21 Genomics of Fish and Shellfish Microbial Pathogens

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Genomics of Fish Bacterial Pathogens

Genome sequence information is a valuable source of information for studying pathogenesis, virulence, immunogenicity, therapeutic targets and diagnosis of fish pathogenic bacteria. In addition, this information increases our understanding of the host–pathogen interaction for developing prevention methods for fish and shellfish infectious diseases in aquaculture. In the microbe genome sequence database of NCBI as of 12 January 2010 (www.ncbi.nlm.nih.gov/genomes/lproks.cgi), the number of completed genome sequences of bacteria including those that are pathogenic and non-pathogenic is 986, those in assembly stage are 1149 and those that are unfinished (under various stages in genome projects) are 1186. Some of these are fish pathogenic bacteria (Table 21.1). Four species, *Aeromonas salmonicida* subsp. *salmonicida* (Reith *et al.*, 2008), *Aliivibrio salmonicida* (Hjerde *et al.*, 2008), *Flavobacterium psychrophilum* (Duchaud *et al.*, 2007) and *Renibacterium salmoninarum* (Wiens *et al.*, 2008) were isolated from fish, and four species, *A. hydrophila* (Seshadri *et al.*, 2006), *Mycobacterium marinum* (Stinear *et al.*, 2008), *Vibrio harveyi* (Genbank accession No. NC_009783, NC_009784) and *V. splendidus* (GenBank accession No. NC_011744, NC_011753) were not taken from fish. Moreover, four species, *Edwardsiella ictaruli*, *E. tarda*, *Lactococcus garvieae* and *Streptococcus iniae* are currently being completed, while two species, *V. anguillarum* (Rodkhum *et al.*, 2006a,b) and *Photobacterium damselae* subsp. *piscicida* (Naka *et al.*, 2005) have been partially sequenced (Table 21.1). Recently, the whole genome sequence of *E. tarda* has been completed (Wang *et al.*, 2009). These bacteria possess a single chromosome of 3.76 Mbp containing 3486 predicted protein-coding sequences. The following section presents the completed genome sequence of four fish pathogenic bacteria, *F. psychrophilum* (Duchaud *et al.*, 2007), *A. salmonicida* subsp. *salmonicida* (Reith *et al.*, 2008), *Al. salmonicida* (Hjerde *et al.*, 2008) and *R. salmoninarum* (Duchaud *et al.*, 2007), and the partial genome sequence of two fish pathogenic bacteria, *P. damselae* subsp. *piscicida* (Naka *et al.*, 2005) and *V. anguillarum* (Rodkhum *et al.*, 2006a,b).

Aeromonas salmonicida subsp. salmonicida

A. salmonicida subsp. *salmonicida* is a Gram-negative bacterium and the causative agent of furunculosis in salmonid fish. Furunculosis has been recognized as an important disease in wild and farmed fish. The genome size of *A. salmonicida* subsp. *salmonicida* strain A449 is 4.7 Mbp encoding

© CAB International 2011. *Fish Diseases and Disorders* Vol. 3, 2nd Edition:
Viral, Bacterial and Fungal Infections (eds P.T.K. Woo and D.W. Bruno)

Table 21.1. Genome sequences of fish pathogenic bacteria.

Species	Genome size (Mbp)	Proteins	References or Groups
Genome sequences of fish isolated bacteria (completed)			
Flavobacterium psychrophilum	2.86	2,432	Duchaud *et al*., 2007
Aeromonas salmonicida subsp. *salmonicida*	4.70	4,388	Reith *et al*., 2008
Aliivibrio salmonicida	3.33 and 1.21	4,175	Hjerde *et al*., 2008
Renibacterium salmoninarum	3.16	3,507	Wiens *et al*., 2008
Edwardsiella tarda	3.76	3,486	Wang *et al*., 2009
Genome sequences of non-fish isolated bacteria (completed)			
Aeromonas hydrophila	4.74	4,122	Seshadri *et al*., 2006
Mycobacterium marinum	6.64	5,423	Stinear *et al*., 2008
Vibrio harveyi	3.76 and 2.20	5,944	NC_009783, NC_009784
Vibrio splendidus	3.30 and 1.68	4,431	NC_011744, NC_011753
Genome sequences of fish pathogenic bacteria (in progress)			
Edwardsiella ictaruli	ND		University of Oklahoma
Lactococcus garvieae	ND		Kitasato University
Streptococcus iniae	ND		Baylor College of Medicine
Genome sequences of fish pathogenic bacteria (partial)			
Photobacterium damselae subsp. *piscicida*	ND		Naka *et al*., 2005
Vibrio anguillarum	ND		Rodkhum *et al*., 2006a,b

4388 genes (Reith *et al*., 2008). Many of the genes are potentially virulence related, including virulence factor secretion system (type II and III secretion systems), adhesion (flagella and pilus), toxins (aerolysin, haemolysin, RTX toxin, endotoxins), secreted enzymes (proteases, nucleases, chitinases, glycerophospholipid cholesterol acyltransferase, esterase, collagenase), iron acquisition (syderophore synthesis and uptake, haem receptors) and quorum sensing-related proteins (Table 21.2). Based on the genomic sequence, Nash *et al*. (2006) developed a DNA microarray containing 2024 genes for *A. salmonicida* subsp. *salmonicida*. They studied the variation of different isolates of the bacteria and found that many known virulence genes were common to all strains tested. Some of these virulence-related genes were knocked down and their pathogenicity against salmonid fish was confirmed. Dacanay *et al*. (2006) demonstrated that the type III secretion system (TTSS) was essential for virulence of *A. salmonicida* subsp. *salmonicida* by using single gene knockout mutant strains. Recently, Boyd *et al*. (2008) examined the virulence of the pili of *A. salmonicida* subsp. *salmonicida*. The bacteria have no visible pili but its genome contains genes for three type IV pilus systems including Tap, Flp and mannose-sensitive haemagglutinin pilus (MSHA). They found that the Tap contributed moderately to the virulence to Atlantic salmon (*Salmo salar*) but Flp made little or no contribution to virulence. Boyd *et al*. suggested that the *A. salmonicida* subsp. *salmonicida* type VI pili were not absolutely required for virulence in Atlantic salmon. Interestingly, Masada *et al*. (2002) reported that the *A. salmonicida* subsp. *salmonicida* type IV pilin (Tap) was required for virulence in rainbow trout (*Oncorhynchus mykiss*). From these studies, it was hypothesized that type IV pilus system in *A. salmonicida* subsp. *salmonicida* had a role in host–pathogen interaction.

Aliivibrio salmonicida

Al. salmonicida (formerly *V. salmonicida*) is a Gram-negative bacterium and the causative agent of coldwater vibriosis in Atlantic

Table 21.2. Identified putative virulence factors in *Aeromonas salmonicida* subsp. *salmonicida* strain A449 by genome analysis.

