



# Completion of the sequence of the *Aspergillus fumigatus* partitivirus 1 genome

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## Abstract

A Portuguese isolate of *Aspergillus fumigatus* was found to contain three double-stranded (ds) RNA elements ranging in size from 1.1 to 1.8 kbp and comprising the genome of a strain of *Aspergillus fumigatus* partitivirus 1 (AfuPV-1) previously thought to contain only the two largest dsRNA elements. The sequence of the smallest dsRNA element is described here, completing the sequence of the AfuPV-1 genome. Sequence analysis of the element revealed an open reading frame encoding a protein of unknown function similar in size and distantly related to elements previously identified in other members of the family *Partitiviridae*.

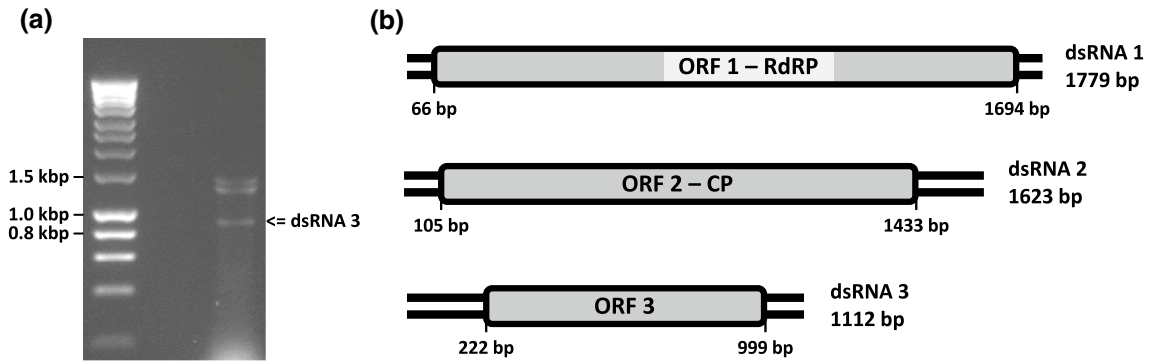
Mycoviruses are widespread in almost all major groups of fungi, and most cause no obvious effects in their hosts, but some do cause obvious symptoms resulting in debilitated virulence, slow growth rate, and poor sporulation [5, 8]. Currently recognized mycoviruses have single-stranded (ss) or double-stranded (ds) RNA, or rarely DNA, as their genetic material [8]. Members of one family, the *Partitiviridae*, are classified into five genera, namely *Alphapartitivirus*, *Betapartitivirus*, *Gammapartitivirus*, *Deltapartitivirus* and *Cryspovirus* [17]. Partitiviruses generally possess two essential dsRNA genome segments (1.3–2.5 kbp in length), each containing a single open reading frame (ORF) [17]. The larger component 1 encodes an RNA-dependent RNA

polymerase (RdRP), and the smaller component 2 encodes the capsid protein (CP) [17]. On occasion, smaller dsRNAs than the two dsRNAs described above encoding proteins of unknown function have been reported for some partitiviruses. Examples include *Ustilagoidea vires* partitivirus 1 (UvPV-1) [18], *Gremmeniella abietina* RNA viruses MS1, MS2 and MS3 (GaRV-MS1, -MS2 and -MS3) [3, 15, 16], *Aspergillus ochraceus* virus (AoV) [10, 11] and *Aspergillus flavus* partitivirus 1 (AfPV-1) [6], but not *Aspergillus fumigatus* partitivirus 1 (AfuPV-1) [2] or a second isolate of UvPV [7]. During our continued monitoring of the incidence of mycoviruses in *Aspergillus fumigatus*, an opportunistic pathogen causing aspergillosis in the immunocompromised human population, we isolated, cloned and sequenced a Portuguese strain of AfuPV-1 termed AfuPV-1A. An examination of the dsRNA profile of AfuPV-1A revealed that it contained three dsRNA components, with the sequences of the two largest dsRNAs being 98–99% identical to those of AfuPV-1. These results confirmed that AfuPV-1A is a strain of AfuPV-1 that can support the replication of a third genomic component termed dsRNA3 and that smaller dsRNA components can apparently be lost and recovered during partitivirus infections. Here, the sequence of the AfuPV-1A dsRNA3 element is reported, completing the sequence of the AfuPV-1A genome. Additionally, relationships between AfuPV-1A dsRNA3 and AfuPV-1 dsRNAs 1 and 2 were investigated together with comparisons to other small dsRNA elements of partitiviruses.

