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Partitiviruses of a fungal forest pathogen have species-specific quantities of genome segments and transcripts

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ABSTRACT

Heterobasidion partitiviruses infect forest pathogenic fungi of the genus *Heterobasidion*. We have studied the amounts of genomes and transcripts of four partitiviruses isolated from four different *Heterobasidion* strains infecting different host trees in Greece, Poland, Finland, and China. Heterobasidion partitiviruses have bisegmented genomes encoding coat protein and RNA-dependent RNA polymerase. Our results show that the coat protein genome segment is generally more abundant in infected mycelia than the RNA-dependent RNA polymerase segment and that this bias persists also at transcript levels. The different virus species all have unique ratios of the genome segments and the ratio is generally stable over different temperatures and hosts. The amounts of transcripts of each virus respond to host growth temperatures in a distinctive and consistent manner. The Heterobasidion partitiviruses studied here affect only rarely the growth of their natural hosts but do influence the growth of a new host more frequently.

Introduction

Partitiviridae is a family of plant, fungal, and protozoan viruses that have segmented dsRNA genomes. *Partitiviridae* that infect fungi are classified into the genus *Partitivirus*. Most of the research on partitivirus molecular biology has been done on Penicillium stoloniferum partitiviruses S (PsV-S) and F (PsV-F), which were found from a common mould *Penicillium stoloniferum* (current name *Penicillium brevicompactum*) (Buck et al., 1969; Ellis and Kleinschmidt, 1967; Kim et al., 2003, 2005). In general, partitivirus genomes encode only coat protein (CP) and RNA-dependent RNA polymerase (RdRp) (Bozarth et al., 1971). The virus particles consist of 120 CP molecules, one RdRp molecule, and one RNA segment (Buck and Kempson-Jones, 1970). Electron cryomicroscope and X-ray crystallography structures of PsV-S, PsV-F, and Fusarium poae partitivirus have been published (Ochoa et al., 2008; Pan et al., 2009; Tang et al., 2010). All the particle structures have the same icosahedral capsid symmetry (triangulation number 2) formed by 60 quasisymmetrical coat protein dimers. The viruses pack both ssRNA and dsRNA into virion but only particles containing dsRNA are transcriptionally active (Buck, 1978). Each virus particle accommodates only one genome segment and Buck and Kempson-Jones (1973) have shown that an isolated pool of PsV-S viruses had twice as many particles containing CP than RdRp segment. Thus the theoretical infectious particle number for bipartite partitiviruses is two, but the genome segment bias lowers the probability that two random PsV-S particles would have both CP and RdRp genome segments compared to the viruses which have equal distribution of the two genome segments. However, like other mycoviruses, partitiviruses are not presumably capable of forming infective extracellular particles but the spread of the viruses is possible only through cell division, spores, or hyphal cell fusion between genetically compatible fungal strains (Nuss, 2011). In host cell cytoplasm partitivirus dsRNA genome stays inside protein-covered particle, which may protect viral dsRNA from RNA interference response as fungi have been shown to elicit RNA interference against partitiviruses and other RNA viruses (Chiba et al., 2013a, 2013b; Segers et al., 2007). Although mycovirus infections are generally symptomless, an increasing number of studies have shown that mycoviruses can have even drastic effects on growth and phenotype of their host fungi (Ahn and Lee, 2001; Bhatti et al., 2011; Hillman et al., 1990; Márquez et al., 2007; Zhou and Boland, 1997). Partitiviruses have also been shown to affect their hosts although somewhat less dramatically (Chiba et al., 2013a; Kanematsu et al., 2010; Potgieter et al., 2013).

Heterobasidion partitiviruses infect both major clusters of the genus *Heterobasidion*: *Heterobasidion annosum sensu lato* (*s.l.*, in the broad sense) and *Heterobasidion insulare s.l.*. *H. annosum s.l.* is a species complex consisting of wood-decaying and pathogenic European, Asian, and North American fungi, whereas fungi belonging to the *H. insulare s.l.* are found only in East Asia and are mostly saprotrophic. Each species of the two groups tend to have

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preference to certain tree species, for example, *H. parviporum* infects mainly Norway spruce and *H. abietinum* favours fir. *H. annosum sensu stricto* (*s.s.*) infects several trees including pine, spruce, and even some hardwoods (Lygis et al., 2004).

15–17% of *Heterobasidion* isolates harbour dsRNA viruses from which less than a third is partitiviruses (Ihrmark et al., 2001; Vainio et al., 2011a). Heterobasidion partitiviruses have been shown at laboratory conditions to pass quite readily from one *Heterobasidion* species to another and some of the virus species have been reported to enhance or/and diminish their hosts' growth rates (Hyder et al., 2013; Ihrmark et al., 2002; Vainio et al., 2010, 2012). For example, HetPV3-ec1 virus studied also in this paper has been shown earlier to affect fungal growth mainly negatively (Vainio et al., 2010). Although already 31 different Heterobasidion partitivirus strains are identified according to GenBank, very little is still known about the molecular biology of these viruses.

The objectives of our research work were to study the amounts of partitivirus RNA in host fungi, how the amounts are affected by temperature and host, and whether the changes in the amounts of viral RNA influence host growth. More specifically, we studied the relative amounts of genome segments and transcripts of four Heterobasidion partitiviruses, HetPV1-ab1, HetPV2-pa1, HetPV12-an1, and HetPV3-ec1, isolated from different species of *H. annosum s.l.* and *H. insulare s.l.* in their natural hosts and in a new host at three different temperatures.

