


Molecular characterization of a novel fusarivirus infecting the plant-pathogenic fungus *Neofusicoccum luteum*

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Abstract Double-stranded RNAs from an isolate of *Neofusicoccum luteum* collected from grapevines were analyzed by high-throughput sequencing. Contig annotations revealed the presence of a potential novel virus belonging to the newly proposed family *Fusariviridae*. Completion of the viral genome sequence was performed. The genome is 6,244 nucleotide long, excluding the poly(A) tail and contains two putative open reading frames (ORFs). The first one encodes a large polypeptide of 1,552 amino acids (aa) with conserved RNA-dependent RNA polymerase and helicase domains typical of viral replicases. The second ORF encodes a putative 475-aa-long polypeptide showing weak homology to the corresponding ORF of *Macrophomina phaseolina* single-stranded RNA virus 1, for which no function is known so far. Phylogenetic analyses indicated that this virus should be considered a novel mycovirus belonging to the proposed family *Fusariviridae*, for which the name “*Neofusicoccum luteum* fusarivirus 1” (NIFV1) is proposed.

Grapevine trunk disease (GTD) is one of the most important problems for the grapevine industry in all major grape-growing countries. Like dieback caused by *Eutypa lata* and Esca syndrome, *Botryosphaeria* dieback, associated with various members of the family *Botryosphaeriaceae* takes a toll on grapevines [1]. Among the possible disease-control strategies, the use of biocontrol agents appears to be a very promising way to prevent the spread of GTD [2]. Despite the fact that fungal viruses are often associated with asymptomatic infections, some of them have been shown to affect the biological properties of their fungal host [3]. Hypovirulence-associated mycoviruses have been characterized from *Botryosphaeriaceae* fungi. In *Botryosphaeria dothidea*, two mycoviruses conferring hypovirulence have been discovered so far: a new member of the family *Chrysoviridae*, *Botryosphaeria dothidea* chrysovirus 1 [4], and a novel double-stranded RNA virus, *Botryosphaeria dothidea* RNA virus 1 [5].

Neofusicoccum luteum is one of the fungal species belonging to the family *Botryosphaeriaceae* that is involved in GTD [6]. In order to better understand the life-history traits of this fungal pathogen, the presence of mycoviruses was investigated. A recent study reported the discovery and characterization of a mitovirus infecting *N. luteum* [7]. In this paper, a novel mycovirus belonging to the proposed family *Fusariviridae* [8, 9] was characterized from an isolate of *N. luteum* and tentatively named “*Neofusicoccum luteum* fusarivirus 1” (NIFV1).

Double-stranded RNA (dsRNA) was purified from mycelium of *N. luteum* (NL-37) that had been collected from symptomatic canes of *Vitis vinifera* in 1996 and cultivated as reported previously [10], and it was analyzed by Illumina sequencing following a previously described multiplexing strategy [7]. After demultiplexing and quality trimming steps, the reads were assembled into 253

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The nucleotide sequence reported in this study has been deposited in GenBank under the accession number KY906213.

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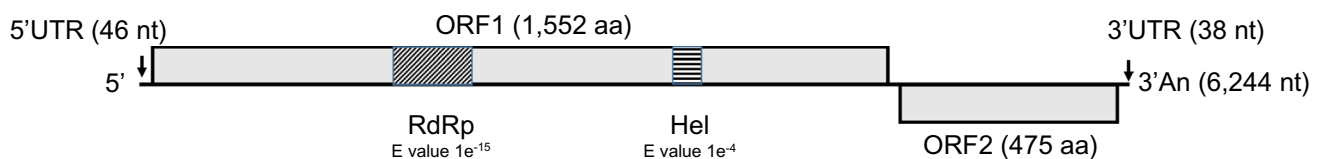
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contigs, which were further analyzed by BlastN and BlastX comparisons with the GenBank nr database. In addition to a contig of 1.2 kb (2.3% of total reads) corresponding to the recently described *Neofusicoccum luteum* mitovirus 1 (NIMV1) [7], a unique contig of ca. 6.2 kb integrating 82.7% of the total reads was found to have significant similarity to *Macrophomina phaseolina* single-stranded RNA virus 1 (MpSRV1; KP900890) [11], a member of the newly proposed family *Fusariviridae*. The two ends of the viral genome were determined using a rapid amplification of cDNA ends (RACE) strategy (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) with internal primers designed from the contig and dsRNA as template. The completed genome sequence has been deposited in the GenBank database (KY906213).