Secretion system	Secreted enzymes
Type II secretion system	Serine protease
Type III secretion system (TTSS)	Zinc metalloprotease
Type VI secretion system	Metalloprotease
Aop H effector	Elastase
Aop effector	Collagenase
Aex effector	Glycerophospholipid cholesterol acyltransferase
Ati 2 putative effector	Phospholipase A1
Putative TTSS effector	Phospholipase C
Adhesion	Extracellular nucleases
Lateral flagella	Amylases
Polar flagellas	Chitinases
Type I pilus	Pullulanase
Tap type IV pilus	Iron acquisition
Flp type IV pilus	Siderophore synthesis and uptake
Msh type IV pilus	Siderophore receptor
Toxins	Haeme receptor
Aerolysin	Haeme uptake protein
Haemolysin	Quorum sensing
RTX toxin	*N*-acyl homoserine lactone synthase
Cytolytic δ-endotoxin	Quorum sensing regulon activator
	AI-2 synthase
	Quorum sensing phosphorelay protein
	Quorum sensing response regulator

salmon, rainbow trout and Atlantic cod (*Gadus morhua*). Similar to the characteristics of family Vibrionaceae, the *Al. salmonicida* strain LFI1238 genome also consists of two chromosomes. The size of the two chromosomes is 3.3 Mbp (chr I) and 1.2 Mbp (chr II) encoding 3070 and 1105 protein coding genes, respectively (Hjerde *et al.*, 2008). Hjerde *et al.* (2008) found several potential virulence factors in the genome, including secretion systems (type I, II and VI), type IV pilus, haemolysins, iron acquisition systems, etc. (Table 21.3). In some *Vibrio* species, haemolysin, particularly the thermostable direct haemolysin (TDH), is known as one of the most important virulence factors. Interestingly, there are five putative haemolysin genes in *Al. salmonicida* genome, although none of the five genes are homologous to TDH (Hjerde *et al.*, 2008). Recently, five different haemolysin genes have also been found in *V. anguillarum* (Rodkhum *et al.*, 2006a). Two of the genes, *VAH2* and *VAH5*, were homologous to those found in the *Al. salmonicida* genome. In the case of *V. anguillarum*, these haemolysin genes are indicated to be two of the virulence factors in rainbow trout. There is a good possibility that the five putative haemolysin genes of *Al. salmonicida* are involved in virulence, but it is necessary to carry out further experiments to confirm this. By using the genome information of *Al. salmonicida*, it is possible to identify or characterize virulence factors and host restrictions.

Flavobacterium psychrophilum

F. psychrophilum is a Gram-negative bacterium and responsible for the coldwater disease, causing considerable economic losses in salmonid fish and ayu aquaculture worldwide. The genome of *F. psychrophilum* strain JIP02/86 (ATCC49511) consists of a 2.9 Mbp circular chromosome with 2432 protein coding genes (Duchaud *et al.*, 2007). Duchaud *et al.* (2007) found several

Table 21.3. Identified putative virulence factors in Aliivibrio salmonicida strain LFI1238 by genome analysis.

Secretion system	Iron acquisition
Type I secretion system	Siderophore synthesis and uptake
Type II secretion system	Siderophore receptor
Type VI secretion system	Haeme receptor
	Haeme uptake protein
Adhesion	Toxins
Polar flagellas	Haemolysin (VAH2)
Msh type IV pilus	Haemolysin (VAH5)
Flp type IV pilus	Haemolysin
	Haemolysin (hlyIII)
LPS and cell capsule	Haemolysin-type calcium-binding protein
LPS biosynthesis systems	Serine protease
	Peptidase

putative virulence factor genes such as toxins (proteases, haemolysin, thiol-activated family of pore-forming toxin), adhesions and secretion systems (Sec-independent transport systems, PorT and PorR). In the case of putative toxins, there are several different types of proteases, including seven genes for metalloprotease, two genes for metallopeptidase and two genes for serine endopeptidase. Interestingly, they have found only a truncated form of the collagenase gene, which is known as a virulence factor in several pathogenic bacteria including *F. psychrophilum* (Duchaud *et al.*, 2007). Duchaud *et al.* (2007) examined this unexpected result using PCR and sequencing of 23 different isolates of *F. psychrophilum*. They revealed that 10 of the 23 strains had the truncated gene, all of which were isolated from rainbow trout. These results suggested that collagenase was not a virulence factor in rainbow trout. They also found the homologue of *V. anguillarum* haemolysin VAH5 in the genome of *F. psychrophilum*, as well as the homologue of the pore-forming toxin thiol-activated cytolysin family (TACY). Such toxins appear to be involved in the pathogenicity of *F. psychrophilum*.

Renibacterium salmoninarum

R. salmoninarum is a Gram-positive bacteria that causes bacterial kidney disease (BKD) in salmonid fish. The genome of *R. salmoninarum* strain ATCC33209 is a 3.2 Mbp circular chromosome encoding 3507 protein-coding genes (Wiens *et al.*, 2008). Wiens *et al.* (2008) reported several virulence-related factors found in the genome, including the haem acquisition systems, capsular synthesis, secretion systems, major soluble antigen (MSA), proteases and haemolysins. Interestingly, there are 68 antibiotic resistance- related genes, including a variety of multidrug transporters, several types of beta lactamase, tetracycline resistance (TetA, TetB and TetP) and macrolide-resistance factors (RlmA, SpoU, MefA, PvsC and KsgA). The MSA is known to be specific to *R. salmoninarum* and it is duplicated in the bacteria's genome. Moreover, some strains have a third copy of the MSA and their virulence is higher than the strains possessing only two copies of MSA gene. Two haemolysins and three haemolysin candidate genes were likewise found.

