[2] Fish and Shellfish Bio-Defense

The Graft-Versus-Host Reaction (GVHR) is a phenomenon of cell-mediated immunity in which CTLs play the major role. The presence of GVHR in a teleost fish has been demonstrated in ginbuna and amago salmon (see review (Nakanishi et al, 2011). Most features of acute GVHD in fish are quite similar to those reported for mammals, suggesting the existence of similar mechanisms. More recently, essential roles of donor-derived $CD8\alpha^+$ T cells together with $CD4^+$ T cells in the induction of acute GVHR/D in teleost have been reported (Shibasaki, 2010).

Glossary

Ig: Immunoglobulin,

MHC: The major histocompatibility complex,

TCR: T cell receptor,

MLC: Mixed leukocyte culture,

CTLs: Cytotoxic T lymphocytes,

NCC: Nonspecific cytotoxic cell,

APC: Antigen presenting cell ,

DCs: Dendritic cells,

GVHR: Graft-Versus-Host Reaction,

GVHD: Graft-Versus-Host Disease

3. SHRIMP BIO-DEFENSE

Ikuo Hirono and Sheryll G. Hipolito

3.1. Synopsis

Because of the importance of penaeid shrimps in world aquaculture, there is much interest in understanding their immune system to improve their resistance to pathogenic microorganisms. Basic knowledge of shrimp immunity is needed to develop strategies for prophylaxis and control of diseases in shrimp aquaculture. Shrimps possess an innate immunity that is composed of both humoral and cellular responses. However, little is known about these systems particularly the mechanisms involved at the molecular level. Here, some recent researches of shrimp immune responses against microbial pathogens are presented.

3.2. Introduction

Shrimps are one of the most important aquaculture species not only for commercial products but also for animal protein source for human consumption. Annual shrimp production is growing year by year after the 1980’s. However, the growing shrimp aquaculture was accompanied by the outbreak of infectious diseases.

Although devoid of an adaptive immune system, shrimp have an innate immune system that combats invading pathogens. This includes phagocytic activity of hemocytes, melanization, antimicrobial proteins and peptides, clotting of hemolymph and unknown unique defense system in shrimp.

3.3. Phenol Oxidase

Prophenol oxidase is one of the most studied immune molecules in shrimp (Table 3.1). It has been cloned from several different penaeid species. Gene silencing/knock down of prophenol oxidase in kuruma shrimp, *Marsupenaeus japonicus,* showed increased bacteria in the haemolymph and increased mortality without artificial microbial challenge (Fagutao et al, 2009). These results suggested that the prophenol oxidase is an important molecule for shrimp survival in normal environmental condition (Fagutao et al, 2009).

Species	References
Marsupenaeus japonicus	Adachi et al., 1999
	Fagutao et al., 2009
Penaeus monodon	Amparyup et al., 2009
	Sritunyalucksana et al., 1999
Litopenaeus vannamei	Lai et al., 2005
	Pan et al., 2008
	Wang et al., 2006
	Yeh et al., 2009
	Okumura, 2007
Penaeus californiensis	Hernández-López et al., 1996
	Gollas-Galvan et al., 1999
	Gollas-Galván et al., 1997
Fenneropenaeus chinensis	Gao et al., 2009

Table 3.1. Prophenol oxidase in penaeid shrimps.

3.4. Antimicrobial Proteins/Peptides

In shrimp, the release of antimicrobial proteins/peptides, more commonly known as AMPs, act as the first line of defense against pathogen invasion (Hancock and Diamond, 2000). A repertoire of penaeid AMPs have been identified and discovered by analysis of expressed sequence tag libraries, microarray studies and proteomic methods. These include anti-lipopolysaccharide factors, penaeidins, crustins, lysozymes, single-whey acidic protein domain containing peptides, bactinectin and stylicins (Tassanakajon et al, 2013). With the advent of RNA interference and recombinant protein technology, functions of AMPs have been discovered and are proven to exhibit a wide range of antimicrobial activities against bacteria, viruses and fungi (Table 3.2). In addition, AMP helps in maintaining a balanced bacterial community in shrimp hemolymph (Kaizu et al, 2012) Clearly, AMPs are involved in major immune reactions and their productions are important against pathogenic microorganism in shrimp.

