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Review

A review on the morphology, molecular characterization, morphogenesis and pathogenesis of white spot syndrome virus

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Abstract

Since it first appeared in 1992, white spot syndrome virus (WSSV) has become the most threatening infectious agent in shrimp aquaculture. Within a decade, this pathogen has spread to all the main shrimp farming areas and has caused enormous economic losses amounting to more than seven billion US dollars. At present, biosecurity methods used to exclude pathogens in shrimp farms include disinfecting ponds and water, preventing the entrance of animals that may carry infectious agents and stocking ponds with specific pathogen-free post-larvae. The combination of these practices increases biosecurity in shrimp farming facilities and may contribute to reduce the risk of a WSSV outbreak. Although several control methods have shown some efficacy against WSSV under experimental conditions, no therapeutic products or strategies are available to effectively control WSSV in the field. Furthermore, differences in virulence and clinical outcome of WSSV infections have been reported. The sequencing and characterization of different strains of WSSV has begun to determine aspects of its biology, virulence and pathogenesis. Knowledge on these aspects is critical for developing effective control methods. The aim of this review is to present an update of the knowledge generated so far on different aspects of WSSV organization, morphogenesis, pathology and pathogenesis.

Keywords: morphogenesis, pathogenesis, review, shrimp aquaculture, viral diseases, white spot syndrome virus.

Introduction

In 1992, a new virus appeared in shrimp farms in northern Taiwan causing disease and massive mortality (Chou, Huang, Wang, Chiang & Lo 1995). In late 1993, the viral agent was first isolated from an outbreak in Japan (Inouye, Miwa, Oseko, Nakano, Kimura, Momoyama & Hiraoka 1994) and within a few years this new pathogenic agent spread to several shrimp farming countries (Flegel 1997; Anonymous 2003). At first, it was thought that different viral agents had simultaneously appeared in different regions and each were given a specific name: hypodermal and haematopoietic necrosis baculovirus (HHNBV) (see Durand, Lightner, Nunan, Redman, Mari & Bonami

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1996), third *Penaeus monodon* non-occluded baculovirus (PmNOB III) (see Wang, Lo, Leu, Chou, Yeh, Chou, Tung, Chang, Su & Kou 1995; Karunasagar, Otta & Karunasagar 1997), rod-shaped nuclear virus of *Marsupenaeus japonicus* (RV-PJ) (Inouye *et al.* 1994; Inouye, Yamano, Ikeda, Kimura, Nakano, Momoyama, Kobayashi & Miyajima 1996), penaeid rod-shaped DNA virus (see Venegas, Nonaka, Mushiake, Nishizawa & Muroga 2000), systemic ectodermal and mesodermal baculovirus (Wongteerasupaya, Vickers, Sriurairatana, Nash, Akarajamorn, Boonsaeng, Panyim, Tassanakajon, Withyachumnarnkul & Flegel 1995; Sahul-Hameed, Anilkumar, Raj & Jayaraman 1998) or white spot baculovirus (Chou *et al.* 1995; Lightner 1996). Later, it was recognized that a single viral agent was responsible for these reports. Eventually an informal consensus was reached to call it white spot syndrome virus (WSSV). This pathogen is now recognized as the most serious for shrimp aquaculture worldwide. The reports of WSSV outbreaks in various shrimp farming countries are presented in Table 1.

In China, production losses of 80% of farmed shrimp were attributed to WSSV (Zhan, Wang, Fryer, Yu, Fukuda & Meng 1998) (Fig. 1a) and in Ecuador, the impact of WSSV on farmed shrimp production was also disastrous (Fig. 1b) (FAO 2006). The spread of WSSV to other shrimp farming countries threatens the development of shrimp aquaculture. In 2000, crabs and crayfish in Australia were apparently found WSSV-positive using polymerase chain reaction (PCR) primers but later these results were proven to be false-positives (Claydon, Cullen & Owens 2004). Australia remains at risk of a WSSV outbreak, given its proximity to Southeast Asia where the pathogen is endemic (Chang, Su, Chen, Lo, Kou & Liao 1999; Chang, Su, Chen & Liao 2003). It is possible that some Asian crustaceans carrying WSSV may reach Australia and spread to shrimp farming areas (Claydon *et al.* 2004). In 2002, WSSV was found in wild crustaceans off the French Mediterranean coast (see Marks 2005). The presence of WSSV in the Mediterranean may hamper the development of shrimp aquaculture, especially in North African

Table 1 Chronology of white spot syndrome virus outbreaks in shrimp farming countries in Asia and America

Year first reported	Country	Reference
1992	Taiwan	Chou *et al.* 1995
1993	China, Japan, Korea	Zhan *et al.* 1998; Inouye *et al.* 1994; Park *et al.* 1998
1994	Thailand, India, Bangladesh	Lo *et al.* 1996a; Karunasagar *et al.* 1997; Mazid & Banu 2002
1995	USA	Lightner 1996; Wang *et al.* 1999a
1996	Indonesia, Malaysia, Sri Lanka	Durand *et al.* 1996; Kasornchandra *et al.* 1998; Rajan *et al.* 2000
1997	Vietnam	Bondad-Reantaso *et al.* 2001
1998	Peru	Rosenberry 2001
1999	Philippines, Ecuador, Colombia, Panamá, Honduras, Nicaragua, Guatemala, Belice	Magbanua *et al.* 2000; Bondad-Reantaso *et al.* 2001; Hossain *et al.* 2001; Wu *et al.* 2001
1999–2000	México	Bondad-Reantaso *et al.* 2001
2002	France, Iran	Dieu *et al.* 2004; Marks 2005
2005	Brazil	APHIS-USDA 2005

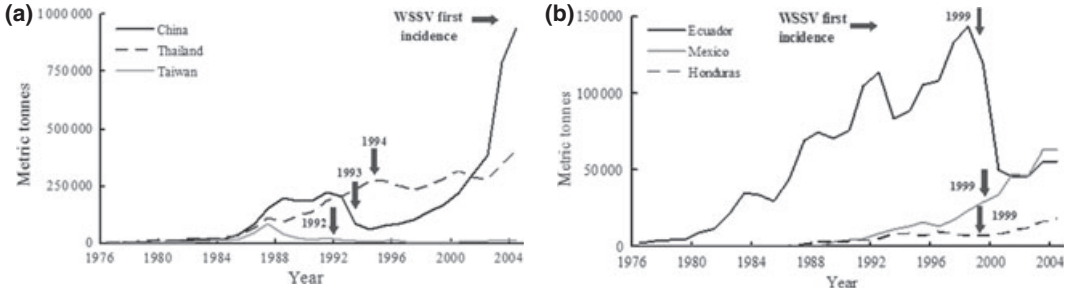

Figure 1 Impact of white spot syndrome virus on farmed-shrimp production in (a) Asian and (b) American countries (FAO 2006). Arrows indicate year of first outbreak reported for each country.

countries. The introduction of WSSV-infected organisms to the areas where the pathogen was previously unknown may be possible through ballast water from cargo ships (Flegel & Fegan 2002) or even frozen commodities (Durand, Tang & Lightner 2000).

Morphology of WSSV

White spot syndrome virus is a bacilliform, non-occluded enveloped virus (Chou *et al.* 1995; Wang *et al.* 1995; Wongteerasupaya *et al.* 1995). Intact enveloped virions range between 210 and 380 nm in length and 70–167 nm in maximum width (Chang, Lo, Wang & Kou 1996; Flegel & Alday-Sanz 1998; Park, Lee, Lee & Lee 1998; Rajendran, Vijayan, Santiago & Krol 1999). A tail-like appendage at one end of the WSSV virion is sometimes observed in negatively stained electron micrographs (Wongteerasupaya *et al.* 1995; Durand *et al.* 1996) (Fig. 2a, b).