Photobacterium damselae subsp. piscicida

P. damselae subsp. *piscicida* (formerly *Pasteurella piscicida*), of the family Vibrionaceae, is the causative agent of pasteurellosis in fish. Since 1969, this disease has economically damaged yellowtail (*Seriola quinqueradiata*) production in Japan. Furthermore, pasteurellosis has also damaged the culture of gilthead sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) in Europe, striped bass (*Morone saxatilis*) in the USA and snakehead (*Channa argus*), cobia (*Rachycentron canadum*) and hybrid striped bass in Taiwan (Daly and Aoki, see Chapter 17, this volume). Several virulence factors have been reported for these bacteria (Daly and Aoki, see Chapter 17), such as iron acquisition system (Magariños *et al.*, 1994), cell surface capsule (Magariños *et al.*, 1996; Arijo *et al.*, 1998), extracellular products (Nakai *et al.*, 1992), haemolysin

(Magariños *et al.*, 1992) and phospholipase (Naka *et al.*, 2007). By partial genome analysis, 2055 protein-coding genes, including several virulence-related genes containing colonization factors (37 genes), exotoxin (5 genes) and lipopolysaccharide biosynthesis-related molecules (6 genes) were identified (Naka *et al.*, 2005). Among the colonization factors, 27, 3 and 4 genes have homology with polar flagellar-related genes, capsule biosynthesis genes and others (accessory colonization factors, SOD, MshA biogeneis and haem receptors), respectively (Naka *et al.*, 2005). With exotoxin genes, two different types of haemolysins, phospholipase, lysophospholipase and hyaluronidase were found. Extracellular products of *P. damselae* subsp. *piscicida* have virulence activity against mouse and rainbow trout and it is thought that phospholipase and haemolysin are involved because they have been detected in the extracellular products. Naka *et al.* (2007) confirmed that phospholipase of *P. damselae* subsp. *piscicida* had haemolytic activity and found the sensitivities of fish and mammal erythrocytes to phospholipase were very different. Phospholipase showed strong haemolytic activities for fish [yellowtail, carp (*Cyprinus carpio*), rainbow trout, red sea bream (*Pagrus major*), sea bass (*Lateolabrax japonicus*) and tilapia (*Oreochromis niloticus*)] erythrocytes but little or no haemolytic activities for mammalian (rabbit, horse and sheep) erythrocytes. However, the haemolytic activity of the phospholipase was detected in all mammalian erythrocytes with the addition of lecithin. These results indicate that the phospholipase is a lecithin-dependent haemolysin (LDH) against mammalian erythrocytes and a direct haemolysin for fish erythrocytes. The different sensitivities between mammalian and fish erythrocytes to phospholipase might be due to differences in the relative amounts of phospholipids (e.g. phosphatidylcholine, phosphatidylethanol amine, phosphatidylserine and phosphatidylinositol) in the membrane of erythrocytes. Other studies have described results similar to those reported here. *V. mimicus* haemolysin (*phl* gene) has little or no ability to lyse mammalian erythrocytes, although it does lyse fish erythrocyte (Lee *et al.*, 2002). *V. parahaemolyticus* thermolabile haemolysin (*tlh* gene) (Taniguchi *et al.*, 1985), which has phospholipase A activity, has high identities with *V. harveyi* and *V. mimicus* and shows LDH activity (Schmiel and Miller, 1999). The amino acid sequences of all these haemolysins from *Vibrio* spp. are similar to the amino acid sequence of *P. damselae* subsp. *piscicida* phospholipase (Taniguchi *et al.*, 1985; Schmiel and Miller, 1999; Lee *et al.*, 2002).

Vibrio anguillarum

V. anguillarum is the aetiological agent of vibriosis or haemorrhagic septicaemia affecting marine and freshwater fish and shellfish. The *V. anguillarum* genome is composed of two chromosomes similar to other Vibrionaceae (Okada *et al.*, 2005). Several virulence factors have been identified in this species, including iron uptake system, polar flagella, metalloproteases, major surface antigens and haemolysins, by individual cloning experiments (Rodkhum *et al.*, 2006b). Rodkhum *et al.* (2006b) identified 2826 genes by partial genome analysis. Among these, 40 putative virulence-related genes were identified, 36 of which were novel in *V. anguillarum*, including a repeat in toxin (RTX) gene cluster, haemolysin gene, enterobactin gene, protease genes, lipopolysaccharide biosynthesis genes, capsule biosynthesis gene, flagellar genes and pilus genes. The RTX gene operons contain a total of five open reading frames (ORFs) arranged in two operons, rtxAC and rtxBD (Rodkhum *et al.*, 2006b). These operons had RTX toxin gene (*rtxA*) and RTX toxin transporter gene (*rtxB*), and RTX toxin activating protein gene (*rtxC*) and RTX toxin transporter gene (*rtxD*), respectively (Li *et al.*, 2008). The gene organization of this RTX gene cluster was highly similar to the RTX gene cluster of *V. cholerae* O1 biovar E1 Tor str. N16961 (Heidelberg *et al.*, 2000) and *V. vulnificus* YJ016 (Ruby *et al.*, 2005). Sixteen genes encoding the components, which might mediate adherence and colonization to the host cells,

were identified. Several capsule and cell surface component genes that were related to virulence in other bacterial pathogens were found, including the virulence gene, vibriobactin-specific 2,3-dihydroxybenzoate-AMP ligase (*angE*) (Liu *et al.*, 2004). This gene was proven to have a redundant function with *angE* gene on virulence plasmid pJM1 of *V. anguillarum* 775 (Alice *et al.*, 2005). Haemolysin has been suspected of being an important molecule involved in haemorrhagic septicaemia, which is a major sign of fish vibriosis. Some types of haemolysin in *V. anguillarum* have been published and some properties of haemolysin have been verified. Five different types of putative haemolysin genes have been identified and characterized, all of which show high similarity to haemolysin genes in other *Vibrio* species, such as *V. cholerae* O1 biovar El Tor (Heidelberg *et al.*, 2000), *V. parahaemolyticus* (Taniguchi *et al.*, 1990; Makino *et al.*, 2003) and *V. vulnificus* (Chen *et al.*, 2003). The role of these novel haemolysin genes in the virulence of *V. anguillarum* was further investigated and compared with the role of haemolysin genes in other *Vibrio* species, and it was shown that each haemolysin gene contributed to the virulence of *V. anguillarum* in juvenile rainbow trout.

Genomics of Fish and Shellfish Viral Pathogens

Mortalities in cultured fish and shellfish due to infectious viral disease have been a serious problem worldwide for several decades. The major viral-causing diseases are listed in Table 21.4. Whole genome analysis of disease-causing viruses is very important to elucidate and understand the mechanism of virus infection into the host animals. Genomics research for fish and shellfish virus began in the mid-1980s, when researchers discovered a small-sized genome from an RNA virus. Currently, 40 whole genome sequences from a total of 20 fish and shellfish viruses (almost half the species in Table 21.4) have already been published in the GenBank database. These genome sizes vary from 3.1 kb to 307.3 kb (Table 21.5), are linear DNA or RNA, except for that of white spot syndrome virus (WSSV), which is circular double-stranded DNA. The genome of RNA virus encoding 2~9 OFRs is small in general (Table 21.6). *Birnaviridae* (e.g. infectious pancreatic necrosis virus and yellowtail ascites virus of the family) and *Nodaviridae* (e.g. red-spotted grouper nervous necrosis virus, *Epinephelus tauvina* nervous necrosis virus, striped jack nervous necrosis virus and *Macrobacterium rosenbergii* nodavirus) have two chromosomes (segment A/B or RNA1/2) and infectious salmon anaemia virus, or isavirus, has nine chromosomes (segments 1~9), encoding a few ORFs within the small size of each segment. On the other hand, the DNA virus genome is much larger in size (>102.6 kb). The WSSV Taiwanese strain (WSSV-TW) has the largest size of all genomes (307.3 kb) among the known infectious virus in fish or shellfish (Table 21.5). The genome structure of *Iridoviridae* [e.g. lymphocystis disease virus (LCDV) and red sea bream iridovirus (RSIV)] could be circularly permuted and terminally redundant (Darai *et al.*, 1985; Tidona and Darai, 1997). The succeeding sections describe the genomics as well as the proteomics of viral haemorrhagic septicaemia virus (VHSV), koi herpesvirus (KHV) and WSSV, which have caused most mass damage to aquaculture.