Family	**Isoform/Species**	**Antimicrobial activity**	**Other activity**	**References**
Crustins	Cru*Fc*	Gram-positive bacteria		Zhang et al., 2007
	Fc-crus 2	Gram-positive bacteria		Sun et al., 2010
	Fc-crus 3	Gram-positive bacteria		Sun et al., 2010
	crustin*Pm*1	Gram-positive bacteria	Agglutination	Krusong et al., 2012; Supungul et al., 2008
	crustin*Pm*5	Gram-positive bacteria		Vatanavicharn et al., 2009
	crustin*Pm*7	Gram-positive bacteria; Gram-negative bacteria	Agglutination	Krusong et al., 2012; Amparyup et al., 2008
	SWD*Fc*	Gram-positive bacteria; Gram-negative bacteria; fungi	Protease inhibitory Activity against subtilisin A and protein K	Jia et al., 2008
	SWD*Pm*	Gram-positive bacteria	Protease inhibitory activity against subtilisin A	Amparyup et al., 2008
	Cruslike*Fc*1	Gram-positive bacteria		Zhang et al., 2007
	*Lv*ABP1	Gram-negative bacteria		Shockey et al., 2009
Penaeidin	*Litvan*Pen2	Gram-positive bacteria; fungi		Destoumieux et al., 1999
	*Litvan*Pen3	Gram-positive bacteria; fungi		Destoumieux et al., 1999
	*Litvan*Pen4	Gram-positive bacteria; Fungi		Cuthbertson et al., 2004
	*Fenchi*Pen5	Gram-negative bacteria; Gram-positive bacteria; Fungi		Kang et al., 2007
	*Penmon*Pen	Gram-positive bacteria		Ho et al., 2004
	*Penmon*Pen3	Gram-positive bacteria; Fungi	Cytokine	Li et al., 2010; Destoumieux et al., 1999
	*Penmon*Pen5	Gram-positive bacteria; Fungi; virus		Woramongkolchai et al., 2011; Hu et al., 2006
Lysozyme	*P. monodon*	Gram-negative bacteria		Supungul et al., 2010
	M. japonicus	Gram-negative bacteria		Kaizu et al., 2012; Bu et al., 2008 ; Hikima et al., 2003
	F. chinensis	Gram-positive bacteria; Gram-negative bacteria		
	L. vannamei	Gram-negative bacteria		Peregrino-Uriarte et al., 2012; Sotelo-Mundo et al., 2003
	F. merguiensis	Gram-positive bacteria; Gram-negative bacteria		Mai et al., 2009
	L. stylirostris	Gram-positive bacteria; Gram-negative bacteria		Mai et al., 2010; de Lorgeril et al., 2008
Anti-lipopoly-saccharide factors	ALF*Pm*2	Gram-positive bacteria; Gram-negative bacteria		Tharntada et al., unpublished data
	ALF*Pm*3	Gram-positive bacteria; Gram-negative bacteria; Fungi; virus	LPS and LTA binding activity	Tharntada et al., 2009; Somboonwiwat et al., 2008; Somboonwiwat et al., 2005

	*Ls*ALF1	Virus		de la Vega et al., 2008
	*Mj*ALF1		LPS neutralizing activity	Nagoshi et al., 2006

Modified from Tassanakajon et al., 2013

Table 3.2. Antimicrobial activities of shrimp AMP families.

3.5. Clotting of Hemolymph

Hemolymph clotting in crustaceans is an integral part of the overall invertebrate immune response and important in the prevention of blood loss during injury and wound healing (Kwok and Tobe, 2006). The shrimp coagulation is believed to rely on the formation of a clottable protein polymer that is catalyzed by the Ca^{2+} dependent covalent linkage of the large dimeric clotting protein by transglutaminase into long chains (Tassanakajon et al, 2013). Transglutaminase and clotting proteins have been identified in several shrimp species (Table 3.3). Phenotypic studies on hemolymph collected from *M. japonicus* where transglutaminase and clotting protein were silenced by RNA interference failed to polymerize/coagulate (Maningas et al, 2008). In addition, tranglutaminase and clotting protein depleted *M. japonicus* resulted to a significantly higher mortality rate after microbial infection (Maningas et al, 2008). Clearly, these two proteins play an important function in blood coagulation and immune response to microbial infection. It was also evidenced that silencing of transglutaminase significantly downregulated some important AMPs like crustin and lysozyme expression suggesting that transglutaminase may also play a role in the regulation some immune-related like AMP expression (Fagutao et al, 2012).