The viral envelope is 6–7 nm thick and is a lipidic, trilaminar membranous structure with two electron-transparent layers divided by an electron-opaque layer (Wongteerasupaya *et al.* 1995; Durand, Lightner, Redman & Bonami 1997; Nadala, Tapay & Loh 1998).

The nucleocapsid is located inside the envelope and is a stacked ring structure composed of globular protein subunits of 10 nm in diameter arranged in 14–15 vertical striations located every 22 nm along the long axis, giving it a cross-hatched appearance (Durand *et al.* 1997; Nadala & Loh 1998). When released from the envelope, the nucleocapsid increases in length indicating that it is tightly packed within the virion. The size of the nucleocapsid varies from isolate to isolate and ranges between 180 and 420 nm in length and 54–85 nm in width, with a 6-nm thick external wall (Kasornchandra, Boonyaratpalin & Itami 1998; Sahul-Hameed *et al.* 1998; Rajendran *et al.* 1999).

A highly electrondense core comprised of the DNA binding protein VP15 and the viral DNA is found inside the nucleocapsid (Durand *et al.* 1997; Wang, White, Redman & Lightner 1999b; van Hulten, Witteveldt, Peters, Kloosterboer, Tarchini, Fiers, Sandbrink, Klein-Langhorst & Vlak 2001a).

Structural proteins

More than 40 WSSV proteins have been characterized (Table 2). Some non-structural proteins are probably involved in transcriptional regulation (VP9) (Liu, Wu, Song, Sivaraman & Hew 2006), virus proliferation (WSV021) (Zhu, Ding & Yang 2007) and/or regulation of DNA replication (WSV477) (Han, Xu & Zhang 2007). At least 38 structural proteins have been located in the WSSV virion. Of these, 21 have been found in the envelope, 10 in the nucleocapsid and five in the tegument (a putative structure located between the envelope and nucleocapsid).

A cell attachment motif that suggests a role in viral entry has been found in the envelope proteins VP31, VP110 and VP281 (Huang, Zhang, Lin, Xu, Hu & Hew 2002a; Tsai, Wang, Leu, Hsiao, Wang, Kou & Lo 2004; Li, Xie & Yang 2005a; Xie, Xu & Yang 2006), the tegument protein VP36A and the nucleocapsid proteins VP664 (Tsai *et al.* 2004; Leu, Tsai, Wang, Wang, Wang, Kou & Lo 2005) and VP136A (Tsai *et al.* 2004; Xie *et al.* 2006). Other proteins such as VP28, VP39B, VP41A,

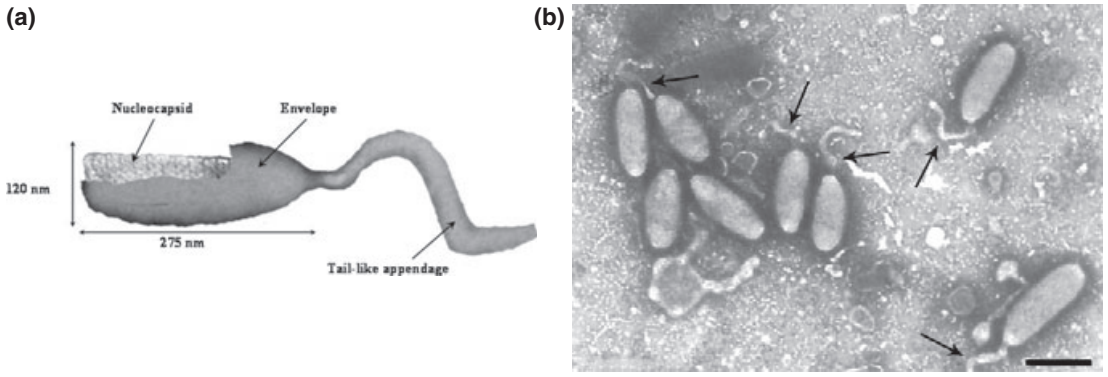

Figure 2 (a) Morphology of the white spot syndrome virus (WSSV) virion. (b) Electron micrograph showing WSSV virions with the tail-like appendage (black arrows) (bar = 250 nm) (picture from Durand *et al.* 1996).

Table 2 List of white spot syndrome virus (WSSV) proteins so far characterized

Protein name	Genbank accession number	Size (aminoacid residues)	Apparent size (kDa)	Putative function	Location in WSSV virion (references)
VP9	2GJIA	79	9	Transcriptional	Non-structural[18]
VP11	AAL89262	433	11	Unknown	Not determined[8]
VP12A (VP95)	AF402996	95	11	Structural	Tegument[8, 9, 21]
VP12B (VP68)	AF411464	68	7	Structural	Envelope[8, 12, 13]
VP13A	AAL89207	100	13	Energy metabolism	Not determined[8]
VP13B (VP16)	AAL89245	117	13	Structural	Envelope[21]
VP14	AAL89217	97	11	Structural	Envelope[21]
VP15	AAL89137	80	15	DNA binding protein	Nucleocapsid/core[8, 11]
VP19	AAL89341	121	19	Structural	Envelope[8, 9, 11]
WSV021	AAL33025	200	23	Regulation virus replication	Non-structural[19]
VP22 (VP184)	AAL89227	891	100	Unknown	Not determined[8]
VP24 (VP208)	DQ902656	208	24	Structural	Nucleocapsid[8, 10, 13]
VP26	EF534253	204	26	Structural	Tegument[8, 10]
VP28	EF534254	204	28	Structural	Envelope[8, 9, 10]
VP31	AY897235	261	31	Cell attachment	Envelope[6, 8, 9]
VP32	AAL89121	278	32	Structural	Envelope[8, 21]
VP35	AY325896	228	26	Structural	Nucleocapsid[1]
VP36A	AAL89002	297	36	Cell attachment	Tegument[8, 9]
VP33 (VP281)	EF534251	281	32	Cell attachment	Envelope[2, 8, 12, 21]
VP38A	AAL89182	309	35	Structural	Envelope[8, 9, 21]
VP38B	AAL89317	321	38	Endonuclease	Not determined[8]
VP39A	AAL89230	419	39	Structural	Tegument[8, 9]
VP39B	AY884234	283	32	Structural	Envelope[8, 15, 21]
VP41A (VP292)	AF411636	292	33	Structural	Envelope[2, 8, 13]
VP41B (VP300)	AF403003	300	34	Structural	Envelope[8, 21]
VP51A	AAL89162	486	51	Structural	Envelope[8, 17, 21]
VP51B (VP384)	AAL89179	384	46	Structural	Envelope[8, 9, 21]
VP51C (VP466)	AAL89232	466	50	Structural	Nucleocapsid[3, 8, 12]
VP53A (VP150)	AAL88935	1301	144	Structural	Envelope[8, 9, 21]
VP53B	AAL89039	968	53	Signal transduction pathway	Not determined[8]
VP53C	AAL89192	489	53	Unknown	Not determined[8]
VP55 (VP448)	AAL88919	448	55	Unknown	Not determined[8]
VP60A (VP56)	AAL89249	465	60	Structural	Envelope[21]
VP60B (VP544)	AAL89342	544	60	Adenovirus fibre-like protein	Nucleocapsid[8, 9, 13, 21]
VP75	AAL89256	786	75	Structural	Nucleocapsid[16]
VP76 (VP73)	AAL89143	675	76	Class 1 cytokine receptor	Nucleocapsid[4, 8, 17, 21]
VP90	AAL89251	856	96	Structural	Envelope[21]
VP95	AAL89370	800	89	Structural	Tegument[21]
VP110	AAL88960	972	110	Cell attachment	Envelope[8, 21]
VP124	AAL89139	1194	124	Structural	Envelope[8, 14, 21]
VP136A	AAL89194	1219	136	Cell attachment	Nucleocapsid[8, 21]
VP136B	AAL89392	1243	136	Unknown	Not determined[8]
VP180 (VP1684)	AAL88920	1684	169	Collagen-like protein	Envelope[8]
VP187	AAL89132	1606	174	Structural	Envelope[7, 21]
VP190	AAL33291	1565	174	Structural	Nucleocapsid[21]
WSV477	DQ121373	208	30	DNA replication	Non-structural[20]
VP664	AAL89287	6077	664	Cell attachment	Nucleocapsid[5, 8, 9]
VP800	AAL02264	800	90	Unknown	Not determined[8]

