Virology 448 (2014) 265–273

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

A dsRNA mycovirus, Magnaporthe oryzae chrysovirus 1-B, suppresses vegetative growth and development of the rice blast fungus ☆

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ARTICLE INFO

Article history:
Received 1 August 2013
Returned to author for revisions
26 August 2013
Accepted 15 October 2013
Available online 5 November 2013

Keywords:
Mycovirus
dsRNA virus
Tentative chrysovirus
Magnaporthe oryzae
Multi-segmented mycovirus
Attenuation
Virus-induced growth inhibition

ABSTRACT

A double-stranded RNA (dsRNA) mycovirus was found in isolate S-0412-II 2a of the rice blast fungus *Magnaporthe oryzae*. Sequence analysis of the five dsRNA segments (dsRNA1 through dsRNA5) revealed that this mycovirus is closely related to Magnaporthe oryzae chrysovirus 1-A (MoCV1-A), tentatively classified as a member of the *Chrysoviridae*; therefore, it was named Magnaporthe oryzae chrysovirus 1-B (MoCV1-B). Virus particles were spherical and composed of the ORF1, ORF3 and ORF4 proteins. MoCV1-B-infected isolate S-0412-II 2a showed a more severe impaired phenotype than the MoCV1-A-infected isolate. In a virus-cured isolate, normal growth was restored, implied that MoCV1-B could be involved in this observed phenotype. An unanticipated result was the occurrence of a fungal isolate lacking dsRNA5. The nonessential dsRNA5 had higher sequence identity (96%) with dsRNA5 of MoCV1-A than with the other dsRNA segments (71–79%), indicating that dsRNA5 could be a portable genomic element between MoCV1-A and MoCV1-B.

Introduction

Mycoviruses have been described in many fungal species (Bao and Roossinck, 2013; Ghabrial et al., 2013; Dawe and Nuss, 2013; Hillman and Cai, 2013). Mycoviruses with dsRNA genomes are classified into four major families based on the sequence of the RNA-dependent RNA polymerase (RdRp), the *Totiviridae* (nonsegmented), *Partitiviridae* (two segments), *Chrysoviridae* (four segments) and *Reoviridae* (10–12 segments), while mycoviruses with single-stranded RNA genomes are classified into three major families, the *Hypoviridae*, *Narnaviridae* and *Barnaviridae* (King et al., 2012). Although most reported mycoviruses are apparently asymptomatic, some affect the phenotype of their fungal hosts (Anagnostakis and Day, 1979). In plant pathogenic fungi, several mycoviruses reduce the virulence of their hosts. For example, mycoviruses acting as a hypovirulence factor on their fungal host have been reported in *Cryphonectria parasitica* (Nuss, 2005), *Rosellinia necatrix* (Chiba et al., 2009; Kanematsu et al., 2004; Kondo et al., 2013) and *Fusarium graminearum* (Chu et al., 2002; Cho et al., 2013), and thus are expected to be agents for biological control of plant diseases.

Magnaporthe oryzae is a filamentous heterothallic ascomycete and the most destructive pathogen of rice worldwide. *M. oryzae* depends on its asexual spores (conidia) for disease establishment and propagation. Recently, we reported on a mycovirus, Magnaporthe oryzae chrysovirus 1 (MoCV1), found in a Vietnamese isolate of *M. oryzae* (Urayama et al., 2010). Since we report a second MoCV1 strain in this paper, the name of the first reported MoCV1 is being changed to Magnaporthe oryzae chrysovirus 1 strain A (MoCV1-A). MoCV1-A substantially impairs growth of host cells and results in altered colony morphology. To investigate potential effects of MoCV1-A gene products on host cells, a yeast heterologous expression system was constructed; overexpression of the open reading frame (ORF) 4 protein in *Saccharomyces cerevisiae* caused remarkable growth inhibition (Urayama et al., 2012). Recent studies on MoCV1-A structural proteins revealed that MoCV1-A has at least two types of viral particles, partially processed MoCV1-A and fully processed MoCV1-A (Urayama et al., 2012). Both of the viruses are isometric particles about 35 nm in diameter, with buoyant densities in CsCl ranging from 1.37 to 1.40 g cm^{-3}. The processed MoCV1-A particles are detectable not

☆The GenBank/EMBL/DDBJ accession numbers for the sequence reported in this paper.