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**(c)**

AfuPV-1	1	-----MCKSNS-	-----	---EASLTKRSSR-LPSVFNHGKAAKCRNLNRCGVRHAPK	42	
UvPV-1	1	-----MVCFPQSG [29]	SLRVNA- [ 5]	VKTEAALQKCSYHPSPRGAGEGKGGKKYHSCRKLKSAAV	88	
GarV-MS1	1	-----MSKTQTN	PVGVRS	-----PPKPKGKIRQPS-	26	
PvAPV-2	1	MNKLKIEDVSPSKDTRAGSPPSIGE [28]	VARKNAE [20]	LESEIARLSKEQQKKFQQLVLRKQSGSKEPRGRSPRKSXP	120	
AoV	1	MIGKNDEDCGPLTPQYLLSRKRYGE	STRKAQR	-----PLHPRGTRNDAAF	45	
PvAPV-10	1	M---NDTKMSSRERRLSDISSSGSK	NTLVDER [ 6]	ACGRKGRFGCGTSYMCPTKRPSRGPKTSPRRSLSPEKEV	75	
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AfuPV-1	43	RNPN	LEQRKFTAFSKALAVRSVLGRRLTREWVKLKHAVRYQCFGPTVP-	LQKQLTMLATRVARICERLVPASI--	115	
UvPV-1	89	ESPQ	-QLKFRRAFASKALVVRTRVLGRRLHRKCEVAVRKKQVFASKADEATRIL	TQVRAEVARLRSILVPAGV--	161	
GarV-MS1	27	ASQL	---RFKKALARALSVRSKPLKRRLSKPEFVQLSAAVKTQIKQETTP-	VNRRLNRVIDIVVRLEEAANSSLS	98	
PvAPV-2	121	SKAE	EQERFQRAIRKASSVRRRLQGRELEHDEWIQLRNAVRKQCSNVKTD	DDFTKRLSVLETRLSRLESQTYASDPG	196	
AoV	46	AKRQ	---RFDKAFKAIAIRVVRVLGRPLTQLEFRRLVSAVRKQIQPRLEN	FVSRVAVNIEKRVKTLLELATPTSVA-	117	
PvAPV-10	76	KQQK [22]	DDDRVSSAIRKSYRVRCLSLKRRHLQDEFDRLCVAVRKQCKLHKKSH	YESLLGEHRRIRIRILERTQPIDVS-	172	
: . : * : : : * * * * * * * * * * * :						
AfuPV-1	116	S---	SVADSIELMYQQLSLLRAQAMELMSERESIA-PSCPHFRKRKVFPT	PSNQLQIGV--KPLGWHSVITYRLQGRDET	189	
UvPV-1	162	L---	QASDSVQILNLQLSVLRQQFMELVSSDNS-T-ETCPHYRKRKYR	APNSDGSFHF--QVPLSELIVWQPRGQVEQ	234	
GarV-MS1	99	LDRPALDA	AVRNLYDRLNLVRSNLLEAMRESSLS-EGCSHVS	KKSVIHLPEPVKVDKI--FPFGICI-SVRI	PSGRQV	173
PvAPV-2	197	V---	QIADKISIFQLQLDIARRQMLELINDQASIS-EGCSHLAKK	VVIKTVEPFEEV----QIKGPGIVGVCNV	PSGSHR	267
AoV	118	----	QLESIAIMFHTQVTVLREQAMRAIQENDVLS-ETC	SHISHKMM--LPVPTDMERLTAHPNLGHL-	TISLKP-TEV	188
PvAPV-10	173	----	ALQDAIENTYSQLEVLRTQLLNAIQDQEENSL	PCTHMKQINVRNSERFQIEHL--IPSFGLI-	FHHFSPTSLR	244
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AfuPV-1	190	LCCGAQLQARS	DAWFSWTGEELRFEHFLEGGDDIPGKE---	QLLRSL SAISP-LSIQGIW	SQILLQYKGSDFS [ 1]	258
UvPV-1	235	LCCPSCLSD	REDTFFTWTEGELPPQFSLSKGEMDPVIS---	PSERAL LNTVPILSIQSV	VTKLCNSQIRSHVA	303
GarV-MS1	174	LCCSRCSGL	RNDAFFEWSGLQ-PSFIDKEGHS	MEVAA--YPLPKSI KNINP-LDFQH	VVTSVLVDVHYSR--	237
PvAPV-2	268	SCCDRCLSD	RDRDFIFESLGLTSPF--RAGSD	SDVDH--LNTTL [10] EGVDP-	MRFQHIWSLFLDKKFRG--	340
AoV	189	LCCDSLAKR	QDSVFLWSGVDLVSLADMMPHL	PREAIAIRWLSNKI SQVHP-LSIQSI	WTAVVNLKYDRTFQ [34]	293
PvAPV-10	245	LACGKCIQK	REDILFYWTGHQLPKFSSLSVSDGT	-----LPTAL KEVSP-LAVP	QIWNQVLDLKYDREI-	306
: * :						