Viral haemorrhagic septicaemia virus (VHSV)

VHSV, which causes one of the devastating diseases of salmonid and marine fish, is a non-segmented, negative single-strand RNA virus of the genus *Novirhabdovirus*, family *Rhabdoviridae*. The whole genome sequence of VHSV has been determined from seven strains (Table 21.5). The size of the whole genome comprises 10,845~11,182 bp, encoding six proteins: the nucleoprotein or nucleocapsid protein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), the non-virion protein (NV) and the viral RNA polymerase (L) (Schütze *et al.*, 1999). These genes are arranged in the order 3′-N-P-M-G-NV-L-5′. Nucleotide and

Table 21.4. Fish and shellfish infectious virus.

Virus name	Genus	Family	Host animal
Fish			
1 Infectious haematopoitic necrosis virus (IHNV)	Novirhabdovirus	Rhabdoviridae	Salmonids
2 Viral haemorrhagic septicaemia virus (VHSV)	Novirhabdovirus	Rhabdoviridae	Salmonids, Japanese flounder
3 Spring viraemia of carp virus (SVCV)	Vesiculovirus	Rhabdoviridae	Carp
4 Perch rhabdovirus	Vesiculovirus	Rhabdoviridae	Perch
5 Pike fry rhabdovirus	Vesiculovirus	Rhabdoviridae	Pike
6 Infectious pancreatic necrosis virus (IPNV)	Aquabirnavirus	Birnaviridae	Salmonids
7 Yellowtail ascites virus (YAV)	Aquabirnavirus	Birnaviridae	Yellowtail, marine fish
8 Epizootic haematopoietic necrosis virus (EHNV)	Ranavirus	Iridoviridae	Redfin perch, rainbow trout, frog
9 Lymphocystis disease virus (LCDV)	Lymphocystivirus	Iridoviridae	Flounder, sea bream, rockfish
10 Red sea bream iridovirus (RSIV)	Megalocytivirus	Iridoviridae	Red sea bream, Murray cod
11 Singapore grouper iridovirus (SGIV)	Ranavirus	Iridoviridae	Grouper
12 White sturgeon iridovirus (WSIV)	Unclassified	Iridoviridae	White sturgeon
13 Carp pox herpesvirus (CyHV-1)	Ictalurivirus	Alloherpesviridae	Carp
14 Haemapoietic necrosis herpesvirus of goldfish (CyHV-2)	Ictalurivirus	Alloherpesviridae	Goldfish
15 Koi herpesvirus/cyprinid herpesvirus 3 (KHV, CyHV-3)	Ictalurivirus	Alloherpesviridae	Carp
16 Ictalurid herpesvirus 1 (CCHV)	Ictalurivirus	Alloherpesviridae	Channel catfish
17 Flounder herpesvirus (FHV)	Ictalurivirus	Alloherpesviridae	Japanese flounder
18 Herpesvirus salmonis (SalHV-1)	Ictalurivirus	Alloherpesviridae	Salmonids
19 *Oncorhynchus masou virus* (OMV, SalHV-2)	Ictalurivirus	Alloherpesviridae	Salmonids
20 Carp nephritis and gill necrosis virus	Unclassified	Alloherpesviridae	Common carp
21 Red-spotted grouper nervous necrosis virus (RGNNV)	Betanodavirus	Nodaviridae	Red-spotted grouper
22 *Epinephelus tauvina* nervous necrosis virus (ETNNV)	Betanodavirus	Nodaviridae	Greasy grouper
23 Striped jack nervous necrosis virus (SJNNV)	Betanodavirus	Nodaviridae	Striped jack
24 Chum salmon virus (CSV)	Aquabirnavirus	Reoviridae	Salmonds, catfish, bivalves
25 Grass carp rhabdovirus	Aquareovirus	Reoviridae	Grass carp
26 Infectious salmon anaemia virus (ISAV)	Isavirus	Orthomyxoviridae	Salmonids, Atlantic cod
27 Erythrocytic inclusion body syndrome virus (EIBSV)	Unclassified		Salmonids
Shellfish			
1 Baculovirus penaei (BP, tetrahedral baculovirus)	Nucleopolyhedro-virus	Baculoviridae	Penaeid shrimps
2 Penaeus monodon-type baculovirus (MBV, spherical baculovirosis)	Nucleopolyhedro-virus	Baculoviridae	Penaeid shrimps
3 Baculoviral midgut gland necrosis virus (BMNV)	Unclassified	Baculoviridae	Penaeid shrimps

(*Continued*)

Table 21.4. *Continued*

Virus name	Genus	Family	Host animal
Shellfish			
4 White spot syndrome virus (WSSV)	Whispovirus	Nimaviridae	Penaeid shrimps
5 Yellow head virus (YHV)	Okavirus	Roniviridae	Penaeid shrimps
6 Taura syndrome virus (TSV)	Cripavirus	Discistroviridae	Penaeid shrimps
7 Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Brevidensovirus	Parvoviridae	Penaeid shrimps
8 Hepatopancreatic parvovirus (HPV)	Unclassified	Parvoviridae	Penaeid shrimps
9 Spawner-isolated mortality virus	Unclassified	Unclassified	Penaeid shrimps, crayfish
10 Mourilyan virus	Unclassified	Bunyaviridae	Penaeid shrimps
11 Infectious myonecrosis virus (IMNV)	Unclassified	Totiviridae	Penaeid shrimps
12 Extra small virus (XSV)	Nodavirus	Nodaviridae	Penaeid shrimps, aquatic insect
13 *Macrobrachium rosenbergii* nodavirus (MrNV)	Unclassified	Nodaviridae	Penaeid shrimps
14 Ostreid herpesvirus type 1 virus (OsHV-1, Pacific oyster herpesvirus)	Ostreavirus	Malacoherpes-viridae	Pacific oyster
15 Haemocyte infection virus	Irido-like viruses		Pacific oyster, pearl oyster
16 Gill necrosis virus (GNV)	Irido-like viruses		Pacific oyster, pearl oyster

deduced amino acid sequences revealed significant homology to corresponding sequences in the VHSV strains: Cod Ulcus, Hededam, 14-58 and 96-43 (Betts and Stone, 2000). Of the VHSV proteins, the G-protein is a good example of a molecule that has been applied successfully as DNA vaccine for the rainbow trout (Lorenzen *et al.*, 1998, 2009). The G-protein is especially capable of inducing protective immunity, having been tested, together with the N-protein, as a DNA vaccine for salmonid fish (Lorenzen *et al.*, 1998), as well as a DNA vaccine for infectious haematopoietic necrosis virus, which is under the same genus *Novirhabdovirus* (Corbeil *et al.*, 1999, 2000). The DNA vaccine of VHSV G-protein is also effective in inducing the immune response of Japanese flounder, giving a high survival rate of >93% (Byon *et al.*, 2005).