References: [1]Chen *et al.* 2002b; [2]Huang *et al.* 2002a,b, 2005; [5]Leu *et al.* 2005; [6]Li *et al.* 2005a, 2006b,a, [8]Tsai *et al.* 2004, 2006; [10]van Hulten *et al.* 2000b; [11]van Hulten *et al.* 2002; [12]Wu *et al.* 2005; [13]Zhang *et al.* 2004; [14]Zhu *et al.* 2005; [15]Zhu *et al.* 2006; [16]Xiao *et al.* 2006; [17]Wu & Yang 2006; [18]Liu *et al.* 2006; [19]Zhu *et al.* 2007; [20]Han *et al.* 2007; [21]Xie *et al.* 2006;.

VP41B, VP51A, VP51B, VP68, VP124, VP150, VP187, VP281, VP292 and a collagen-like protein (Li, Chen & Yang 2004) have been located in the envelope (van Hulten, Witteveldt, Snippe & Vlak 2001b; van Hulten *et al.* 2001a; Huang *et al.* 2002a; van Hulten, Reijns, Vermeesch, Zandbergen & Vlak 2002; Zhang, Huang, Tang, Zhuang & Hew 2004; Wu, Wang & Zhang 2005; Zhu, Xie & Yang 2005; Li, Zhu, Xie & Yang 2006a; Xie *et al.* 2006; Zhu, Li & Yang 2006); whereas the proteins VP35 (Chen, Leu, Huang, Chou, Chen, Wang, Lo & Kou 2002a), VP466 (Huang, Zhang, Lin, Xu & Hew 2002b), VP15 (van Hulten *et al.* 2002), VP51, VP76 (Wu & Yang 2006) and others (Xiao, Zhang, Dai, Yuan, Wang, Zhang, Xu & Dai 2006) have been located in the nucleocapsid and may have

different putative functions (van Hulten *et al.* 2001a; Yang, He, Lin, Li, Pan, Zhang & Xu 2001).

In vivo neutralization assays using antibodies against different structural proteins showed a significant delay of shrimp mortality, indicating that proteins such as VP28 (van Hulten *et al.* 2001b; Yi, Wang, Qi, Yao, Qian & Hu 2004), VP68, VP281, VP466 (Wu *et al.* 2005) and even VP24 (Xie & Yang 2006), might have an important role in virus penetration. Recently, a 25-kDa membrane protein from shrimp haemocytes was found to bind to recombinant VP28 or WSSV virions. This protein has high homology to the small GTP-binding protein Rab7. *In vivo* neutralization assays with anti-Rab7 antibody inhibited the binding of WSSV virions to the cells and significantly reduced mortality upon WSSV challenge (Sritunyalucksana, Wannapapho, Lo & Flegel 2006). In crayfish, neutralization assays with the envelope proteins VP31, VP33 (also known as VP36B) and the tegument protein VP36A strongly inhibited WSSV replication, indicating that these proteins have an important role in infection (Li, Yuan, Cai, Gu & Shi 2006b).

The role of different WSSV proteins in infection has recently been studied by RNA interference. In *Litopenaeus vannamei* (=*P. vannamei*), long double-stranded (ds) RNA corresponding to VP19 induced a specific antiviral response that inhibited WSSV infection and significantly reduced mortality (Robalino, Bartlett, Shepard, Prior, Jaramillo, Scura, Chapman, Gross, Browdy & Warr 2005). In *Fenneropenaeus chinensis* (=*P. chinensis*), long ds RNA corresponding to VP28, VP281, WSSV protein kinases (PK) and an unrelated ds RNA from the green fluorescence protein (GFP) induced higher survival of WSSV-challenged shrimp. The highest survival rates were found in shrimp treated with ds RNA from VP28 and PK (Kim, Kosuke, Nam, Kim & Kim 2007). A complete inhibition of a WSSV infection in shrimp was achieved by three consecutive injections of small short interfering RNA (siRNA) against VP28 in *Marsupenaeus japonicus* (=*P. japonicus*) (Xu, Han & Zhang 2007).

Genome and classification

The WSSV genome is a circular, ds DNA molecule with an A+T content of 59% homogeneously distributed. The genome size varies according to the viral isolate [Thailand 293 kbp (see Fig. 3), China 305 kbp, Taiwan 307 kbp] (van Hulten *et al.*

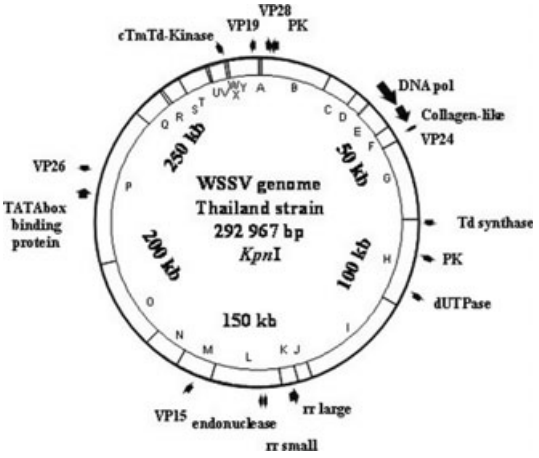

Figure 3 General structure of the white spot syndrome virus genome (Thailand strain).

2001a; Yang *et al.* 2001; Chen, Wang, Huang, Peng, Chen, Lin, Chen, Dai, Yu & Wang 2002b).

This viral genome is one of the largest sequenced so far, being only smaller than a huge virus (mimivirus) infecting an amoeba (1 181 404 bp), a canarypox (359 853 bp), a virus from the brown alga *Ectocarpus siliculosus* (335 593 bp) and a virus from *Paramecium bursaria* (PBCV-1) (330 743 bp) (van Hulten *et al.* 2001b; Raoult, Audic, Robert, Abergel, Renesto, Ogata, La Scola, Suzan & Claverie 2004; http://www.giantvirus.org).