**(d)**

5'-UTRs

dsRNA1	CGCAAAG--ACCCUU-----GUCUUUACCGCCUUGCG-----
dsRNA2	CGAAAAGAACCCUUUUUGACUCCAGUGUAUCUGGAGGGUUUACGCUAAAUUUAAAACCGUAAAACUUUUUUU-----
dsRNA3	CGAAAUUU--UUCCUCUCUCCUGAGGGGGUGGGAUGAGAAUGUAGGAAAUUUCCUAGGUGUUAAGUUUAUAGACACUGGCCG
	* *
dsRNA1	-----GGUUUUCUUGCCUC-----CUUCUCUCUUGAGCG-----
dsRNA2	-----UCCUCGAGCCUC-----CUUCUCU-----AAGCG-----
dsRNA3	ACGCCACGGCUUAUAGCUCGUGGCCCAUGACAGCUCUCCACGUAUGCGGAUCAACAUACGUUACAGUUUUGGAGUGUUGCAUCAC
	* *
dsRNA1	-----UAA
dsRNA2	-----ACC
dsRNA3	ACUCGCUCGGUUUCUUGGCCUGGUUAUUGAGAACUUUUCUAC

3'-UTRs

dsRNA1	-----GAGUGUCUUUCUUGCAGAACGCAGGUCUU--
dsRNA3	UCUCAG-----AUCGCU--AUUCACGUCUGAGCCUUAGAUCGACAGGCUUU-
dsRNA2	ACAUAGCCAGUACUUGAAGAAACCAGUUUUCUGGUCAGCCAAACCUUACCACUAGAUGUCUGGCCUUUUAUCGGUAGCCUAGUGUUG
	* *
dsRNA1	CCUGUUUUAUCAGGAGGA---UGUAACGUGCCGUUUU-----CGGCGUUUCCGUUAUUAUCCA-
dsRNA3	GGUGUCGACGAGAUGGA---CGAA---GGUGUUUUUC-----CAGCUUUUCGUAUCUACCCGU
dsRNA2	CCUGUCUAUCAGGAAGACCCUGUAUCGGGCCAUCUAUUUCCUAUCAAGGAGUGGGUUAUACGUGGCCUUUUAUCGGUACGUGUUCAC
	* *
dsRNA1	-----
dsRNA3	CCCAAUAGAUCCGU
dsRNA2	UGUAAAUCCA-----

**Fig. 1** Genomic organisation of AfuPV-1 and comparisons of the amino acid sequences of putative proteins encoded by dsRNA3 elements of representative partitiviruses. **(a)** Agarose gel electrophoresis of dsRNA elements extracted from *A. fumigatus* isolate 12/43. Molecular sizes are indicated on the left with numbers. **(b)** Schematic representation of the genome organisation of AfuPV-1A. AfuPV-1 dsRNAs 1, 2 and 3 each contain a single ORF (grey boxes) flanked by 5'- and 3'-UTRs (black lines). The dsRNA1 is 1779 bp long and contains an ORF that encodes a putative RdRp with a molecular mass (*Mr*) of 63 kDa. The dsRNA2 is 1623 bp long and contains an ORF that encodes a putative CP with a *Mr* of 48 kDa. The dsRNA3 is 1112 bp long and contains an ORF that encodes a protein of unknown function with a *Mr* of 29 kDa. **(c)** BLAST COBALT alignment of the amino acid sequences of the putative proteins of unknown function encoded by the smallest genomic dsRNA3 elements of *Aspergillus fumigatus partitivirus-1A* (AfuPV-1A; LR743535), *Ustilaginoidea virens partitivirus 1* (UvPV-1; AGO04405), *Gremmeniella abietina* RNA virus-MS1 (GaRV-MS1; AII16003), *Plasmopara viticola* associated partitivirus 2 (PvAPV-2; QHD64806), *Aspergillus ochraceus* virus (AoV; AYP71820) and *Plasmopara viticola* associated partitivirus 10 (PvAPV-10; QHD64798). Accession numbers are shown in brackets above. In the sequence alignment, asterisks signify identical amino acid residues, colons signify highly conserved residues, and single dots signify less-conserved but related residues. **(d)** Alignment of the 5'- and 3'-UTRs of the coding strands of the three genomic segments of AfuPV-1A, where asterisks indicate identical nucleotides in the three elements and single dots signify conserved purines or pyrimidines

