Sequence determination of a satellite RNA isolated from *Aspergillus foetidus*

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Abstract

Aspergillus foetidus virus (AfV) contains at least two distinct particle types designated as AfV-fast (F) and AfV-slow (S). AfV-S contains AfV-S1, a victorivirus, AfV-S2, an unclassified satellite RNA, and AfV-S3, a previously uncharacterized dsRNA element. Here we describe the complete sequence of AfV-S3, which is a short non-coding RNA with no known homologs. AfV-S3 is predicted to form extended secondary structure, shares a 5'-terminus with AfV-S2 and is a satellite RNA possibly dependent on both AfV-S1 and AfV-S2. This work concludes the sequencing of the *A. foetidus* virome.

Introduction

Double-stranded (ds) RNA mycoviruses have been described in yeasts, mushrooms and filamentous fungi; they are classified into six families based on their virion structure and genome composition, but some still remain unassigned to a genus or family. Satellite RNAs, which rely on their helper virus for maintenance, are commonly associated with fungal viruses. The most prominent and well-studied example is the killer-toxin-encoding dsRNA that accompany the Saccharomyces cerevisiae L-A totivirus [17], while a number of satellite RNAs dependent on various members of the *Partitiviridae* family have also been noted [6, 10, 14].

In the genus *Aspergillus*, the presence of dsRNA mycoviruses from the families *Totiviridae*, *Partitiviridae* and *Chrysoviridae* has been reported, and mixed infections are common [4, 8, 16]. More specifically, *Aspergillus foetidus* isolate IMI 41871 has been found to harbour at least two different types of isometric virus particles, designated as A. foetidus virus-fast (AfV-F) and -slow (AfV-S), based on their relative electrophoretic mobility [3, 15]. The quadripartite polyadenylated dsRNA virus present in AfV-F virions is similar to Alternaria alternata virus 1, both members of a putative novel mycovirus family [12], while AfV-S virions contain AfV-S1, a member of the genus *Victorivirus* in the family *Totiviridae* [11], AfV-S2, a putative satellite RNA similar to an unclassified RNA from the fungus

Rosellinia necatrix [13] and AfV-S3, an uncharacterized small dsRNA element, approximately 0.4 kbp in length. In this paper, we report the complete sequence of the AfV-S3 dsRNA element.

Provenance of the virus material

Aspergillus foetidus (Thom and Raper) isolate IMI 41871 (CBS 618-78), originating from Carl A. Neuberg in Berlin, Germany, and probably isolated in the 1940s, was grown in a 60-litre fermenter.

Purification of virus particles was performed as described previously [2] and two different types of virus particles designated AfV-F and AfV-S, according to their electrophoretic mobilities, were separated by rate zonal sucrose density gradient centrifugation [5] and stored in 50% glycerol at 4^{o}C. The AfV-S dsRNA elements were extracted using phenol/Sevag treatment and separated by electrophoresis on a 1% (w/v) agarose gel containing Tris-acetate-EDTA (TAE) buffer and 500 ng/ml ethidium bromide (Fig. 1a). The smallest segment was extracted from the gel using the MinElute Gel Extraction Kit (Qiagen) and was used as a template in a modified protocol of RNA ligase-mediated rapid amplification of cDNA ends [7]. Due to the small size of the dsRNA element (*ca.* 0.4 kbp), no sequence-specific primers were required and the whole element was amplified, cloned using the pGEM-T Easy vector system (Promega) and introduced into competent *Escherichia coli* XL10-Gold cells (Agilent). Four different clones of the element were sequenced. One of them was used as a template for the production of a digoxygenin (DIG)-labelled probe with the DIG Northern Starter Kit (Roche) and the origin of the clones was confirmed by northern blotting, hybridization and chemiluminescent detection according to the manufacturer's instructions (Fig. 1b).

Sequence properties

The sequence of the AfV-S3 dsRNA element has been deposited in the GenBank/EMBL/DDBJ databases with accession number LN614706. AfV-S3 consists of a single dsRNA of 439 bp, which has a GC content of 53%. Sequence similarity searches of the GenBank and EMBL databases using the BLAST program [1], revealed no statistically significant homology between AfV-S3 and other known sequences, including the other AfV-S components and AfV-F. Additionally, AfV-S3 contains no open reading frames (ORFs) of significant length in either strand; both strands were found to contain short ORFs potentially encoding polypeptides less than 9 kDa in mass that lack significant sequence similarity with proteins in the databases. Therefore, it is unlikely that these polypeptides are actually produced.

Secondary structure analysis of the AfV-S3 dsRNA using the mfold server [18] indicated that *ca.* 54% of the ribonucleotides are involved in the formation of secondary structures and predicted the presence of stem-loop structures (Fig. 1c), which are common in mycoviruses and considered to be

implicated in RdRp recognition and RNA replication [9]. Interestingly, the first seven nucleotides of the AfV-S3 5'-terminus (5'-GGGATTT-3') are identical to those of AfV-S2, suggesting that AfV-S3 may be using the RNA-dependent RNA polymerase encoded by AfV-S2 to replicate. AfV-S1, AfV-S2 and AfV-S3 dsRNA elements are all derived from the AfV-S virus particles, which consist of a capsid protein most probably produced by AfV-S1, a victorivirus and the only AfV-S component to encode a structural protein. Therefore, AfV-S3 may be a satellite RNA dependent on two different viruses; AfV-S2 for its replication and AfV-S1 for its encapsidation.