Sequence analysis shows that the WSSV genome contains between 531 and 684 open reading frames (ORFs) with an ATG initiation codon. Of these, 181–184 ORFs are likely to encode functional proteins with sizes between 51 and 6077 amino-acids, which represent 92% of the genetic information contained in the genome (van Hulten *et al.* 2001b; Yang *et al.* 2001). About 21–29% of such ORFs have been shown to encode WSSV proteins or share identity with other known proteins. These proteins include enzymes involved in nucleic acid metabolism and DNA replication such as DNA polymerase (Chen *et al.* 2002b), a non-specific nuclease (Witteveldt, van Hulten & Vlak 2001; Li, Lin & Yang 2005b), a small and a large subunit of ribonucleotide reductase (van Hulten, Tsai, Schipper, Lo, Kou & Vlak 2000a; Tsai, Lo, Van Hulten, Tzeng, Chou, Huang, Wang, Lin, Vlak & Kou 2000a), thymidine kinase, thymidylate kinase, a chimeric thymidine–thymidylate kinase (Tsai, Yu, Tzeng, Leu, Chou, Huang, Wang, Lin, Kou & Lo 2000b), a thymidylate synthase (Li *et al.* 2004), a dUTPase (Liu & Yang 2005) and two PK (van

Hulten & Vlak 2001; van Hulten *et al.* 2001b; Yang *et al.* 2001). Other proteins with a putative function include a collagen-like protein (Li *et al.* 2004), flagellin, a chitinase, a pupal cuticle-like protein, a cell surface flocculin, a kunitz-like proteinase inhibitor, a class 1 cytokine receptor, a *sno*-like peptide and a chimeric anti-apoptotic protein (van Hulten *et al.* 2001b; Yang *et al.* 2001; Marks 2005). Three ORFs (151, 366 and 427 of the Thailand isolate) may encode putative proteins involved in WSSV latency (Khadijah, Neo, Hossain, Miller, Mathavan & Kwang 2003).

Recently, it was found that WSSV also has three immediate early (IE) genes (ORFs 126, 242 and 418 of the Taiwan isolate). These genes are transcribed independently of any viral protein synthesized *de novo* by the host cell machinery and are directly expressed *in vitro*. These IE genes may be important to determine host range and also can function as regulatory trans-acting factors during infection (Liu, Chang, Wang, Kou & Lo 2005).

Transcriptional analysis of genes coding for proteins required in DNA replication and nucleotide metabolism are synthesized early during virus replication. Early transcribed WSSV genes in general have a TATA box 20–30 nucleotides upstream of the transcription initiation site (TIS) (A/C)TCANT (Chen *et al.* 2002b; Liu *et al.* 2005; Marks 2005). Structural proteins are synthesized later during infection and generally have a degenerate TIS motif (A/TNAC/G) located 25 nucleotides downstream of an A/T rich region; which is similar to the TIS motif found in arthropods (Tsai *et al.* 2004; Marks 2005). WSSV has an internal ribosome entry site (IRES) element. WSSV IRES efficiently co-expressed a glutathione S-transferase and a GFP protein arranged in a dicistronic mRNA *in vitro* (Han & Zhang 2006).

Sequence analysis of the DNA polymerase and the organization of several ORFs known to encode WSSV structural proteins were different from those of known baculoviruses, demonstrating that WSSV is not closely related to this virus group (Nadala *et al.* 1998; van Hulten, Goldbach & Vlak 2000b; Chen *et al.* 2002a,b; Huang *et al.* 2002a,b; van Hulten *et al.* 2002; Tsai *et al.* 2004; Zhan, Wang, Chen, Xing & Fukuda 2004; Zhang *et al.* 2004; Huang, Xie, Zhang & Shi 2005; Leu *et al.* 2005; Marks 2005; Xie & Yang 2005; Zhu *et al.* 2005). As WSSV is a distinct new virus, it has been assigned to its own virus family *Nimaviridae* (van Hulten & Vlak 2001, 2002; Vlak, Bonami, Flegel, Kou, Lightner, Lo, Loh & Walker 2005).

Genetic, antigenic and virulence variability in WSSV strains

The genome of three WSSV isolates has been fully sequenced: Thailand 293 kbp (van Hulten *et al.* 2001b), China 305 kbp (Yang *et al.* 2001) and Taiwan 307 kbp (Chen *et al.* 2002a). The nucleotide identity between these isolates is 99.3% (Marks 2005).

In silico restriction analysis with the enzyme *Kpn*I predicts 27 fragments for the Chinese and Taiwanese isolates and 25 for the Thai isolate (Fig. 3). Nine fragments of 0.3, 0.5, 0.7, 4.2, 4.7, 5.3, 5.4, 8.3 and 10.8 kbp are identical in size for all three isolates. Two fragments of approximate sizes of 9 and 20 kbp, respectively, are missing in the Thai isolate. The remaining 14–16 fragments vary in size from 1.2 to 18 kbp between the isolates.

Experimental restriction analysis with *Hin*dIII in several WSSV isolates also found differences in restriction fragment length polymorphism (RFLP) between a Chinese isolate (*F. chinensis*), two isolates from Indonesia (*P. monodon*) and one from the USA (*Farfantepenaeus setiferus*). The latter two isolates were more similar to each other (Nadala & Loh 1998).

Other WSSV isolates from China (*F. chinensis*), India (*P. monodon*), Thailand (*P. monodon* and *L. vannamei*) and the USA (crayfish, *Orconectes punctimanus* from Washington and *L. vannamei* from South Carolina and Texas) were compared by dot blot hybridization using a DNA probe from a Taiwanese isolate. With this method, negative results or a very faint signal were found in some samples from India, Thailand and Texas. This finding suggests important differences between these isolates. Further RFLP analysis of PCR products from 10 different primer sets showed that the Texas isolate was very different from the others (Lo, Hsu, Tsai, Ho, Peng, Kou & Lightner 1999).

Different regions of the WSSV genome display important sequence variations which can be used to establish the origin of a WSSV isolate and its spread within a certain area (Dieu, Marks, Siebenga, Goldbach, Zuidema, Duong & Vlak 2004; Hoa, Hodgson, Oanh, Phuong, Preston & Walker 2005) and also to differentiate isolates in the field (Wongteerasupaya, Pungchai, Withyachumnarnkul, Boonsaeng, Panyim, Flegel & Walker 2003; Marks

2005). Such variability may also induce false-negative results when using certain PCR primers (Claydon *et al.* 2004; Kiatpathomchai, Taweetungtragoon, Jittivadhana, Wongteerasupaya, Boonsaeng & Flegel 2005). An unstable region of 9.6 kbp of the Chinese WSSV genome appears to undergo spontaneous deletions of different sizes depending on the host species. This observation has led to the suggestion that such deletions may play an important function in WSSV virulence (Lan, Lu & Xu 2002).

The protein profiles of the six WSSV isolates described in Lo *et al.* (1999) and isolates from India and Korea were very similar as all of them displayed at least three major structural proteins (VP28, VP24 and VP19). An additional band corresponding to VP15 was found in four isolates. The sequence of the amino-terminal portion of these proteins was identical between isolates (Wang, Poulos & Lightner 2000; Rajendran, Mukherjee, Vijayan, Jung, Kim & Oh 2004).