The viral genome is 6,244 nt long excluding the polyA tail and, in addition to two short non-coding regions at the 5' and 3' ends (46 and 38 nt long, respectively), contains two open reading frames (ORFs), encoding polypeptides of 1,554 and 475 aa, respectively (Fig. 1a). Conserved

RNA-dependent RNA polymerase (RNA_dep_RNAP, cd01699) and RNA helicase (Helicase_C, pfam00271) domains typical of single-stranded positive-sense RNA viruses were detected in the ORF1-encoded polypeptide using the conserved domain search program on the NCBI website, with significant e-values ($1e^{-15}$ and $1e^{-4}$, respectively; Fig. 1a). Moreover, a phylogenetic analysis of the putative replicase showed that it clusters with very high bootstrap confidence with members of the newly proposed family *Fusariviridae* (Fig. 1b). The amino acid sequence identity level of the putative replicase and the corresponding proteins of *Fusariviridae* members is limited and reaches a maximum of 55% when compared with the MpSRV1 replicase (Table 1). The ORF2-encoded protein is even more distant, with only 38% aa sequence identity to the MpSRV1 ORF2 product (data not shown). No conserved domain was detected in this protein, raising the question of its role. The genomic organization of fusariviruses tolerates some flexibility in the number of ORFs, although most of them contain two ORFs (Table 1). In the

(a)



(b)

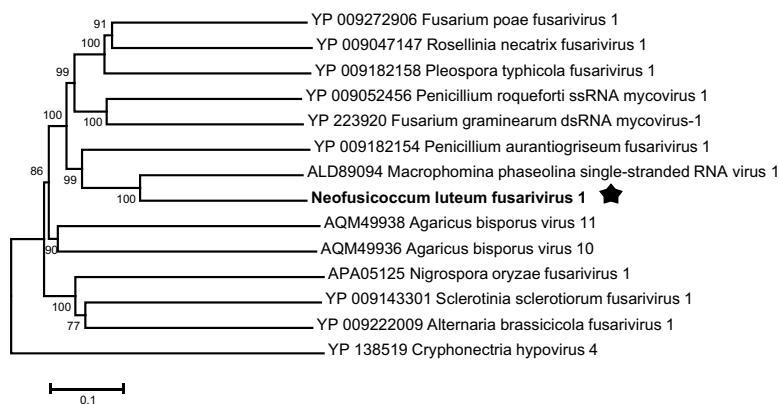


Fig. 1 Genomic organization and phylogenetic relationships of *Neofusicoccum luteum* fusarivirus 1 (NIFV1). (a) Schematic representation of the genome organization of NIFV1. Open reading frames (ORF) are represented by boxes. The sizes of the deduced proteins and of the 5' and 3' untranslated regions (UTR) are indicated in amino acids and nucleotides, respectively. Conserved RNA-dependent RNA polymerase (RdRp) and RNA helicase (Hel) domains in the ORF1-encoded protein are represented by striped shading, with the e-values associated with their identification using the conserved domain search program on the NCBI website. (b) Neighbor-joining phylogenetic tree constructed using the complete amino acid

sequences of RNA-dependent RNA polymerases from known and putative *Fusariviridae* members. The tree was constructed using strict identity distance, and the statistical significance of branches was evaluated by bootstrap analysis (1,000 replicates). Only values higher than 70% are indicated. The scale bar represents 10% amino acid sequence divergence. The sequence of the NIFV1 is indicated by a black star. Some viruses included in the phylogenetic analysis are not yet classified as *Fusariviridae* members in NCBI database, but sequence analysis suggests that they could be considered putative fusariviruses