Koi herpesvirus (KHV)

KHV (alternatively termed cyprinid herpesvirus type-3, CyHV3) has been responsible for outbreaks resulting in high mortalities in the common carp (*C. carpio*) and its variety, the koi carp, both in fish farms and in the natural environment worldwide (McGeoch *et al.*, 2006). KHV classified within the *Alloherpesviridae* family, has the longest known genome among the species of the *Herpesvirales* order, with a double-stranded DNA of about 295 kb, containing 22 kb terminal direct repeat (Hutoran *et al.*, 2005; Waltzek *et al.*, 2005; Aoki *et al.*, 2007). The whole genome sequences of three KHV strains from Japan (strain TUMST1), the USA (strain KHV-U) and Israel (strain KHV-I) have been determined (Table 21.5). From these sequences, 15 KHV genes have been found to have clear homologues with the distantly related channel catfish virus (icutalurid herpesvirus type-1, CyHV1), indicating that the fish herpesvirus is considerably more divergent than the mammalian herpesvirus (Aoki *et al.*, 2007). These genome sequences are interpreted as having arisen from a wild-type parent encoding 156 unique protein-coding genes, eight of which are duplicated in the terminal repeat. Thirty-one kinds of proteins identified in KHV-J genome are listed in Table 21.6. Interestingly, the

Table 21.5. Completed genome sequences of fish and shellfish viruses.

Virus name	Genus	Family	Accession no.	Genome shape (strain/isolation)	Genome type	Length (bp)	Total genome size (bp)	References
Fish virus								
1 Koi herpesvirus (KHV, CyHV-3)	Unclassified	Alloherpesviridae	NC_009127, DQ657948	Complete genome (strain KHV-U, USA)	dsDNA, linear		295,146	Aoki *et al.*, 2007
			AP008984	Complete genome (strain TUMST1, Japan)	dsDNA, linear		295,271	Aoki *et al.*, 2007
			DQ177346	Complete genome (strain KHV-I, Israel)	dsDNA, linear		295,138	Aoki *et al.*, 2007
2 Ictalurid herpesvirus 1 (CCHV)	Ictalurivirus	Alloherpesviridae	NC_001493, M75136	Complete genome (strain Auburn 1, ATCC:VR-665)	dsDNA, linear		134,226	Davison, 1992
3 Lymphocystis disease virus (LCDV)	Lymphocystivirus	Iridoviridae	NC_001824, L63545	Complete genome (LCDV-1)	dsDNA, linear*		102,653	Tidona and Darai, 1997
			NC_005902, AY380826	Complete genome (isolate China, LCDV-C)	dsDNA, linear*		186,250	Zhang *et al.*, 2004
4 Red sea bream iridovirus (RSIV)	Megalocytivirus	Iridoviridae	ND**	Complete genome	dsDNA, linear*		112,415	Nakajima and Kurita, 2005
5 Singapore grouper iridovirus (SGIV)	Ranavirus	Iridoviridae	AY521625	Complete genome	dsDNA, linear		140,131	Song *et al.*, 2004
6 Infectious pancreatic necrosis virus (IPNV)	Aquabirnavirus	Birnaviridae	NC_001915	Segment A	dsRNA, linear	3,097	5,881	Duncan and Dobos, 1986
			NC_001916	Segment B	dsRNA, linear	2,784		Duncan *et al.*, 1991
			AY283780	Segment A (strain AM-98)	dsRNA, linear	3,088	3,088	Zhang and Suzuki, 2004
7 Yellowtail ascites virus (YAV), marine birnavirus (MABV)	Aquabirnavirus	Birnaviridae	NC_004168	Segment A (strain Y-6)	dsRNA, linear	2,976	5,711	Suzuki *et al.*, 1998
			NC_004176	Segment B (strain Y-6)	dsRNA, linear	2,735		Zhang and Suzuki, 2004
8 Infectious haematopoitic necrosis virus (IHNV)	Novirhabdovirus	Rhabdoviridae	NC_001652, L40883	Complete genome (strain WRAC)	ssRNA, linear		11,131	Morzunov *et al.*, 1995

(*Continued*)

Table 21.5. *Continued*

Virus name	Genus	Family	Accession no.	Genome shape (strain/isolation)	Genome type	Length (bp)	Total genome size (bp)	References
Fish virus								
9 Viral haemorrhagic septicaemia virus (VHSV)	Novirhabdovirus	Rhabdoviridae	NC_000855, Y18263	Complete genome (strain Fi 13)	ssRNA, linear		11,158	Schutze *et al.*, 1999
			AB490792	Complete genome (strain JF00Ehi1)	ssRNA, linear		11,182	Unpublished
			EU481506	Complete genome (strain FA281107)	ssRNA, linear		11,065	Unpublished
			Z93414	Complete genome (strain Cod Ulcus)	ssRNA, linear		10,845	Stone *et al.*, 1997
			Z93412	Complete genome (strain Hededam)	ssRNA, linear		10,845	Stone *et al.*, 1997
			AF143863	Complete genome (strain 14-58)	ssRNA, linear		10,845	Betts and Stone, 2000
			AF143862	Complete genome (strain 96-43)	ssRNA, linear		10,845	Betts and Stone, 2000
10 Spring viraemia of carp virus (SVCV)	Vesiculovirus	Rhabdoviridae	NC_002803, U18101	Complete genome	ssRNA, linear		11,019	Bjorklund *et al.*, 1996
			EU177782	Complete genome (isolate BJ0505-2)	ssRNA, linear		11,047	Unpublished
			DQ491000	Complete genome (isolate A2)	ssRNA, linear		10,990	Unpublished
			AJ318079	Complete genome (strain ATCC VR-1390)	ssRNA, linear		11,019	Hoffmann *et al.*, 2002
11 Red-spotted grouper nervous necrosis virus (RGNNV)	Betanodavirus	Nodaviridae	NC_008040	Segment RNA 1 (strain SGWak97)	ssRNA, linear	3,105	4,539	Iwamoto *et al.*, 2004
			NC_008041	Segment RNA 2 (strain SGWak97)	ssRNA, linear	1,434		Iwamoto *et al.*, 2004
12 *Epinephelus tauvina* nervous necrosis virus (ETNNV)	Betanodavirus	Nodaviridae	NC_004137	Segment RNA 1 (strain Singapore)	ssRNA, linear	3,103	4,536	Tan *et al.*, 2001