Several WSSV isolates from the USA (*F. setiferus* and *L. vannamei*), Panama, China (*F. chinensis* and *M. japonicus*), Indonesia (*P. monodon*), Japan (*M. japonicus*), Thailand, Malaysia, Taiwan or different isolates from India were shown to have low antigenic variability using polyclonal or monoclonal antibodies (from whole WSSV virions or raised against full or truncated recombinants of VP28) in different immunoassays such as immunodot assays (Nadala & Loh 2000; You, Nadala, Yang, van Hulten & Loh 2002), Western blot (Shih, Wang, Tan & Chen 2001; Yoganandhan, Syed Musthaq, Narayanan & Sahul Hameed 2004), indirect immunofluorescence (IIF) (Poulos, Pantoja, Bradley-Dunlop, Aguilar & Lightner 2001),

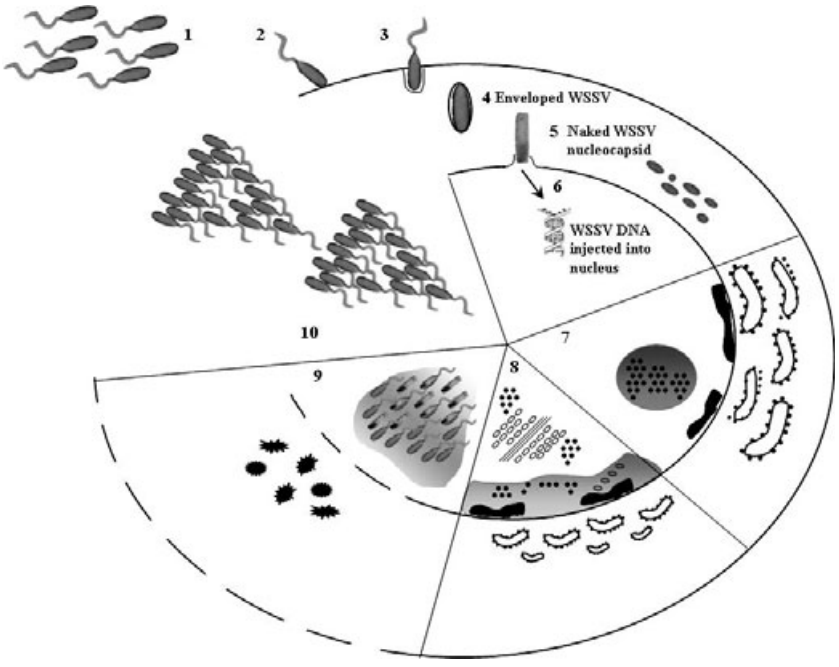

Figure 4 A proposed model of the morphogenesis of white spot syndrome virus (WSSV). (1) Infectious WSSV particles. (2) An infectious WSSV virion attaches to a susceptible cell using envelope proteins with a cell attachment motif. (3) WSSV enters the cell. (4) The envelope of the WSSV virion probably fuses with the endosome and the naked nucleocapsid is transported to the nucleus, in a similar way as in baculoviruses (see Lua & Reid 2000). (5) The naked WSSV nucleocapsid attaches to the nuclear membrane and the WSSV genome is released into the nucleus. (6) The WSSV genome replication starts. In the cytoplasm, the mitochondria start degenerating (Wang *et al.* 1999a). (7) In the nucleus the early virogenic stroma appears composed of loose granular material. Cellular chromatin accumulates near the nuclear membrane and the rough endoplasmic reticulum (RER) becomes enlarged and active. (8) The marginated chromatin is transformed in a dense ring zone (shaded area). The virogenic stroma is less dense and starts forming vesicles that will form the viral envelope. The vesicles are probably formed with membranous material found in the ring zone, as in baculoviruses (see Lua & Reid 2000). A viral nucleosome is also observed as a filamentous structure in the virogenic stroma. This structure contains proteins that will form the nucleocapsid. (9) New WSSV particles are assembled in the nucleus within an electron-dense inclusion. The empty envelopes are filled with a nucleocapsid. In cytoplasm, organelles become disintegrated and the cellular and nuclear membranes are disrupted (Wang *et al.* 1999a). (10) WSSV virions are completely formed and ready to be released from the disrupted cell to begin the cycle in other susceptible cells.

immunohistochemistry (IHC) (Anil, Shankar & Mohan 2002) or enzyme-linked immunosorbent assay (Zhang, Xu & Xu 2001).

Differences in virulence of six WSSV isolates were found in post-larvae of *L. vannamei* and juveniles of *F. duorarum* inoculated *per os*. Virulence was determined as the time required to induce 100% mortality in *L. vannamei*. The Texas isolate was the most virulent while the Washington isolate (from crayfish) was the least virulent. The shrimp *F. duorarum* is known to be more resistant to WSSV infection. In this species, cumulative mortality was 60% with the Texas isolate and 35% with the WSSV isolate from crayfish (Wang *et al.* 1999b). Another study showed that differences in virulence and competitive fitness may be dependent on the genome size. A putative ancestral WSSV isolate (WSSV-TH-96-II) with the largest genome size recorded (312 kbp), showed a lower virulence [median lethal time (LT_{50}) = 14 days] and competitive fitness compared with another WSSV isolate (WSSV-TH) with a smaller genome size (292 kbp) (LT_{50} = 3.5 days). This study indicated that WSSV isolates with a smaller genome size may represent an advantage for virus replication (Marks 2005).

Morphogenesis

The stages of WSSV morphogenesis have been characterized and are directly related to the development of cellular lesions (Durand *et al.* 1997; Wang, Hassan, Shariff, Zamri & Chen 1999a; Tsai, Wang, Leu, Wang, Zhuang, Walker, Kou & Lo 2006) (see Fig. 4).

Stage 1: the early stage of cell infection. Infected cells show slightly hypertrophied nuclei. A viral nucleosome appears before the formation of viral particles. It is composed of viral proteins organized in fibrillar fragments. In the cytoplasm, the endoplasmatic reticulum (ER) becomes enlarged with abundant free ribosomes.

Stage 2: in the nucleus, the fibrillar material induces the formation of circular membranes that are soon filled with viral core material starting viral assembly. At this stage, Cowdry-A type inclusions appear as a translucent zone between the virogenic stroma and the very electron-dense marginated chromatin. The nuclei become hypertrophied and rounded.

Stage 3: in the nucleus, the nucleocapsids appear with low electron density and gradually grow from one end towards the other, still open. The central intranuclear inclusion appears smaller than in cells in stage 2 and is more electron dense because of the presence of abundant viral particles. When the marginated chromatin disappears, the nuclear membrane is disrupted and the marginal transparent zone is fused with the lucent cytoplasm. Most organelles are abnormal, disintegrated or forming membranous structures.

Stage 4: in the nucleus, the nucleocapsid is completed with 12–14 rings of globular protein units arranged in a stacked series. Each nucleocapsid has one round and one square end. The nucleocapsid becomes completely enclosed by the envelope.

Stage 5: a late stage of viral morphogenesis. The viral particles become ovoid in shape and a long tail-like projection derived from the envelope is observed. The inner material of the tail is separated from the nucleocapsid. Afterwards, the nucleocapsids become shorter, thicker and more electron dense because of the packing of the viral DNA-VP15 complex.

Stage 6: the final phase of morphogenesis. The mature virions are elliptical with complete smooth envelopes enclosing an electron-dense nucleocapsid and with a tail-like projection at the last enclosed end. Sometimes assembly of nucleocapsids occurs completely separated from the envelopes and later they are wrapped by the envelopes. At this final stage, infected cells are severely damaged and disrupted. Void spaces are observed in tissues as cells disintegrate.

