed a lower tolerance for a decrease in salinity (Lotz et al. 2005).

De la Vega et al. (2007) examined the stressed-induced gene expression profile in *P. monodon* under hypoxic, hyperthermic, and hypoosmotic conditions by utilizing cDNA technology. Of the 3,853 probes on the chip, 145 were responsive to at least one stress treatment and 83% were unknown molecules. Generally, hemocyanin showed significant differential expression in response to osmotic stress and thermal stress, but not hypoxic stress. In shrimp, following a long-time recovery period after hyperosmotic stress (wherein the salinity was reduced from 35 to 10 ppt), down-regulation of several different hemocyanin clones on a chip were identified, whereas up-regulated expression levels were observed when stress immediately follows. In addition, hemocyanin had higher expression level after higher water temperature treatment (35°C) following a long-time recovery period. Because of antimicrobial function (against virus, bacteria, and fungi), the up-regulation of hemocyanin by elevated temperature appears to decrease the susceptibility of shrimp after changing the salinity (Bray et al. 1994; Lotz et al. 2005; Liu et al. 2006a; Wang and Chen 2006; Granja et al. 2003; 2006; García-Carreño et al. 2008).

Hypoxic stress, causing changes in the expression of several immune-related genes, was observed, such as the up-regulation of transglutaminase and suppression of three WAP family proteins (crustin and protease inhibitors) and Kunitz domain-containing protease inhibitors. As described previously, the WAP domain contains protease inhibitors that play an important role in the host defense system against invasion of shrimp pathogens (Zhang et al. 2007; Amparyup et al. 2008; Chen et al. 2008a, b; Smith et al. 2008). Because hypoxic stress suppresses WAP family proteins, it should make shrimp more susceptible to pathogens.

As shown by de la Vega et al. (2007), shrimp physical condition, the immune response, and environmental stress are closely related.

Conclusion

Microarray has been an effective tool in elucidating and understanding the mechanisms of shrimp responses to viral and bacterial infection, antibiotic treatment, and even environmental stress at the molecular level. It is therefore

clear that the technique will become an indispensable tool in the study of shrimp in both the short- and long-term future and should reveal breakthrough information that could lead to novel applications for the continuous development of shrimp culture and the shrimp industry in general.

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