			NC_004136	Segment RNA 2 (strain Singapore)	ssRNA, linear	1,433		Tan *et al.*, 2001
13 Striped jack nervous necrosis virus (SJNNV)	Betanodavirus	Nodaviridae	NC_003448	Segment RNA 1	ssRNA, linear	3,107	4,528	Iwamoto *et al.*, 2001
			NC_003449	Segment RNA 2	ssRNA, linear	1,421		Iwamoto *et al.*, 2001
14 Infectious salmon anaemia virus (ISAV)	Isavirus	Orthomyxoviridae	NC_006505	Segment 1 (isolate 810/9/99)	ssRNA, linear	2,169	12,716	Unpublished
			NC_006503	Segment 2 (isolate CCBB)	ssRNA, linear	2,185		Clouthier *et al.*, 2002
			NC_006502	Segment 3 (isolate CCBB)	ssRNA, linear	2,046		Clouthier *et al.*, 2002
			NC_006501	Segment 4 (isolate CCBB)	ssRNA, linear	1,787		Clouthier *et al.*, 2002
			NC_006500	Segment 5 (isolate CCBB)	ssRNA, linear	1,504		Clouthier *et al.*, 2002
			NC_006499	Segment 6 (isolate CCBB)	ssRNA, linear	1,323		Clouthier *et al.*, 2002
			NC_006498	Segment 7 (isolate CCBB)	ssRNA, linear	966		Clouthier *et al.*, 2002
			NC_006497	Segment 8 (isolate CCBB)	ssRNA, linear	736		Clouthier *et al.*, 2002
Shellfish virus								
1 White spot syndrome virus (white spot bacilliform virus, WSSV)	Whispovirus	Nimaviridae	NC_003225, AF332093	Complete genome (isolate WSSV-CH)	dsDNA, circular		305,107	Yang *et al.*, 2001
			AF440570	Complete genome (isolate WSSV-TW)	dsDNA, circular		307,287	Tsai *et al.*, 2004
			AF369029	Complete genome (isolate WSSV-TH)	dsDNA, circular		292,967	van Hulten *et al.*, 2001
2 Infectious myonecrosis virus (IMNV)	Unclassified	Totiviridae	NC_007915, AY570982	Complete genome (isolate Brazil)	dsRNA, linear		7,560	Poulos *et al.*, 2006
			EF061744	Complete genome (isolate Indonesia)	dsRNA, linear		7,561	Unpublished

(*Continued*)

Table 21.5. *Continued*

Virus name	Genus	Family	Accession no.	Genome shape (strain/ isolation)	Genome type	Length (bp)	Total genome size (bp)	References
Shellfish virus								
3 Taura syndrome virus (TSV)	Cripavirus	Discistroviridae	NC_003005, AF277675	Complete genome	ssRNA, linear		10,205	Mari *et al.*, 2002
			DQ21279	Complete genome (isolate Venezuela)	ssRNA, linear		10,095	Cote *et al.*, 2008
			AY997025	Complete genome (isolate Th04Lv)	ssRNA, linear		10,205	Srisuvan *et al.*, 2005
			DQ104696	Complete genome (isolate ZHZC3TSV)	ssRNA, linear		10,202	Unpublished
4 Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Brevidensovirus	Parvoviridae	NC_002190, AF218266	Complete genome	ssDNA, linear		4,075	Mari *et al.*, 1993
			EF633688	Complete genome (isolate Fujian)	ssDNA, linear		3,833	Unpublished
5 Hepatopancreatic parvovirus (HPV)	Unclassified	Parvoviridae	NC_007218, DQ002873	Complete genome	ssDNA, linear		6,321	Sukhumsirichart *et al.*, 2006
			NC_011545, FJ410797	Complete genome (isolate India)	ssDNA, linear		6,222	Unpublished
6 *Macrobrachium rosenbergii* nodavirus (MrNV)	Unclassified	Nodaviridae	NC_005094, AY222839	Segment RNA-1	ssRNA, linear	3,202	4,377	Sri Widada *et al.*, 2003
(host: *Macrobrachium rosenbergii*)			NC_005095, AY222840	Segment RNA-2	ssRNA, linear	1,175		Sri Widada *et al.*, 2003

*The structure of iridoviridae genome could be circularly permuted and terminally redundant (Tidona and Darai, 1997); **ND: no accession number in GenBank database.

Table 21.6. Protein sequences coded in the published genome sequences of fish and shellfish infectious viruses.

Virus name	Virus protein	Accession No. (amino acid sequence)	Accession No. (genome)
Fish virus 1 KHV, CyHV-3	ORF1L~ORF8L	YP_001096040~YP_001096047	NC_009127; DQ177346
	ORF9~ORF156	YP_001096048~YP_001096191	
	ORF1R~ORF8R	YP_001096192~YP_001096199	
	Secreted (soluble) TNFR	ORF4L/R,12	
	G protein-coupled receptor	ORF16	
	Deoxynucleotide (deoxyguanosine) kinase	ORF19	
	Small subunit of ribonucleotide reductase	ORF23	
	Predicted membrane glycoprotein	ORF25,26,27,30,40,65, 99,115,116,124, 126,131,136,138,139,146, 148,149	
	Similar to bacterial NAD-dependent epiderase/dehydratase	ORF28	
	Similar to eukaryotic DUF614 proteins	ORF31	
	Similar to a family of Singapore grouper iridovirus proteins	ORF32	
	ATPase subunit of terminase	ORF33	
	Multiple membrane-spanning (multipule transmembrane) protein	ORF29,39,64,81-83,114,153	
	RING-finger protein (SPRY protein; TRIM-like protein)	ORF41,128,144,150	
	Primase	ORF46	
	Similar to protein kinase	ORF48	
	Zinc-binding protein	ORF54	
	Thymidylate kinase	ORF55,140	
	OUT-like cysteine protease domain	ORF62	
	Similar to myosin and related protein	ORF68	
	DNA helicase	ORF71	
	Capsid triplex protein 2	ORF72	
	Capsid protease and scaffolding protein	ORF78	
	DNA polymerase	ORF79	
	Major capsid protein	ORF92	
	Trypsin-like serine protease	ORF94	
	Uracil-DNA glycosylase	ORF98	
	Serine-threonine protein kinase	ORF104	
	Among the least convincing protein-coding regions	ORF105	
	Contains double-stranded nucleic acid-binding domain	ORF112	
	dUTP diphosphatase (dUTPase); deoxyuridine triphosphatase	ORF123	
	Predicted membrane protein	ORF132	
	Interleukin-10	ORF134	
	Ribonucleotide reductase large subunit	ORF141	