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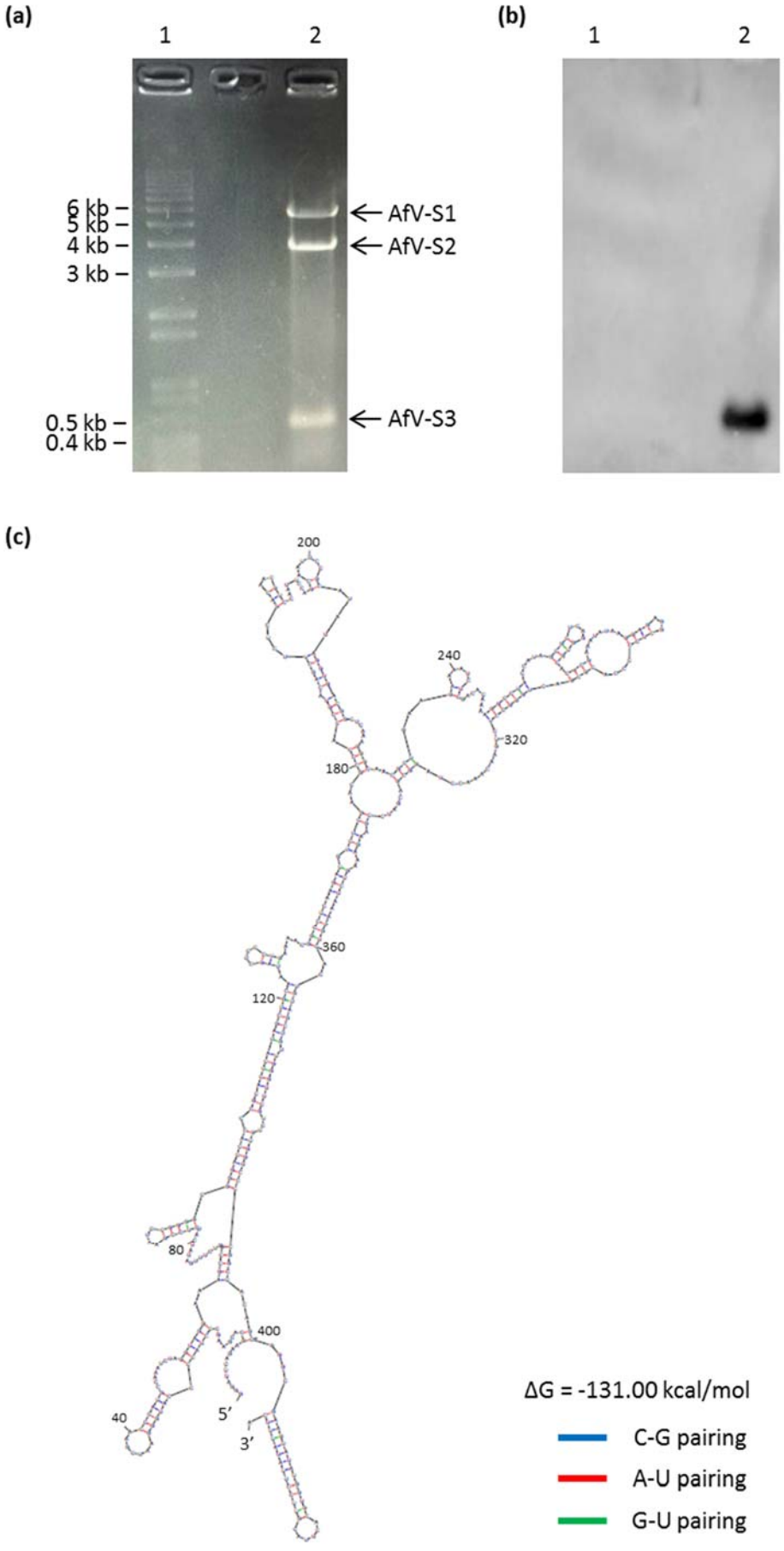
(a)
1
2
6 kb
5 kb
4 kb
3 kb
0.5 kb
0.4 kb
AfV-S1
AfV-S2
AfV-S3
(b)
1
2
(c)
200
240
320
180
360
120
80
400
40
5′
3′
ΔG = -131.00 kcal/mol
C-G pairing
A-U pairing
G-U pairing

Fig. 1 (a) Agarose electrophoresis of AfV-S viruses from *A. foetidus* isolate IMI 41871 (lane 2). AfV-S1, AfV-S2 and AfV-S3 are indicated by arrows. Lane 1 contains the 1 kb Plus DNA Ladder (Invitrogen), the sizes of with is shown to the left of the gel. **(b)** Northern blot hybridization of AfV-S3 using a DIG-labelled probe derived from its cloned sequence. **(c)** Schematic representation of the minimum free energy structure of the AfV-S3 sequence, as predicted by mfold.