## Provenance of the virus material

*A. fumigatus* 12-43 was originally isolated from bronchial secretions of a hospitalised patient in Portugal [12]. This was the only *A. fumigatus* isolate found to harbor dsRNA elements following the screening of 48 isolates in total: 12 environmental, 12 avian, 12 from cystic fibrosis patients, and 12, including isolate 12-43, from non-cystic fibrosis patients [12–14]. The *A. fumigatus* isolate 12-43 was grown in liquid Czapek-Dox complete medium at 25 °C with shaking. Purification of virus particles was performed as described previously [9], and dsRNA was extracted from the particles using phenol/Sevag treatment. Three viral dsRNA elements were separated by electrophoresis on a 1% (w/v) agarose gel containing Tris-acetate-EDTA (TAE) buffer and 500 ng of ethidium bromide per ml (Fig. 1a). The two larger dsRNAs 1 and 2 of the *A. fumigatus* isolate 12-43 were 1779 and 1623 bp in size, respectively, and were 99% and 98% identical in sequence to those previously reported to constitute the genome of AfuPV-1 [2]. The additional smaller dsRNA is referred to as “AfuPV-1A dsRNA3” in this study. AfuPV-1A dsRNA3 was extracted from the gel using a MinElute Gel Extraction Kit (QIAGEN) and its dsRNA nature was confirmed by its resistance to DNase 1 and S1 nuclease (Promega). Subsequently, the dsRNA was denatured with methyl mercuric hydroxide, and this served as a template for reverse transcription and PCR amplification using random primers [5]. The sequence was completed using sequence specific primers, genome walking, and RNA ligase-mediated

rapid amplification of cDNA ends [4]. All products were cloned using the pGEM-T Easy Vector system (Promega) and introduced into competent *Escherichia coli* XL10-Gold cells (Agilent). At least three different clones covering the same part of the genome were sequenced. The effects of AfuPV-1A on the phenotype and pathogenicity of its host are currently unknown but are assumed to be similar to those described previously by Bhatti *et al.* [2] who observed an abnormal colony phenotype, slow growth, and lighter-than-normal pigmentation.

## Sequence properties

AfuPV-1A dsRNA3 is 1112 bp in length, has 50% GC content and possesses a single ORF that encodes a hypothetical protein of unknown function comprising 258 amino acids (aa) with a molecular mass of 29.274 kDa (Fig. 1b). A BLASTp [1] search using the non-redundant protein sequences database updated on 6 April 2020 revealed that the amino acid sequence of the protein encoded by the AfuPV-1A dsRNA3 ORF was 41.5%, 32.6% and 31.5% identical to that of the corresponding protein of unknown function encoded by the smallest genomic dsRNA3 elements of UvPV-1 (303 aa), AoV (293 aa) and GaRV-MS1 (237 aa), respectively. It also demonstrated 33.2% and 30.3% sequence identity to dsRNA3 of the recently reported *Plasmopara viticola* associated partitivirus 2 (PvAPV-2; 340 aa) and *Plasmopara viticola* associated partitivirus 10 (PvAPV-10; 306 aa), respectively (Fig. 1c). Neither GaRV-MS2 nor GaRV-MS3 was included in these alignments, as they are considered to be strains of GaRV-MS1 [3, 16]. The AfuPV-1A dsRNA3 ORF is flanked by 5'- and 3'-UTRs 222 and 113 bp in length, respectively. A comparison between the 5'-UTRs of AfuPV-1A dsRNAs 1, 2 and 3 illustrated that they differed in length but showed some conserved nucleotides at the 5' terminus (CGC/AAAAA/UG/U; Fig. 1d). This is consistent with observations for multi-component RNA viruses, where the 5'-terminal sequences are essential for recognition by the viral RdRP during viral RNA replication [6]. Such conserved sequences proximal to the 5' termini of partitiviruses are thought to be involved in RdRP recognition for RNA packaging and/or replication [17]. The 3'-UTRs differ in length, and there is little sequence conservation (Fig. 1d). *In silico* analysis of the entire sequence of AfuPV-1A dsRNA3, including both UTRs, revealed the presence of stem-loop and panhandle structures (results not shown), but their significance in replication is unknown [17]. It is not known why some members of the family *Partitiviridae* possess a bipartite genome while others appear to be tripartite, as is the case now for AfuPV-1A. It is curious that the smallest dsRNA element was not isolated in our initial screens of dsRNA elements in *A. fumigatus*, but the appearance and

disappearance of mycovirus elements in some instances is thought to be related to fungal growth conditions and variable virus distribution in mycelia. In the absence of reverse genetics to investigate whether AfuPV-1A dsRNA3 and similar dsRNAs in other partitiviruses are essential, any possible roles in replication remain unknown.

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