Cystoviridae

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Summary

The family Cystoviridae includes enveloped viruses with a tri-segmented dsRNA genome enclosed in one or two concentric, icosahedrally-symmetric protein shells. The innermost protein layer is a polymerase complex responsible for genome packaging, replication and transcription. Cystoviruses infect Gram-negative bacteria, primarily plant-pathogenic Pseudomonas syringae strains.

Table 1. Cystoviridae. Characteristics of members of the family Cystoviridae.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical member</td>
<td>Pseudomonas phage phi6 (Segment S, M12921; Segment M, M17462; Segment L, M17461), species Pseudomonas virus phi6, genus Cystovirus</td>
</tr>
<tr>
<td>Virion</td>
<td>Enveloped virions (~85 nm) with typically two concentric, icosahedrally symmetric protein layers: the nucleocapsid surface shell (T=13) and the polymerase complex core (T=1). Spikes protrude from the virion surface</td>
</tr>
<tr>
<td>Genome</td>
<td>Three segments of linear, double-stranded RNA, totaling 12.7 – 15.0 kbp. All segments encode several viral proteins.</td>
</tr>
<tr>
<td>Replication</td>
<td>Single-stranded genomic precursor molecules are packaged into the viral polymerase complex. The packaged RNA molecules are replicated and transcribed within the particle</td>
</tr>
<tr>
<td>Translation</td>
<td>Viral proteins are translated from polycistronic messenger RNAs</td>
</tr>
<tr>
<td>Host range</td>
<td>Gram-negative bacteria, mostly Pseudomonas species</td>
</tr>
<tr>
<td>Taxonomy</td>
<td>One genus (Cystovirus) and seven species</td>
</tr>
</tbody>
</table>

Currently, there is only one genus (Cystovirus) in the family. Therefore the description of the genus Cystovirus is the same as the family description.

Virion

Morphology

The enveloped virions are spherical, about 85 nm in diameter and covered by spikes (Figure 1. Cystoviridae). The envelope surrounds an isometric nucleocapsid, about 58 nm in diameter. However, Pseudomonas phage phi8 is lacking the nucleocapsid shell and has a single protein shell surrounding the genome. The nucleocapsid surface shell (if present) follows T=13 icosahedral symmetry. Turret-like extrusions of the underlying polymerase complex span the nucleocapsid surface shell layer at the five-fold symmetry positions (Figure 1. Cystoviridae). The dodecahedral polymerase complex is about 50 nm in diameter. In the polymerase complex major capsid protein dimers are arranged on a T=1 icosahedral lattice (Butcher et al., 1997, Huiskonen et al., 2006, Sun et al., 2017).
Physicochemical and physical properties

The molecular mass of Pseudomonas phage phi6 virion is $9.9 \times 10^6$ and the nucleocapsid is $4.0 \times 10^6$. Virion $S_{20,w}$ is about 405S. The buoyant density of the virion is 1.27 g cm$^{-3}$ in CsCl, 1.22 g cm$^{-3}$ in Cs$_2$SO$_4$ and 1.24 g cm$^{-3}$ in sucrose. Virions are sensitive to detergents, ether and chloroform but stable between pH 6.0 and 9.5.

Nucleic acid

Virions contain three segments of linear, double-stranded RNA: Segment L (6.4–7.1 kbp), Segment M (3.6–4.7 kbp) and Segment S (2.3–3.2 kbp). The complete genome is 12.7–15.0 kbp and has a GC content of 53.4–58.8%. Virion particles contain a single copy of each of the three genome segments. The genome constitutes approximately 10% of the virion weight. The genome is organized into five concentric layers arranged into nearly coaxial spools (Ilca et al., 2019).

Proteins

Proteins constitute about 70% of the virion weight. The viral genome (Figure 2. Cystoviridae) encodes structural (Figure 1. Cystoviridae) and non-structural proteins. The envelope contains several integral membrane proteins, including the fusogenic protein P6 and major envelope protein P9. Receptor binding spike (P3) is anchored to the envelope via P6 (Sinclair et al., 1975, Gottlieb et al., 1988a) (Bamford et al., 1987). In some cystoviruses the spike is composed of several different polypeptides. Protein P8 forms the nucleocapsid surface shell (Figure 1. Cystoviridae) (Sun et al., 2017) or is associated with the membrane (phi8, (Hoogstraten et al., 2000, Jäälinoja et al., 2007)). Protein P5 is a lytic enzyme (Mindich and Lehman 1979, Dessau et al., 2012). Protein P2 is the viral replicase and transcriptase and is located in the interior of the P1 shell (Ilca et al., 2015, Makeyev and Bamford 2000, Butcher et al., 2001). The turret-like extrusions of the polymerase complex are made by hexamers of P4 protein (Sun et al., 2017). P4 is a nucleoside triphosphatase required for genome packaging and transcription (Pirttimaa et al., 2002, Sun et al., 2013). Minor capsid protein P7 is an assembly factor (Poranen et al., 2001). Non-structural protein P12 is a morphogenetic protein that is probably involved in envelope assembly (Johnson and Mindich 1994). Some cystoviruses also encode other non-structural proteins.