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0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.10.022

only in host cells but also in cell-free culture supernatants, and MoCV1-A dsRNAs can be detected within mycelia of the MoCV1-A-free isolate after co-incubation with MoCV1-A particles (Urayama et al., 2010). Phylogenetic analysis based on RdRp protein sequences showed that MoCV1-A forms a separate clade with Fusarium graminearum mycovirus-China 9 (FgV-ch9) (Darissa et al., 2011), Fusarium graminearum virus 2 (FgV2) (Yu et al., 2011), Aspergillus mycovirus 1816 (AsV1816) (Hammond et al., 2008) and Agaricus bisporus virus 1 (AbV1) (Van der Lende et al., 1996) in the family *Chrysoviridae*. While typical chrysoviruses, such as *Penicillium chrysogenum virus* (PcV) (Jiang and Ghabrial, 2004) and *Helminthosporium victoriae 145S virus* (Hv145S) (Bruenn, 2002), have four dsRNA segments, MoCV1-A, FgV-ch9 and FgV2 have five genomic dsRNA segments.

In this paper, we report a new MoCV1-A-related mycovirus, Magnaporthe oryzae chrysovirus 1 strain B (MoCV1-B), found in Vietnamese isolates of *M. oryzae* (Le et al., 2010). Five dsRNA segments ranging from 2.8 kbp to 3.5 kbp and multiform structural proteins were detected in purified MoCV1-B viral particles, which were also observed in MoCV1-A. However, a MoCV1-B-infected isolate (S-0412-II 2a) showed more severe growth defects than a MoCV1-A-infected isolate (S-0412-II 1a). During cultivation of the host fungus, we unexpectedly isolated derivative MoCV1-B, which lacks dsRNA5. The sequence of the dsRNA5 segment had extremely high similarity in MoCV1-A and MoCV1-B. We compare the effects of MoCV1-B on its host fungus and discuss the evolutionary relationship between MoCV1-A and MoCV1-B.

Results

Isolate S-0412-II 2a of M. oryzae shows a severely impaired growth phenotype

In a previous study, we found several mycovirus-infected isolates of *M. oryzae* with dsRNA genome segments ranging from 2.8 to 3.5 kbp (Urayama et al., 2010, 2012). Isolates S-0412-II 1a and S-0412-II 2a, which contained such dsRNA genomes, were picked from lesions on different rice plants in the same field. Isolate S-0412-II 2a exhibited an extremely abnormal phenotype, including loss of aerial hyphae formation and extremely reduced pigmentation, showing albino mycelia on PDA medium (Fig. 1A and C, lane 1). This phenotype was more severe than that of S-0412-II 1a, which is infected by MoCV1-A (Fig. 1B and C, lane 2) (Urayama et al., 2010). Native PAGE demonstrated that the dsRNAs of isolate S-0412-II 2a consisted of five segments, the same number as MoCV1-A (Fig. 1C). For convenience, the numbers assigned to the dsRNA genomes in S-0412-II 2a were based on sequence similarities to the MoCV1-A dsRNA genomes.

We then examined dsRNA sequence similarities among six dsRNA-harboring isolates of *M. oryzae* (Fig. 1D) by northern blotting using a subclone of the MoCV1-A dsRNA4 cDNA as probe (nt 1026–2405, AB560764). As shown in Fig. 1E, signals were detected in the dsRNAs extracted from S-0412-II 1a (lane 1, control MoCV1-A), T-0412-II 2a (lane 3) and S-0412-II 1c (lane 5), while signal was barely detected in the dsRNAs extracted from S-0412-III 1a (lane 2), T-0412-II 2b (lane 4) and S-0412-II 2a (lane 6). When we used subclones of MoCV1-A dsRNA1, 2 or 3 as probes, similar patterns of signal intensity were observed (data not shown), suggesting that the mycoviral dsRNA sequences of T-0412-II 2a and S-0412-II 1c are very similar to those of MoCV1-A, while those of isolates S-0412-III 1a, T-0412-II 1a and S-0412-II 2a are not. Thus, the mycoviral dsRNA sequences in isolate S-0412-II 2a (Fig. 1 A and C, lane 1) are different from those of MoCV1-A in isolate S-0412-II 1a (Fig. 1B and C, lane 2).