(*Continued*)

Table 21.6. *Continued*

Virus name	Virus protein	Accession No. (amino acid sequence)	Accession No. (genome)
Fish virus			
2 CCHV	ORF1~ORF79	NP_041092~NP_041169	NC_001493
	ORF1R~ORF14R	NP_041170~NP_041183	
	Deoxyribonucleoside kinase	ORF5	
	RING-finger protein	ORF9,11,12	
	Membrane glycoprotein	ORF10, 46	
	Protein kinase	ORF14-16,73,74	
	Membrane protein	ORF19, 51	
	Helicase	ORF25	
	Capsid triplex protein 2	ORF27	
	Putative capsid maturational protease	ORF28	
	Major capsid protein	ORF39	
	Subtilisin-like proprotein convertase	ORF47	
	dUTP diphosphatase	ORF49	
	Capsid triplex protein 1	ORF53	
	DNA polymerase catalytic subunit	ORF57	
	Major envelope protein	ORF59	
	Putative terminase ATPase subunit	ORF62	
	Putative primase	ORF63	
	Tegument protein	ORF65	
	Tegument-associated protein	ORF72	
	(Deoxy)nucleoside-phosphate kinase	ORF76	
	Putative Zn-binding protein	ORF78	
3 IPNV	Hypothetical protein	NP_047195	NC_001915 (segment A)
	Polyprotein	NP_047196	
	Viral protein 1 (RNA polymerase)	NP_047197	NC_001916 (segment B)
4 YAV	Protein VP5	YP_899471	NC_004168 (segment A)
	Polyprotein	NP_690805	
	Putative RNA-dependent RNA polymerase	NP_690835	NC_004176 (segment B)
5 LCDV	Proliferating cell nuclear antigen	NP_078615	NC_001824
	DNA methyltransferase	NP_078617	
	DNA-depenent RNA polymerase largest subunit	NP_078624	
	Putative antimutator GTP pyrophosphohydrolase MutT	NP_078631	
	DNA-directed RNA polymerase subunit 2	NP_078633	
	Ribonucleotide reductase small subunit	NP_078636	
	VLTF2-like late transcription factor	NP_078638	
	Papain-like proteinase	NP_078647	
	Hypothetical immediate-early protein	NP_078648	
	Galactose-binding lectin	NP_078654	
	Virion assembly protein, NTPase	NP_078656	
	Collagen-like protein	NP_078660	
	Myristylated membrane protein A	NP_078665	

(*Continued*)

Table 21.6. *Continued*

Virus name	Virus protein	Accession No. (amino acid sequence)	Accession No. (genome)
Fish virus			
	Uncharacterized LCDV1 paralogue family 1	NP_078666, NP_078668, NP_078728	
	Apoptosis regulation Bcl-2 family protein	NP_078671	
	Phosphotransferase	NP_078677, NP_078729	
	Putative NIF/NLI interacting factor	NP_078678	
	Uncharacterized conserved domain linked to protein kinase domain	NP_078689	
	Tristetraprolin-like zinc finger protein C3H	NP_078696	
	Thiol oxidoreductase	NP_078699	
	Ariadne-2 homologue	NP_078700	
	Hypothetical LCDV1 paralogue family 2	NP_078702, NP_078704	
	Early iridovirus protein	NP_078713	
	D5 family NTPase involved in DNA replication	NP_078717	
	SWI/SNF2 family helicase	NP_078720	
	DNA polymerase family B	NP_078724	
	Deoxynucleoside kinase	NP_078725	
	Ribonuclease III	NP_078726	
	Major capsid protein	NP_044812	
	Hydroxysteroid dehydrogenase	NP_078739	
	Membrane (myristylated) protein	NP_078745	
	Putative replication factor and/or DNA binding/packing protein	NP_078747	
	TNF/TNFR- and CUB-domains protein	NP_078749	
	Transcription factor SII homologue	NP_078754	
	Ribonucleotide reductase large subunit	NP_078756	
	Putative filamentous protein	NP_078764	
	Putative XPG/RAD2-type nuclease	NP_078767	
6 SGIV	ORF001~ORF00162 (L/R)	AAS18016~AAS18177	AY521625
	3-beta-hydroxy-delta-5-C27-steroid oxidoreductase	ORF003R	
	Ribonucleoside-diphosphate reductase beta subunit	ORF047L	
	CARD-like protein	ORF048L	
	dUTPase	ORF049L	
	D5 family NTPase	ORF052L	
	NTPase	ORF060R	
	Ribonucleoside-diphosphate reductase alpha subunit	ORF064R	
	Deoxynucleoside kinase	ORF067L	
	Thiol oxidoreductase	ORF070R	
	Major capsid protein	ORF072R	
	DNA-directed RNA polymerase II second largest subunit	ORF073L	
	Tyrosine kinase	ORF078L, 081L	
	RNase III	ORF084L	
	Transcription elongation factor TFIIS	ORF085R	

(*Continued*)

Table 21.6. *Continued*

Virus name	Virus protein	Accession No. (amino acid sequence)	Accession No. (genome)
	Putative immediate-early protein	ORF086R	
	DNA repair protein RAD2	ORF097L	
	Ubiqutin/ribosomal protein	ORF102L	
	DNA-dependent RNA polymerase II largest subunit	ORF104L	
	Putative replication factor	ORF116R	
	DNA polymerase	ORF128R	
	ATPase	ORF134L	
	NTPase/helicase	ORF146L	
	Phosphotransferase	ORF150L	
	Helicase	ORF152R	
7 IHNV	Nucleocapsid protein	NP_042676	NC_001652
	Polymerase-associated protein	NP_042677	
	Matrix protein	NP_042678	
	Glycoprotein	NP_042679	
	Non-virion protein	NP_042680	
	RNA polymerase	NP_042681	
8 VHSV	Nucleoprotein	NP_049545	NC_000855
	Phosphorylated protein	NP_049546	
	Matrix protein	NP_049547	
	Glycoprotein	NP_049548	
	Non-virion protein	NP_049549	
	Large protein	NP_049550	
9 SVCV	Nucleocapsid protein	NP_116744	NC_002803
	Phosphoprotein	NP_116745	
	Matrix protein	NP_116746	
	Glycoprotein	NP_116747	
	Polymerase	NP_116748	
10 RGNNV	Protein A (RNA-dependent RNA polymerase)	YP_611155	NC_008040 (RNA1)
	Protein B	YP_611156	
	Coat protein	YP_611157	NC_008041 (RNA2)
11 ETNNV	RNA-dependent RNA polymerase	NP_689433	NC_004137 (RNA1)
	Hypothetical protein	NP_689434	
	Coat protein	NP_689432	NC_004136 (RNA2)
12 SJNNV	Protein A	NP_599247	NC_003448 (RNA1)
	Protein B	NP_599248	
	Coat protein	NP_599249	NC_003449 (RNA2)
13 ISAV	PB2 polymerase	YP_145807	NC_006505 (segment 1)
	Putative PB1 protein	YP_145804	NC_006503 (segment 2)
	Putative nucleocapsid protein	YP_145803	NC_006502 (segment 3)