Table 1 Genome features of putative members of the family *Fusariviridae* and amino acid sequence identity values between ORF1-encoded proteins and the corresponding proteins of *Neofusicoccum luteum* fusarivirus 1

Virus name and accession number	Genome size (nt)	Number of ORFs	Amino acid sequence identity to ORF1-encoded protein (%)	Reference
Macrophomina phaseolina single-stranded RNA virus 1, KP900890	6,356	2	55.0	[11]
Penicillium aurantiogriseum fusarivirus 1, NC028467	6,139	2	37.9	[15]
Fusarium poae fusarivirus 1, NC030868	6,379	2	36.1	[16]
Fusarium graminearum virus-DK21, NC006937	6,621	4	35.6	[14]
Penicillium roquefortii ssRNA mycovirus 1, NC024699	6,002	2	35.7	GenBank
Pleospora typhicola fusarivirus 1, NC028470	6,733	2	34.6	[15]
Rosellinia necatrix fusarivirus 1, NC024485	6,286	2	34.1	[8]
Agaricus bisporus virus 11, KY357496	6,981	2	28.3	GenBank
Agaricus bisporus virus 10, KY357495	7,033	2	27.5	GenBank
Sclerotinia sclerotiorum fusarivirus 1, NC027208	7,769	4	26.4	[12]
Alternaria brassicicola fusarivirus 1, NC029056	6,656	3	26.3	[13]
Nigrospora oryzae fusarivirus 1, KU980909	7,018	2	25.4	[9]

case of the new virus reported in this study, no ORFs other than those shown in Fig. 1a were detected. As for other reported fusariviruses, homology searches failed to detect any viral coat protein domain. Since the family *Fusariviridae* has not been approved and ratified so far by the International Committee on Taxonomy of Viruses, there are no species or genus demarcation criteria defined for this family; however, considering the high divergence of the new virus reported here from other fusariviruses and other viral sequences in the GenBank database, we suggest that the virus infecting *N. luteum* NL37 should be considered a member of a novel species belonging to the proposed family *Fusariviridae*, for which the name of *Neofusicoccum luteum* fusarivirus 1 (NIFV1) is proposed.

The *N. luteum* NL37 strain in which NIFV1 was detected was found to be co-infected with a recently described mitovirus (NIMV1) [7], suggesting that mycoviruses are not rare in members of this *Botryosphaeriaceae* species. Questions remain about their effect on the host phenotype. Some of the putative fusariviruses characterized so far are thought to be latent. This is the case for *Rosellinia necatrix* fusarivirus 1, *Sclerotinia sclerotiorum* fusarivirus 1, and *Alternaria brassicicola* fusarivirus 1, infections with which have been shown to have no biological effect on their host [8, 12, 13]. Only one example of a hypovirulence-associated fusarivirus has been described so far: *Fusarium graminearum* virus DK-21, which has been shown to alter several virulence traits of infected *Fusarium graminearum* [14]. No biological data are available for the eight remaining putative

fusariviruses, which were characterized only molecularly [9, 11, 15, 16]. Concerning the *N. luteum* fusarivirus described here, the NL37 strain of *N. luteum* in which it was detected is multiply infected by at least a mitovirus [7], further complicating any efforts to associate a particular phenotype with the presence of a specific virus. Additional experiments are clearly needed to investigate the effect of viral infection on biological traits of the host, such as a comparative study using cured or virus-free fungal strains.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

Ethical approval This article does not contain any studies with animals or human participants performed by any of the authors.