Lipids

Virions are composed of 20% lipids by weight. There is enough lipid to cover about one-half of the envelope surface area (the rest being protein). Viral lipids are derived from the host plasma membrane (Laurinavičius et al., 2007). The lipid compositions of the viral envelope and the host plasma membrane are similar.

Genome organization and replication

Distinct noncoding regions at the termini of the three genome segments contain signals for genome packaging and replication (Gottlieb et al., 1988a).
Virions adsorb either to the rough lipopolysaccharide layer or to pili of the host bacterium (Bamford et al., 1976, Roine et al., 1998, Mindich et al., 1999) (Figure 2. Cystoviridae). The viral envelope fuses with the host outer membrane (Bamford et al., 1987) and the nucleocapsid-associated lytic enzyme locally digests the peptidoglycan layer (Mindich and Lehman 1979). Delivery of the viral polymerase complex into the host cytoplasm involves an endocytic-like process at the host plasma membrane (Poranen et al., 1999). The viral genome is transcribed by the virion-associated RNA-dependent RNA polymerase within the polymerase complex. Early in the infection approximately equal amounts of messenger RNA molecules are produced from the L, M and S segments (Coplin et al., 1975, Emori et al., 1983). Later in the infection cycle transcripts of the M and S segments typically predominate. Transcription is semi-conservative and produces full-length copies of the genome segments (Usala et al., 1980, Van Etten et al., 1980).

Virus mRNAs are polycistronic. Translation of L segment transcripts produces the early proteins, which assemble to form empty polymerase complexes (Figure 3. Cystoviridae) (Gottlieb et al., 1988b). These package the three positive-strand transcripts (Frilander and Bamford 1995). Negative-strand synthesis then takes place inside the polymerase complex. RNA packaging and replication induce structural changes in the polymerase complex (expansion) (Nemecek et al., 2011). Transcription by these polymerase complexes produces messages for late protein synthesis. The nucleocapsid surface shell assembles on the polymerase complex (Figure 3. Cystoviridae) (Poranen et al., 2001, Olkkonen et al., 1991). Nucleocapsid acquires protein P5 and the envelope. Spikes are assembled on the envelope surface. Mature virions are released upon virus-induced host cell lysis (Bamford et al., 1976).

**Figure 3. Cystoviridae.** Schematic presentation of the Pseudomonas phage phi6 life cycle. (A) Adsorption. (B) Envelope fusion. (C) Peptidoglycan digestion. (D–E) Endocytic uptake of the nucleocapsid. (F) Early transcription. (G) Polymerase complex assembly. (H) ssRNA packaging and replication. (I) Late transcription. (J) Nucleocapsid shell assembly. (K) Translocation of the viral envelope and assembly of spikes. (L) Host cell lysis and release of mature virions.

**Biology**

Cystoviruses are lytic bacteriophages that induce host cell lysis at the end of the viral reproduction cycle. Natural hosts are Gram-negative plant or human pathogenic bacteria.

**Derivation of names**

*Cysto:* from Greek *kystis,* “bladder”, “sack”.

**Relationships within the family**

Phylogenetic relationships among members of the family *Cystoviridae* are shown in Figure 4. *Cystoviridae.*
Figure 4. Cystoviridae. Phylogenetic analysis of cystoviruses based on nucleotide sequence comparisons of the genome segments L (A), M (B) and S (C). Phylogenetic trees were constructed using Mega 7.0 software (Kumar et al., 2016) with maximum likelihood method. Bootstrap values greater than 50% (1000 replicates) are shown at branch points. This phylogenetic tree and corresponding sequence alignment are available to download from the Resources page.

Relationships with other taxa

In terms of genome replication strategy, cystoviruses resemble eukaryotic double-stranded RNA viruses (Mertens 2004, Poranen and Bamford 2012). The structure, organization and functions of the polymerase complex are the major similarities among members of families Cystoviridae, Chrysoviridae, Reoviridae, Totiviridae, Partitiviridae, Megabirnaviridae, Quadriviridae and Picobirnaviridae. The T=13 architecture of the surrounding capsid layer is also shared by cystoviruses and reoviruses.