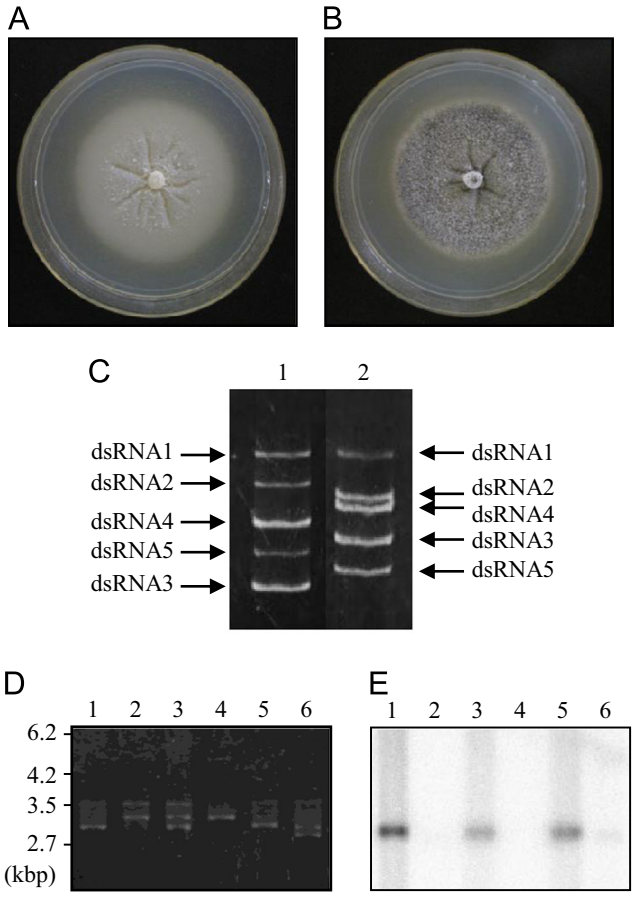

Fig. 1. Comparisons of colony morphology and dsRNAs of the S-0412-II 2a isolate and other isolates. (A, B) Comparison of colony morphology of the S-0412-II 2a isolate (MoCV1-B-infected) (A) and the S-0412-II 1a isolate (MoCV1-A-infected) (B). Both isolates were inoculated in the center of a PDA plate and incubated for 10 days at 25 °C. (C) Migration patterns of dsRNAs purified from S-0412-II 2a (MoCV1-B-infected, lane 1) and S-0412-II 1a (MoCV1-A-infected, lane 2). dsRNA was electrophoresed in 5% (w/v) polyacrylamide gels, and stained with ethidium bromide. (D, E) Agarose gel electrophoresis of dsRNAs purified from MoCV1-A-infected S-0412-II 1a (lane 1), S-0412-III 1a (lane 2), T-0412-II 2a (lane 3), T-0412-II 1a (lane 4), S-0412-II 1c (lane 5), and S-0412-II 2a (MoCV1-B-infected, lane 6), after staining with ethidium bromide (D) or the same samples after northern blotting (E). Northern blotting of purified dsRNAs extracted from *M. oryzae* Vietnam isolates using a cDNA probe derived from MoCV1-A-dsRNA4 (1026–2405 nt).

Nucleotide sequences of the five dsRNA segments in S-0412-II 2a

The complete nucleotide sequences of the five dsRNA segments in S-0412-II 2a were determined from a series of cDNA clones spanning the entire length of each dsRNA. The nucleotide sequences were significantly similar to MoCV1-A dsRNAs using the MegaBLAST algorithm of Zhang et al. (2000), and we named this mycovirus MoCV1-B. The sequences of these dsRNA segments have been deposited in GenBank/EMBL/DDBJ with accession numbers AB824667–AB824671. These five dsRNAs were named dsRNA1 to dsRNA5 based on sequence similarities to those of MoCV1-A (see Tables S1 and S2 in the Supplemental information). The genetic organization of the five dsRNAs is shown in Fig. 2A. Both the 5'- and 3'-terminal regions were conserved among the five dsRNAs of MoCV1-B and those of MoCV1-A (Fig. 2B). Comparison of nucleotide sequences of MoCV1-A and MoCV1-B revealed moderately high levels of mutual identities for dsRNA1 (79.6%), dsRNA2 (76.3%), dsRNA3 (71.4%) and dsRNA4 (72.4%), but very high levels for dsRNA 5 (96.2%) (see Table S1 in the Supplemental information). While MoCV1-A dsRNA3 and dsRNA4 carry adenine-rich regions of about 130 and 100 bp at their 3'-untranslated