Results

Each Heterobasidion partitivirus has a unique ratio of CP to RdRp genome segments

To study the relative amounts of CP and RdRp genome segments the virus hosts were grown at 20 °C and 25 °C. DsRNA was first isolated by affinity chromatography and then the viral dsRNA genome segments were isolated from agarose gel followed by reverse transcription and quantitative PCR (Fig. 1A). Genomic dsRNAs were isolated from a gel to exclude possible viral ssRNA transcripts which could interfere with the quantification. The relative amount of RdRp segment is calculated as a proportion of CP genome segments (Fig. 1B). Three out of the four viruses had more CP than RdRp genome segments and the CP to RdRp ratio remained the same in the two temperatures. In detail, HetPV1-ab1 had about three times, HetPV2-pa1 twice, and HetPV12-an1 ten times more of CP than RdRp segments. HetPV3-ec1 from *H. ecrostosum*, 05166, was an exception to the general trend, since it had more of RdRp than of CP segments and the CP to RdRp ratios varied in the two temperatures. On average the amount of RdRp segments exceeded that of CP 125 times at 20 °C and 12 times at 25 °C. Furthermore, the standard deviation of three separate measurements of CP to RdRP ratios was especially large for HetPV3-ec1. The standard deviation for HetPV3-ec1 was almost 30% at 20 °C and 70% at 25 °C from the mean value, whereas the standard deviation for the other viruses ranged from about 2–25%. This may

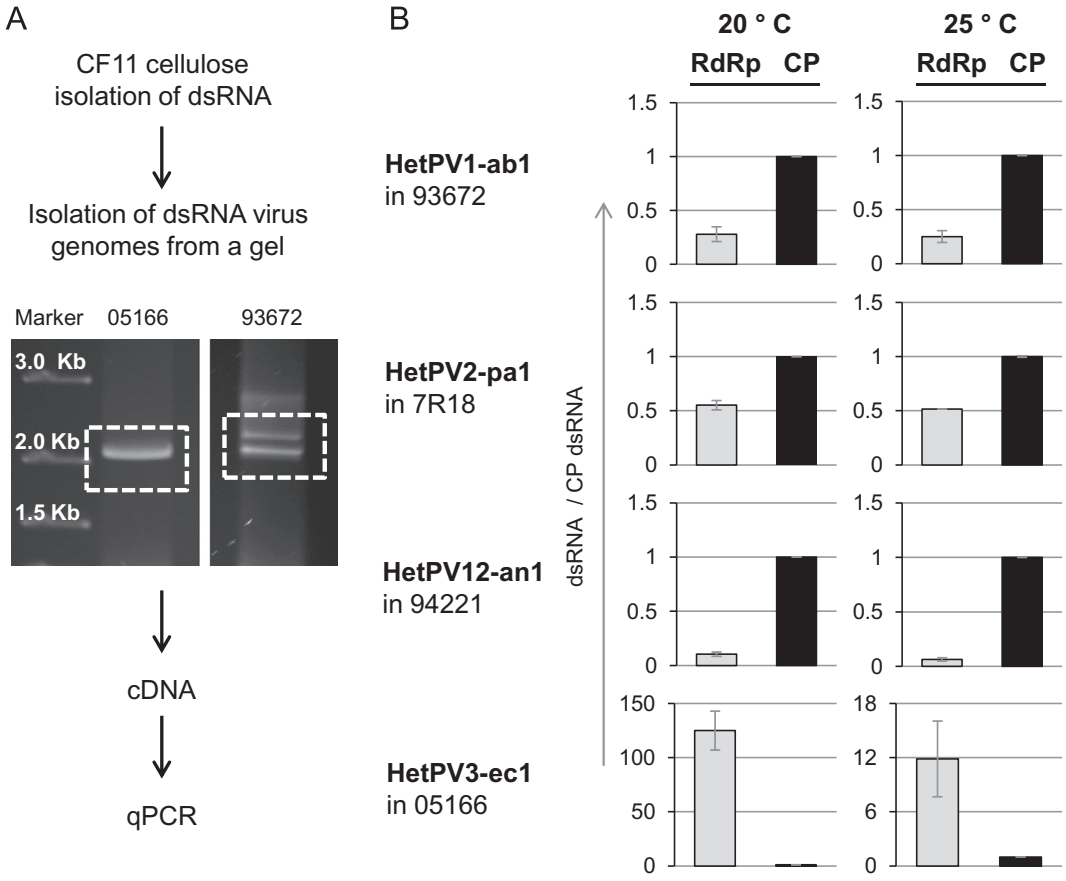

Fig. 1. Each Heterobasidion partitivirus species has a unique ratio of the dsRNA genomes segments. For quantitative PCR the virus genomes were isolated by affinity chromatography (CF11 cellulose) and gel electrophoresis, after which cDNA was prepared from the isolated dsRNA (A). Representative agarose gels showing the areas (indicated by dashed white squares) that were excised to isolate the viral dsRNAs. The example shows HetPV3-ec1 and HetPV1-ab1 dsRNA isolated from hosts 05166 and 93672, respectively, grown at 20 °C. The size marker indicates sizes in kilobases for dsDNA. (B) Quantitative-PCR analysis of the relative amounts of HetPV1-ab1, HetPV2-pa1, HetPV12-an1, and HetPV3-ec1 genomes isolated from their natural host fungi grown at 20 °C and 25 °C. The amount of CP dsRNA was set to one (black column) and used to calculate the relative amounts of RdRP (grey column). The error bars represent standard deviation of three separate experiments.