Clinical signs and pathology

Under culture conditions, many Asian and American penaeid species infected with WSSV display obvious white spots or patches of 0.5–3.0 mm in diameter embedded in the exoskeleton (Lo, Leu, Ho, Chen, Peng, Chen, Chou, Yeh, Huang, Chou, Wang & Kou 1996a; Kasornchandra *et al.* 1998; Wu, Namikoshi, Nishizawa, Mushiake, Teruya & Muroga 2001; T.W. Flegel, pers. comm.) (Fig. 5). The exact mechanism of white spot formation is not known. It is possible that a WSSV infection may induce the dysfunction of the integument resulting in the accumulation of calcium salts within the cuticle and giving rise to white spots (Wang *et al.* 1999a). Other signs of disease include a reddish discolouration of body and appendages because of the expansion of chromatophores (Lightner, Hasson, White & Redman 1998; Nadala *et al.*1998), a

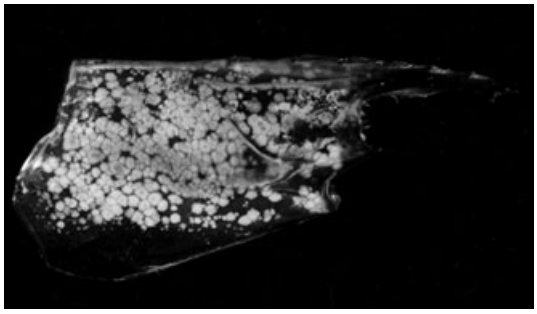

Figure 5 Presence of white spots in the carapace of farmed *Penaeus monodon* infected with white spot syndrome virus.

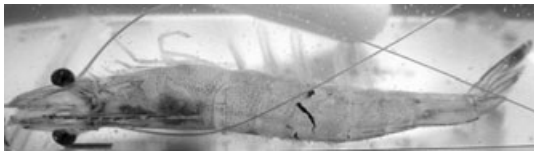

Figure 6 Experimentally white spot syndrome virus-infected moribund *Litopenaeus vannamei* with empty gut.

reduction in feed uptake (Chou *et al.* 1995; Durand *et al.* 1996; Flegel 1997), preening and response to stimulus (Wongteerasupaya *et al.* 1995; Durand *et al.* 1997) (Fig. 6), loose cuticle (Lo, Ho, Peng, Chen, Hsu, Chiu, Chang, Liu, Su, Wang & Kou 1996b), swelling of branchiostegites because of accumulation of fluid (Otta, Shubha, Joseph, Chakraborty, Karunasagar & Karunasagar 1999), enlargement and yellowish discolouration of the hepatopancreas (Sahul-Hameed *et al.* 1998), and thinning and delayed clotting of haemolymph (Wang *et al.* 2000; Kiatpathomchai, Boonsaeng, Tassanakajon, Wongteerasupaya, Jitrapakdee & Panyim 2001).

In the field, WSSV-infected shrimp gather near the pond edge and display clinical signs 1 or 2 days before the first mortalities occur (Kou, Peng, Chiu & Lo 1998). Cumulative mortality may reach 100% within 10 days after the onset of disease (Karunasagar *et al.* 1997; Lotz & Soto 2002). In grow-out ponds, juvenile shrimp of all ages and sizes are susceptible to the disease but massive mortality usually occurs 1 or 2 months after stocking (Kasornchandra *et al.* 1998).

By histopathology, WSSV infection is characterized by cells with hypertrophied nuclei showing amphophilic intranuclear inclusions and marginated chromatin (Durand *et al.* 1997; Wang *et al.* 2000) (Fig. 7). These intranuclear inclusions are markedly distinct and bigger than the Cowdry A-type inclusions characteristic of the infectious hypodermal and haematopoietic necrosis virus (Wongteerasupaya *et al.* 1995). Infected nuclei become progressively more basophilic and enlarged (Chang *et al.* 1996; Durand *et al.* 1996, 1997; Lo *et al.* 1996b; Flegel 1997; Wang, Lo, Chang & Kou 1998a; Otta *et al.* 1999; Takahashi, Kondo, Itami, Honda, Inagawa, Nishizawa, Soma & Yokomiso 2000). In the late stages of infection, karyorrhexis and cellular disintegration may occur, leading to the formation of necrotic areas characterized by vacuolization (Karunasagar *et al.* 1997; Kasornchandra *et al.* 1998; Wang *et al.* 1999a).

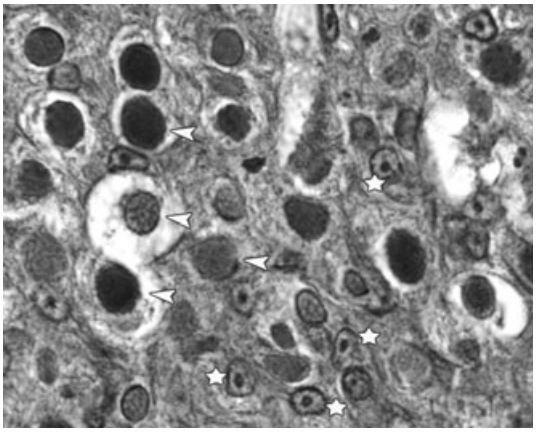

Figure 7 Histopathological lesions of white spot syndrome virus (WSSV) infection in cells of stomach. Stars indicate cells at early stages of WSSV replication containing Cowdry-A type-like intranuclear inclusions. Arrowheads indicate cells at late stages of WSSV replication showing hypertrophied nuclei with amphophilic intranuclear inclusions (×1000).

Pathogenesis

Experimental methods of WSSV inoculation that simulate natural routes of virus entry have been developed. These inoculation methods are: (i) waterborne, by immersing animals in water containing WSSV cell-free suspensions (Chou, Huang, Lo & Kou 1998; Supamattaya, Hoffman, Boonyaratpalin & Kanchanaphum 1998) and (ii) feeding WSSV-infected tissues to the animals for a single time or once daily for up to 7 days (Chang, Chen & Wang 1998; Lightner *et al.* 1998; Wang, Tsai & Chen 1998b; Rajan, Ramasamy, Purushothaman & Brennan 2000). The latter route is considered the most important in natural and culture conditions

