

Introduction

Cypoviruses (CPVs) belong to the genus *Cypovirus* in the family *Reoviridae* (Payne and Mertens, 1983; Regenmortel *et al.*, 2000). Wide range of insects belonging to the order Diptera, Hymenoptera and Lepidoptera are infected by *cypoviruses* (Belloncik & Mori, 1998). Viral infection is characterized by the production of large numbers of occlusion bodies called polyhedra in the cytoplasm of infected cells. The genomes of CPVs are usually composed of 10 double stranded dsRNA segments (S1-S10) (Payne and Mertens, 1983) although, in some cases such as AmCPV-4 (Qanungo *et al.*, 2000), BmCPV-1 (Arella *et al.*, 1988) and TnCPV-15 (Rao *et al.*, 2000) presence of an extra small dsRNA as 11th segment is observed.

Each dsRNA is composed of a plus-stranded mRNA and its complementary minus strand in an end to end base paired configuration except for a protruding 5' cap on the plus strand. The exact mechanism of viral entry into the host cell is still unknown. Recent reports suggested that virion particles enter columnar cells by binding through the receptor present in the cell membrane (Tan *et al.*, 2003). mRNA is synthesized by endogenous RNA transcription, capped, and released from the intact capsid. Upon protein synthesis, the viral capsid is assembled in virogenic stroma (Tan *et al.*, 2003).

On the basis of electrophoretic migration patterns of the dsRNA segments in agarose or acrylamide gels, they have been classified into 16 different types (Mertens *et al.*, 2005). Among the family *Reoviridae*, complete sequences of dsRNA genomes have been reported for members of the genera *Orthoreovirus*, *Rotavirus*, *Orbivirus*, *Phytoreovirus* and other putative members of *Fijivirus* and *Cypovirus* (Duncan, 1999; Estes and Cohen, 1989; Roy and Gorman, 1990; Suzuki, 1995; Nakasimha *et al.*, 1996). From the *Cypovirus*, complete nucleotide sequence of type 14 *Lymnatria dispar* CPV (LdCPV-14) (Rao *et al.*, 2001), type 1 *Dendrolimus punctatus* CPV (DpCPV-1) (Zhao *et al.*, 2003 a, b), type 15 *Trichoplasia ni* CPV (TnCPV-15) (Rao *et al.*, 2000), type 1 *Bombyx mori* CPV (BmCPV-1) (Hagiwara *et al.*, 1998 a, b; 2000; 2001; 2002; Ikeda *et al.*, 1998) and *Choristoneura occidentalis* (CoCPV) (Graham *et al.*, 2008) have been reported and deposited in the Genbank. The elucidation of these cypovirus sequences has led to a better understanding of the possible functions that each segmented dsRNA may play in viral replication or pathogenesis. In case of BmCPV, segment 1, 3, 4, 6, 7, 10 encodes structural proteins VP1, VP2, VP3, VP4, VP5, polyhedrin and segment 2, 5, 8, 9 encodes viral non-structural proteins RNA dependent RNA polymerase, p101, p44, NS5, respectively (Hagiwara *et al.*, 1998 a, b; 2000; 2001; 2002; Ikeda *et al.*, 1998). Segments 1, 3, 4, 6, 7 and 10 of DpCPV encode viral structural proteins while segments 2, 5, 8, 9 and 10 encode non-structural proteins (Zhao *et al.*, 2003 a, b). Besides these, polyhedrin genes of several other cypoviruses such as *Euxoa scandens* CPV (EsCPV)

(Fossiez *et al.*, 1989), *Orgyia pseudotsugata* CPV (OpCPV), *Heliothis armigera* CPV (HaCPV) (Galinski *et al.*, 1994), *Choristoneura fumiferana* CPV (CfCPV) (Echeverry *et al.*, 1997) and *Uranotaenia sapphirina* CPV (UsCPV) (Shapiro *et al.*, 2005) have been molecularly characterized but no sequence homology has been found among them.

Antheraea mylitta, the Indian non-mulberry saturniidae silkworm, produces an exotic variety of silk called tasar silk. Due to wild in nature of *Antheraea mylitta*, viral infection destroys a major population of these silkworms (Jolly *et al.*, 1974). Qanungo *et al.*, in 2000 reported that a Type IV CPV, called AmCPV containing 11 dsRNA segments is the causative agent of the viral infection. Molecular cloning and characterization of AmCPV genome segment 2, 6, 7, 8, 9 and 10 of this CPV shows that segment 2 (S2) codes for viral RDRP (Ghorai *et al.*, 2010), segment 6 (S6) codes for viral structural proteins having ATPase activity (Chavali *et al.*, 2008), segment 7 (S7) codes for a structural proteins (Chavali *et al.*, 2007;), segment 9 (S9) codes for viral non-structural protein NSP38 having RNA binding property (Qanungo *et al.*, 2002) and segment 10 (S10) codes for polyhedrin (Sinha-Datta *et al.*, 2005), respectively. No other genome segments of this CPV have been characterized although these viruses destroy a major population of these economically important insects (silkworms) each year. To understand the role of each genome segment of AmCPV in virus replication and pathogenesis complete characterization of all its genome segments is necessary.

In the present work an attempt has been made to molecularly characterize the genome segments 1 (S1), 3 (S3), 8 (S8) and 11 (S11) of AmCPV by cloning, sequencing and expressing of these genes in *E. coli* and insect cell. It has been shown by immunoblot analysis that S1, S3 and S8 encode 141 kDa, 137 kDa and 60 kDa viral structural proteins, respectively. S11 does not contain any ORF and its function remains to be determined. By electron microscopic analysis and electrophoretic mobility shift assay it has also been shown that S3 and S1 encoded proteins helps in the formation of capsid and maintenance of its stability, respectively, whereas S8 encoded protein binds to viral RNA for its replication and/or packaging.