(*Continued*)

Table 21.6. *Continued*

Virus name	Virus protein	Accession No. (amino acid sequence)	Accession No. (genome)
	Putative PA protein	YP_145802	NC_006501 (segment 4)
	Putative acetylesterase	YP_145801	NC_006500 (segment 5)
	Putative HA protein	YP_145800	NC_006499 (segment 6)
	P4	YP_145798	NC_006498 (segment 7)
	P6	YP_145796	NC_006497 (segment 8)
	P7	YP_145797	
Shellfish virus 1 WSSV	wsv001 (VP28)	AAL33534	AF332093 (WSSV-CH)
	wsv002~wsv245	AAL33006~AAL33249	
	wsv246	AAL33535	
	wsv247~wsv286	AAL33250~AAL33289	
	wsv287	AAL33536	
	wsv288~wsv436	AAL33290~AAL33438	
	wsv437	AAL33533	
	wsv438~wsv531	AAL33439~AAL33532	
	Putative protein kinase	wsv083	
	Putative CREB-binding protein	wsv100	
	Putative dUTP pyrophoshatase	wsv112	
	Putative ribonucleoside-diphosphate reductase large chain	wsv172	
	Putative ribonucleotide reductase R2 subunit	wsv188	
	Putative deoxyribonuclease I	wsv191	
	Putative nuclear protein	wsv214	
	Putative serine/threonine protein kinase	wsv289	
	Putative transcription initiation factor IID	wsv303	
	Putative thymidylate kinase	wsv395	
	Putative DNA polymerase III catalytic subunit	wsv514	
2 IMNV	Structural protein (coat protein)	YP_529548	NC_007915
	Non-structural protein (gag-pol fusion protein / RNA polymerase)	YP_529549	
3 TSV	Non-structural polyprotein	NP_149057	NC_003005
	Capsid protein precursor	NP_149058	
4 IHHNV	Hypothetical protein (ORF 2)	NP_039237	NC_002190
	Non-structural protein	NP_039238	
	37 kDa coat protein	NP_039239	
5 HPV	Non-structural protein 2	YP_271914	NC_007218
	Non-structural protein 1	YP_271915	
	Structural protein	YP_271916	
6 MrNV	RNA-dependent RNA polymerase	NP_919036	NC_005094 (RNA-1)
	B2 protein	NP_919037	
	Capsid protein	NP_919038	NC_005095 (RNA-2)

predicted 27 membrane proteins (including transmembrane protein or glycoprotein) of KHV are major components of the virion envelope and appear to be relevant for the virus host cells as well as the host immune response (Table 21.6). The vertebrate immune components containing two soluble types of tumour necrosis factor receptor (TNFR) and interleukin-10 are also found in the KHV genome. The KHV envelope protein pORF81 has already been detected by Western blot and immunoelectron microscopy (IEM) using rabbit anti-pORF81 serum (Rosenkranz *et al.*, 2008).

White spot syndrome virus (WSSV)

White spot syndrome virus (WSSV, alternatively named white spot bacilliform virus [WSBV]) is an acute disease pathogen of major economic importance in cultured penaeid shrimp worldwide. The virus is not only present in shrimp but also occurs in other fresh, brackish and seawater crustaceans, including crabs and crayfish (Lo *et al.*, 1996). WSSV is a large, double-stranded circular DNA virus assigned to the genus *Whispovirus* (family *Nimaviridae*), which is not related to any known viruses. The whole genome sequence of WSSV has been determined from three strains isolated from China (WSSV-CH, Yang *et al.*, 2001), Taiwan (WSSV-TW, Tsai *et al.*, 2004) and Thailand (WSSV-TH, van Hulten *et al.*, 2001; Marks *et al.*, 2004). The genome sizes of these strains are 292,967 bp (WSSV-TH), 305,107 bp (WSSV-CH) and 307,287 bp (WSSV-TW) (shown in Table 21.5). The three genomes share an overall nucleotide identity of 99.32%. Compared with WSSV-CN, major differences include a 12-kb deletion in WSSV-TH that is located at 275,235–287,285 (the nucleotide coordinates are from WSSV-CN), a variable region at 267,203–268,046, and an insertion of a 1.3 kb transposase sequence in WSSV-TW at 204,978–204,979 (Marks *et al.*, 2004, 2005). The WSSV genome has nine homologous repeat regions, each of which contains numerous imperfect palindromic repeats (250 bp in size) (Yang *et al.*, 2001; van Hulten *et al.*, 2001; Marks *et al.*, 2004). The number of non-overlapping ORFs (≧ 60 amino acids) ranges from 181 (in the WSSV-CN genome) to 184 (in the WSSV-TH genome). Some genes encode proteins with identifiable function. These proteins include DNA replicating enzymes (DNA polymerase, ribonucleotide reductase subunits, dUTPase, thymidylate synthase, thymidine-thymidylate kinase) and protein-modifying proteins (protein kinase) (Table 21.6). Proteomic studies on purified virions have led to the identification of over 50 structural proteins (Huang *et al.*, 2002; Tsai *et al.*, 2004; Zhang *et al.*, 2004; Xie and Yang, 2006; Li *et al.*, 2007). Together with other techniques, such as Western blot analysis and IEM, these structural proteins could be classified into envelope, tegument and nucleocapsid proteins (Tsai *et al.*, 2006; Xie *et al.*, 2006). The structural proteins include a very large gene, *vp664*, that encodes a major nucleocapsid protein of about 664 kDa (Leu *et al.*, 2005). Shotgun proteomic analysis of WSSV infected epithelium identified 11 novel proteins that were presumed to be non-structural (Wu *et al.*, 2007). Functional studies have meanwhile discovered several WSSV genes, including latency-related genes (Khadijah *et al.*, 2003), immediately early genes (Liu *et al.*, 2005), ubiquitination-related genes (Wang *et al.*, 2005; He *et al.*, 2006) and anti-apoptosis genes (Wang *et al.*, 2004; He *et al.*, 2006). However, most ORFs of WSSV are still unassigned.

Acknowledgements

Special thanks to Professor Chu-Fang Lo (Institute of Zoology, Taiwan National University) for giving us advice and comments on the white spot syndrome virus.

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