Related, unclassified viruses

<table>
<thead>
<tr>
<th>Virus name</th>
<th>Accession number</th>
<th>Virus abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas phage phi7*</td>
<td>S, AF125682; M, AF125681; L, AF125680</td>
<td>phi7</td>
</tr>
<tr>
<td>Pseudomonas phage phi9*</td>
<td>S, AF125679; M, AF125678; L, AF125677</td>
<td>phi9</td>
</tr>
<tr>
<td>Pseudomonas phage phi10*</td>
<td>S, AF125676; M, AF125675; L, AF125674</td>
<td>phi10</td>
</tr>
<tr>
<td>Pseudomonas phage phi11*</td>
<td></td>
<td>phi11</td>
</tr>
<tr>
<td>Pseudomonas phage phi14*</td>
<td></td>
<td>phi14</td>
</tr>
</tbody>
</table>

Virus names and virus abbreviations are not official ICTV designations.

*Either none, or only partial sequences are available for these isolates (Mindich et al., 1999).
Genus: \textit{Cystovirus}

Since only one genus (\textit{Cystovirus}) is currently recognized in the family \textit{Cystoviridae}, the family description above corresponds to the genus description. For clarity, the additional information that can be found on the genus page is also presented below.

Species demarcation criteria

Species are demarcated based upon nucleotide sequence distances for the L, M and S segments being greater than 0.05 for comparisons between members of different species; nucleotide sequence distances are $> 0.35$ for comparisons between members of all species except for \textit{Pseudomonas virus phi6} and \textit{Pseudomonas virus phiNN}, for which distances are $> 0.15$ (Figure 4. \textit{Cystoviridae}).

Member species

<table>
<thead>
<tr>
<th>Species</th>
<th>Virus name</th>
<th>Isolate</th>
<th>Accession number</th>
<th>RefSeq number</th>
<th>Available sequence</th>
<th>Virus Abbrev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>★ Pseudomonas virus phi6</td>
<td>Pseudomonas phage phi6</td>
<td>L: M17461; M: M17462; S: M12921</td>
<td>L: NC_003715; M: NC_003716; S: NC_003714</td>
<td>Complete genome</td>
<td>phi6</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phi8</td>
<td>Pseudomonas phage phi8</td>
<td>L: AF226851; M: AF226852; S: AF226853</td>
<td>L: NC_003299; M: NC_003300; S: NC_003301</td>
<td>Complete genome</td>
<td>phi8</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phi12</td>
<td>Pseudomonas phage phi12</td>
<td>L: AF034425</td>
<td>L: NC_004173; M: NC_004175; S: NC_004174</td>
<td>Complete genome</td>
<td>phi12</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phi13</td>
<td>Pseudomonas phage phi13</td>
<td>L: AP261666; M: AP261667; S: AP261668</td>
<td>L: NC_004172; M: NC_004171; S: NC_004170</td>
<td>Complete genome</td>
<td>phi13</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phi2954</td>
<td>Pseudomonas phage phi2954</td>
<td>L: FJ608825; M: FJ608824; S: FJ609825</td>
<td>L: NC_012091; M: NC_012092; S: NC_012093</td>
<td>Complete genome</td>
<td>phi2954</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phiNN</td>
<td>Pseudomonas phage phiNN</td>
<td>L: KJ857164; M: KJ857165; S: KJ857166</td>
<td>L: NC_042068; M: NC_042069; S: NC_042070</td>
<td>Complete genome</td>
<td>phiNN</td>
<td></td>
</tr>
<tr>
<td>★ Pseudomonas virus phiYY</td>
<td>Pseudomonas phage phiYY</td>
<td>L: KX074201; M: KX074202; S: KX074203</td>
<td>L: NC_042071; M: NC_042072; S: NC_042073</td>
<td>Complete genome</td>
<td>phiYY</td>
<td></td>
</tr>
</tbody>
</table>

Virus names, the choice of exemplar isolates, and virus abbreviations, are not official ICTV designations.
Authors: Cystoviridae

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Resources: Cystoviridae

Sequence alignments and tree files:

Figure 1A. Cystovirus:
- Tree file (newick format)
- Alignment file (MEGA format)

Figure 1B. Cystovirus:
- Tree file (newick format)
- Alignment file (MEGA format)

Figure 1C. Cystovirus:
- Tree file (newick format)
- Alignment file (MEGA format)
Further reading: Cystoviridae


References: Cystoviridae


Mindich, L., Qiao, X., Qiao, J., Onodera, S., Romantschuk, M. & Hoogstraten, D. (1999). Isolation of additional bacteriophages with genomes...


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