Table 3 White spot syndrome virus host range

Animal	Species	Type of infection		Detection method	Country (references)
		Natural	Experimental		
Penaeid shrimp	*Farfantepenaeus aztecus*		X	Histopathology	USA[10]
	F. duorarum		X	Histopathology	USA[10]
	Fenneropenaeus chinensis	X	X	Histopathology, ISH, PCR	China, Korea, Thailand[13, 25, 26]
	F. indicus	X	X	Histopathology, PCR, TEM	India, Indonesia, Thailand[14, 15]
	F. merguiensis	X	X	Histopathology, PCR, IIF	Malaysia, Thailand[3, 4, 18, 19]
	Litopenaeus setiferus		X	Histopathology	USA[10]
	L. stylirostris	X	X	Histopathology	USA, Latin America[10, 13]
	L. vannamei	X	X	Histopathology, ISH, TEM	USA, Latin America[10, 13]
	Marsupenaeus japonicus	X	X	Histopathology, PCR, TEM	China, Japan, India[11, 13, 23, 24, 26]
	Metapenaeus dobsonii	X	X	Histopathology, PCR, TEM	India[4, 16]
	M. ensis	X	X	ISH, PCR	Taiwan[1, 11, 23, 24]
	M. monoceros		X	PCR	India[16]
	Penaeus monodon	X	X	Histopathology, ISH, PCR	At least eight Asian countries[1, 11, 14, 15, 23, 24, 26]
	P.penicillatus	X		ISH, PCR	Taiwan[11, 23]
	P. semisulcatus	X	X	ISH, PCR	India, Taiwan[11, 15, 23]
	Parapenaeopsis stylifera	X		PCR	India[4]
	Solenocera indica	X		PCR	India[4]
	Trachypenaeus curvirostris	X	X	ISH, PCR	Taiwan[23, 24]
Caridean shrimp	*Alpheus* sp.		X	PCR	Thailand[11]
	Callianassa sp.		X	PCR	Thailand[11]
	Exopalaemon orientalis		X	ISH, PCR	Taiwan[23, 24]
	Palaemon sp.	X		ISH, PCR	Taiwan[11]
	P. adspersus		X	TEM, ISH, PCR, dot-blot	France[2]
	Macrobrachium idella		X	Histopathology, WB	India [5]
	M. lamerrae		X	Histopathology, WB	India[17]
	M. rosenbergii	X	X	Histopathology, ISH, PCR	India, Taiwan[4, 11, 15, 23, 24]
Lobster	*Panulirus homarus*		X	Histopathology	India[15]
	P. longipes	X	X	ISH, PCR	Taiwan[24]
	P. ornatus	X	X	Histopathology, ISH, PCR	India, Taiwan[15, 23]
	P. penicillatus		X	ISH, PCR	India, Taiwan[1, 23]
	P. polyphagus	X	X	Histopathology	India[15]
	P. versicolor	X	X	ISH, PCR	Taiwan[1, 23]
	Scyllarus arctus		X	TEM, ISH, PCR, Dot-blot	France[2]
Crayfish	*Astacus astacus*		X	PCR	Sweden[7]
	A. leptodactylus		X	TEM, ISH, PCR, Dot-blot	France[2]
	Cherax destructor		X	Histopathology, Dot-blot	Australia[3]
	C. quadricarinatus		X	Histopathology, ISH, TEM	China[20]
	Pacifastacus leniusculus		X	Histopathology, ISH	Sweden[6]
	Procambarus clarkii		X	ISH, PCR	China, Taiwan[1, 5, 23]
	Orconectes limosus		X	TEM, ISH, PCR, Dot-blot	France[2]
Crab	*Atergatis integerrimus*		X	PCR	India[19]
	Calappa philarigus	X	X	Histopathology, ISH, PCR	India, Taiwan[9, 19]
	Callinectes lophos		X	ISH, PCR	Taiwan[23]
	Cancer pagurus		X	TEM, ISH, PCR, Dot-blot	France[2]
	Carcinus maenas		X	TEM, ISH, PCR, Dot-blot	France[2]
	Charybdis annulata	X	X	Histopathology, PCR	India[4, 19]
	C. cruciata		X	PCR	India[4]
	C.feriatus	X	X	Histopathology, ISH, PCR	India, Taiwan[9, 11, 23]
	C. granulata		X	ISH	Taiwan[1, 23]
	C. lucifera	X	X	Histopathology, PCR	India[12, 19]
	C. natatus	X	X	Histopathology, ISH PCR	India, Taiwan, Thailand[9, 19]
	Demania splendida		X	PCR	India[19]
	Doclea hybrida		X	Histopathology, PCR	India[19]
	Gelasimus marionis nitidus	X		PCR	India[4]
	Grapsus albolineatus		X	Histopathology, PCR	India[19]
	Halimede ochtodes		X	Histopathology, PCR	India[19]
	Helice tridens	X		PCR	Taiwan, Thailand[9, 11]
	Liagore rubronaculata		X	Histopathology, PCR	India[19]
	Liocarcinus depurator		X	TEM, ISH, PCR, Dot-blot	France,India[8, 15]
	L. puber		X	TEM, ISH, PCR, Dot-blot	France, India[8, 15]
	Lithodes maja		X	Histopathology, PCR	India[19]

Table 3 (Continued)

Animal	Species	Type of infection		Detection method	Country (references)
		Natural	Experimental		
Crab	*Macrophthalmus sulcatus*	X		PCR	India[4]
	Matuta miersi		X	Histopathology, PCR	India[19]
	M. planipes	X		PCR	India[12]
	Menippe rumphii		X	PCR	India[19]
	Metapograpsus sp.		X	Histopathology	India, Taiwan[15]
	Metapograpsus messor	X		PCR	India[4]
	Paradorippe granulata		X	Histopathology, PCR	India[19]
	Paratelphusa hydrodomous		X	Histopathology, PCR,	India[18]
	P. pulvinata		X	Histopathology, PCR,	India[18]
	Parthenope prensor		X	Histopathology, PCR	India[19]
	Phylira syndactyla		X	Histopathology, PCR	India[19]
	Podophthalmus vigil		X	Histopathology, PCR	India[19]
	Portunus pelagicus	X	X	Histopathology, ISH, TEM	Taiwan, Thailand[9, 11, 21]
	P. sanguinolentus	X	X	Histopathology, ISH, PCR	India, Taiwan[1, 9, 11, 19, 24]
	Sesarma sp.		X	Histopathology, ISH, PCR	India, Thailand[8, 15]
	S. oceanica	X		PCR	India[12]
	Scylla serrata	X	X	Histopathology, ISH, PCR	India, Taiwan, Thailand[8, 9, 11, 15, 19, 21]
	S. tranquebaricca		X	Histopathology	India[15]
	Thalamite danae		X	Histopathology, PCR	India[19]
	Uca pugilator		X	Histopathology, ISH	Thailand[8]
Other	Sergestoidea, *Acetes* sp.	X	X	Histopathology, ISH, PCR	Thailand[21]
	Cirripedia *Balanus* sp.	X	X	PCR	Mexico, Thailand[11, 16]
	Branchiopoda Cladocera	X		PCR	Mexico[16]
	Branchiopoda *Artemia* sp.	X		PCR	India[12]
	Stomatopoda, *Squilla mantis*	X		PCR	India[4]
	Copepoda	X		PCR	Mexico, Thailand[11, 16]
	Chaetognata	X		PCR	Mexico[16]
	Rotifera	X		PCR	China[25]
	Polychaeta, *Marphysa* sp.	X		PCR	India[22]
	Coleoptera Ephydridae	X		PCR	Taiwan[11]

References: [1]Chang *et al.* 1998; [2]Corbel *et al.* 2001; [3]Edgerton 2004; [4]Hossain *et al.* 2001; [5]Huang *et al.* 2001; [6]Jiravanichpaisal *et al.* 2001, 2004; [8]Kanchanaphum *et al.* 1998; [9]Kou *et al.* 1998; [10]Lightner *et al.* 1998; [11]Lo *et al.* 1996b; [12]Lo *et al.* 1999; [13]Lu *et al.* 1997; [14]Rajan *et al.* 2000; [15]Rajendran *et al.* 1999; [16]Ramírez-Douriet *et al.* 2005; [17]Sahul-Hameed *et al.* 2000; [18]Sahul-Hameed *et al.* 2001; [19]Sahul-Hameed *et al.* 2003; [20]Shi *et al.* 2000; [21]Supamattaya *et al.* 1998; [22]Vijayan *et al.* 2005; [23]Wang *et al.* 1998a; [24]Wang *et al.* 1998b; [25]Yan *et al.* 2004; [26]Zhan *et al.* 1998;.
PCR, polymerase chain reaction; ISH, *in situ* hybridization; TEM, transmission electron microscopy; IIF, indirect immunofluorescence.

(Chou *et al.* 1998; Wu *et al.* 2001; Lotz & Soto 2002; Pramod-Kiran, Rajendran, Jung & Oh 2002).