References

1. Wunderlich N, Ash G, Steel C, Rahman H, Savocchia S (2010) *Botryosphaeriaceae* associated with bunch rot of grapes in South Eastern Australia. *Phytopathol Mediterr* 49:106
2. Yacoub A, Gerbore J, Magnin N, Chambon P, Dufour MC, Corio-Costet MF, Guyoneaud P, Rey P (2016) Ability of *Pythium oligandrum* strains to protect *Vitis vinifera* against *Phaeomoniliella chlamydospora*, a pathogen involved in Esca, by inducing plant resistance. *Biol Control* 92:7–16

3. Ghabrial SA, Castón JR, Jiang D, Nibert ML, Suzuki N (2015) 50-plus years of fungal viruses. *Virology* 479–480:356–368
4. Wang LP, Jiang JJ, Wang YF, Hong N, Zhang F, Xu WX, Wang GP (2014) Hypovirulence of the phytopathogenic fungus *Botryosphaeria dothidea*: association with a coinfecting Chrysovirus and a Partitivirus. *J Virol* 88:7517–7527
5. Zhai L, Xiang J, Zhang M, Fu M, Yang Z, Hong N, Wang G (2016) Characterization of a novel double-stranded RNA mycovirus conferring hypovirulence from the phytopathogenic fungus *Botryosphaeria dothidea*. *Virology* 493:75–85
6. Phillips AJL (2002) Botryosphaeria species associated with diseases of grapevines in Portugal. *Phytopathol Mediterr* 41:3–18
7. Marais A, Nivault A, Faure C, Theil S, Comont G, Candresse T, Corio-Costet MF (2017) Determination of the complete genomic sequence of *Neofusicoccum luteum* mitovirus 1 (NLMV1), a novel mitovirus associated with a phytopathogenic *Botryosphaeriaceae*. *Arch Virol* 162:2477–2480
8. Zhang R, Liu S, Chiba S, Kondo H, Kanematsu S, Suzuki N (2014) A novel single-stranded RNA virus isolated from a phytopathogenic filamentous fungus, *Rosellinia necatrix*, with similarity to hypo-like viruses. *Front Microbiol* 5:e360
9. Zhong J, Zhao SQ, Li GF, Pang XD, Deng XJ, Zhu HJ, Gao BD, Zhang Q (2016) A novel fusarivirus isolated from the phytopathogenic fungus *Nigrospora oryzae*. *Virus Genes* 52:891–895
10. Bellée A, Comont G, Nivault A, Abou-Mansour E, Coppin C, Dufour MC, Corio-Costet MF (2017) Life traits of four *Botryosphaeriaceae* species and molecular responses of different genotypes and cultivar of *Vitis vinifera*. *Plant Pathol* 66:763–776
11. Marzano SL, Nelson BD, Ajayi-Oyetunde O, Bradley CA, Hughes TJ, Hartman GL, Eastburn DM, Domier LL (2016) Identification of diverse mycoviruses through metatranscriptomics: characterization of the viromes of five major fungal plant pathogens. *J Virol* 90:6846–6863
12. Liu R, Cheng J, Fu Y, Jiang D, Xie J (2015) Molecular characterization of a novel positive-sense, single-stranded RNA mycovirus infecting the plant pathogenic fungus *Sclerotinia sclerotiorum*. *Viruses* 7:2470–2484
13. Zhong J, Shang HH, Zhu CX, Zhu JZ, Zhu HJ, Hua Y, Gao BD (2016) Characterization of a novel single-stranded RNA virus, closely related to fusariviruses, infecting the plant pathogenic fungus *Alternaria brassicicola*. *Virus Res* 217:1–7
14. Chu YM, Jeon JJ, Yea SJ, Kim YH, Yun SH, Lee YW, Kim KH (2002) Double-stranded RNA mycovirus from *Fusarium graminearum*. *Appl Environ Microbiol* 68:2529–2534
15. Nerva L, Ciuffo M, Vallino M, Margaria P, Varese GC, Gnani G, Turina M (2016) Multiple approaches for the detection and characterization of viral and plasmid symbionts from a collection of marine fungi. *Virus Res* 219:22–38
16. Osaki H, Sasaki A, Nomiya K, Tomioka K (2016) Multiple virus infection in a single strain of *Fusarium poae* shown by deep sequencing. *Virus Genes* 52:835–847