Coagulation/clotting component	Species	References
Transglutaminase	*Litopenaeus vannamei*	Yeh et al., 2009
	Fenneropenaeus chinensis	Liu et al., 2007
	Marsupenaeus japonicus	Yeh et al., 2006
	Penaeus monodon	Chen et al., 2005; Yeh et al., 2006
Clotting proteins	*Marsupenaeus japonicus*	Cheng et al., 2008
	Litopenaeus vannamei	Cheng et al., 2008
	Farfantepenaeus paulensis	Perazzolo et al., 2005
	Penaeus monodon	Yeh et al., 1999

Table 3.3. Transglutaminase and clotting proteins identified in shrimps.

3.6. Other Shrimp Immune-Related Genes

In addition to the phenol oxidase system, antimicrobial peptides/proteins and blood clotting system, other immune-related molecules were also identified in penaeid shrimps including proteinases/proteinase inhibitors, heat shock proteins, apoptotic tumor-related proteins, pattern recognition receptors or pattern recognition proteins, and proteins involved in signaling transduction and oxidative stress. These proteins work by inhibiting bacterial or viral activities, protection against stress, elimination of leftover, damaged or infected harmful cells, microbe recognition, activation of signaling pathways involve in immune responses and in maintaining normal aerobic metabolism.

Glossary

AMPs: Antimicrobial Proteins or Peptides

4. SHELLFISH BIO-DEFENSE

Keisuke G. Takahashi, Naoki Itoh and Makoto Osada

4.1. Synopsis

Human has exploited shellfish as important bio-resources for multiple purposes; for example, seafood and pearl production. Aquaculture of shellfish is one of the most important fishery industries worldwide. Therefore, interest in shellfish immunity has developed due to the importance of aquaculture and their role in the aquatic environment. Shellfish, as well as other invertebrates, do not possess adaptive immunity. Therefore, to combat infection, shellfish rely on an innate immune system, which is comprised of multiple bio-defense reactions employing circulating hemocytes and multiple defense molecules. Circulating hemocytes, which possess strong migration ability in response to invading microorganisms and subsequently actively phagocytose these invaders, are the most responsible in bio-defense in shellfish. Humoral defense factors comprise molecules of two types, those which act in bio-defense with the recognition of pathogenic microorganisms and those that mediate microbial killing and macromolecular degradation.

4.2. Introduction

Shellfish belongs to the phylum Mollusca and is mainly comprised of bivalves and gastropods. The Phylum Mollusca is one of the largest and numerous groups in the animal kingdom. Shellfish and microorganisms coexist in the biosphere in numerous ways. Thus, bivalves have evolved sensitive mechanisms for recognizing pathogens and an array of strategies to defend themselves against attacks by microorganisms such as bacteria, fungi, and parasites. An oft-asked question is how invertebrates including shellfish survive against pathogenic microorganisms without an adaptive immune system. Indeed, invertebrates do not have lymphocytes and do not produce antibodies (Loker et al, 2004; Rowley and Powell, 2007). They have only an innate immune system that comprises hemocytes and non-specific humoral defense molecules (Bachère et al, 2004; Song et al, 2010). Therefore, to combat infection, bivalves rely on multiple bio-defense reactions. The point of bio-defense mechanisms is to recognize and eliminate various types of pathogens (Loker et al, 2004; Rowley and Powell, 2007; Bachère et al, 2004; Song et al, 2010). Circulating hemocytes, which possess strong migratory ability in response to invading microorganisms and subsequently actively phagocytose these invaders, are the most responsible factor in bio-defense in shellfish (Cheng, 1996; Hine, 1999). Humoral defense factors comprise molecules of two types, those which act in bio-defense with recognition and binding to typical microbial pathogen-associated molecular patterns (PAMPs), and those which mediate microbial killing and macromolecular