The portals of WSSV entry into the shrimp have not yet been clearly identified. According to experimental data on feeding shrimp with WSSV-infected tissues, the primary sites of WSSV replication in early juvenile *P. monodon* are the subcuticular epithelial cells of the stomach and cells in the gills, in the integument and in connective tissue of the hepatopancreas as determined by *in situ* hybridization (ISH) (Chang *et al.* 1996). Another study on *M. japonicus* indicated that epithelial cells in the midgut trunk may be a transient site of WSSV replication which would allow the virus to cross the underlying basal lamina (Di Leonardo, Bonnichon, Roch, Parrinello & Bonami 2005). In *P. monodon*, a WSSV challenge by immersion showed that haemocytes migrating to gills and midgut were WSSV-negative at late stages of infection [48–72 h post-inoculation (hpi)]. Many WSSV-positive cells were found in gills and only a few in midgut epithelium. Electron microscopy showed that epithelial cells in the midgut were VP28-positive in supranuclear vacuoles early during infection (8 hpi), suggesting lysis of WSSV particles. VP28-positive nuclei were never seen in the epithelial cells of the midgut (Arts, Taverne-Thiele, Savelkoul & Rombout 2007).

Recently, the infectivity titres of a WSSV stock solution was determined by oral route challenge (Escobedo-Bonilla, Wille, Alday-Sanz, Sorgeloos, Pensaert & Nauwynck 2005) and a standardized oral inoculation procedure that delivered an exact amount of virus titre to all inoculated shrimp was developed (Escobedo-Bonilla, Wille, Alday-Sanz, Sorgeloos, Pensaert & Nauwynck 2006). With this standardized inoculation technique, the primary

sites of WSSV replication as determined with IHC were the epithelial cells in the foregut, cells in the gills, and only with a high dose (10 000 SID_{50}), also cells in the antennal gland (Escobedo-Bonilla, Wille, Alday-Sanz, Sorgeloos, Pensaert & Nauwynck 2007).

The mechanism of viral spread from the primary replication sites to other target organs has been controversial. Some studies have indicated that WSSV infects haemocytes in crayfish and travels throughout the body in these cells to reach distant target organs (Wang, Liu, Seah, Lam, Xiang, Korzh & Kwang 2002; Di Leonardo *et al.* 2005). Other studies have shown by ISH, IHC and IIF that circulating haemocytes in freshwater prawns and shrimp are refractory to WSSV infection (van De Braak, Botterblom, Huisman, Rombout & van der Knaap 2002; Shi, Wang, Zhang, Xie, Li, Chen, Edgerton & Bonami 2005; Escobedo-Bonilla *et al.* 2007), thus indicating that WSSV might reach other target organs through haemolymph circulation in a cell-free form (Escobedo-Bonilla *et al.* 2007). It is possible that these mechanisms of spread may be host species-dependent.

White spot syndrome virus targets cells of organs of ectodermal and mesodermal origin, including those of the epidermis, gills, foregut, hindgut (Wongteerasupaya *et al.* 1995; Chang *et al.* 1996), antennal gland, lymphoid organ (Durand *et al.* 1996; Chang *et al.* 1998), muscle, eye-stalk, heart (Kou *et al.* 1998), gonads (Lo, Ho, Chen, Liu, Chiu, Yeh, Peng, Hsu, Liu, Chang, Su, Wang & Kou 1997), haematopoietic cells and cells associated with the nervous system (Rajendran *et al.* 1999; Wang *et al.* 1999b). Epithelial cells of organs of endodermal origin such as the hepatopancreas, anterior and posterior midgut caeca and midgut trunk are refractory to WSSV infection (Sahul-Hameed *et al.* 1998). In the late stages of infection, the epithelia of the stomach, gills and integument may become severely damaged (Chang *et al.* 1996; Wang *et al.* 1999a). This may cause multiple organ dysfunctions and probably leads to death.

A number of techniques such as two-dimensional gel electrophoresis, expressed sequence tags (Wang, Wang, Leu, Kou, Wang & Lo 2007), microarray chips (Wang, Li, Dong, Zhang, Zhang & Xiang 2006), suppression subtractive hybridization (Zhao, Yin, Weng, Guan, Li, Xing, Chan & He 2007) and differential hybridization (He, Qin & Xu 2005) are useful tools to better understand the host response to mechanisms of WSSV virulence and pathogenesis. These methods measure the altered abundance of host and/or viral mRNA and/or protein expression levels after WSSV infection. Molecules with important biological functions that showed variations in response to WSSV infection included those involved in energy production, nucleic acid synthesis, calcium homeostasis and/or cellular signalling. Many such molecules may be useful as biomarkers and probably could be used to identify targets to control virus replication (Wang *et al.* 2007).

Host range

White spot syndrome virus has a broad host range within decapod crustaceans. At least 18 cultured and/or wild penaeid shrimp (Wongteerasupaya, Wongwisansri, Boonsaeng, Panyim, Pratanpipat, Nash, Withyachumnarnkul & Flegel 1996; Durand *et al.* 1997; Lu, Tapay, Loh, Gose & Brock 1997; Chou *et al.* 1998; Lightner *et al.* 1998; Park *et al.* 1998), eight caridean species (Sahul-Hameed, Charles & Anilkumar 2000; Shi, Huang, Zhang, Chen & Bonami 2000; Pramod-Kiran *et al.* 2002), seven species of lobster (Chang *et al.* 1998; Rajendran *et al.* 1999), seven species of crayfish (Wang *et al.* 1998a; Corbel, Zuprisal, Shi, Huang, Sumartono, Arcier & Bonami 2001; Jiravanichpaisal, Bangyeekhun, Söderhäll & Söderhäll 2001; Edgerton 2004; Jiravanichpaisal, Soderhall & Soderhall 2004), 38 crab species (Lo *et al.* 1996a; Kanchanaphum, Wongteerasupaya, Sitidilokratana, Boonsaeng, Panyim, Tassanakajon, Withyachumnarnkul & Flegel 1998; Kou *et al.* 1998; Sahul-Hameed, Yoganandhan, Sathish, Rasheed, Murugan & Jayaraman 2001; Sahul-Hameed, Balasubramanian, Syed Musthaq & Yoganandhan 2003), six non-decapod crustacean species (Supamattaya *et al.* 1998; Otta *et al.* 1999; Hossain, Chakraborty, Joseph, Otta, Karunasagar & Karunasagar 2001), members of the phyla Chaetognata and Rotifera (Yan, Dong, Huang, Yu & Feng 2004; Ramírez-Douriet, De Silva-Dávila, Méndez-Lozana, Escobedo-Urias, Leyva-Arana & López-Meyer 2005; Yan, Dong, Huang & Zhang 2007), polychaete worms (Supak, Boonnat, Poltana, Kanchanaphum, Gangnonngiw, Nash & Withyachumnarnkul 2005; Vijayan, Stalin Raj, Balasubramanian, Alavandi, Thillai Sekhar & Santiago 2005) and some aquatic insect larvae (Lo *et al.* 1996b; Flegel 1997; Ramírez-Douriet *et al.* 2005) have been found to be WSSV-positive by PCR (Table 3). Although many of these species have

been confirmed to support WSSV replication under experimental conditions, some other species collected from the wild have only been found WSSV-positive by PCR. This indicates that many such species are not necessarily WSSV natural hosts, but may only be mechanical carriers. This seems to be the case at least for polychaete worms (T.W. Flegel, pers. comm.).

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