Molecular characterization of a novel Victorivirus (Gharbivirales: Totiviridae) infecting Metarhizium anisopliae

Matheus Silva Camargo (mtsc.camargo@gmail.com)
Centro de Biotecnologia da UFRGS https://orcid.org/0000-0002-8230-6702
Felipe Geremia
Centro de Biotecnologia da UFRGS
Nicolau Sbaraini
UWA: University of Western Australia
Charley Christian Staats
Centro de Biotecnologia da UFRGS
Márcio Silva Filho
USP ESALQ: Universidade de Sao Paulo Escola Superior de Agricultura Luiz de Queiroz
Augusto Schrank
Centro de Biotecnologia da UFRGS https://orcid.org/0000-0002-5248-4620

Research Article

Keywords:

Posted Date: December 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2369947/v1

Version of Record: A version of this preprint was published at Archives of Virology on February 9th, 2023.
See the published version at https://doi.org/10.1007/s00705-023-05716-7.

Abstract

In this study, we described the occurrence and complete genome of a novel Victorivirus infecting *Metarhizium anisopliae*, named "*Metarhizium anisopliae* victorivirus 1" (MaVV1). The genome is 5,353 bp in length and contains two ORFs, encoding a Coat Protein and an RNA-dependent RNA-polymerase (RdRp), which are overlapped in an octanucleotide (AUGAGUAA). Among characterized viruses, the identified ORFs showed similarity with *Ustilaginoidea virens RNA virus L* (68.23%) and *Ustilaginoidea virens RNA virus 1* (58.11%), both belonging to Totiviridae family. Phylogenetic analysis based on RdRp sequences revealed the MaVV1 placement in the Victorivirus genus. This is the first genome sequence report of a virus belonging to the genus Victorivirus infecting the entomopathogenic fungus *M. anisopliae*.

Full Text

The genus *Metarhizium* encompass cosmopolitan entomopathogenic fungi widely used to control pests of agricultural importance [1]. Besides the entomopathogenic life cycle, these species can also be found associated with plants, as endophytes; or in soil and litter, as saprophytes [2]. The biological control potential of the genus *Metarhizium* has been explored and has great relevance in countries where the agricultural economy is predominant, such as Brazil [1]. Notably, several isolates of *Metarhizium* harbor potential mycoviruses [3–5]. However, the function and importance of these viral segments in fungal biology are still unexplored.

Mostly of the knowledge about mycoviruses is derived from phytopathogenic-infecting viral sequences, due to the agricultural importance of the hosts. Moreover, several mycoviruses have been linked to hypovirulent-phenotypes [6, 7]. However, recently, virome approaches have also been applied to understand the diversity of viral sequences in entomopathogens, such as *Beauveria bassiana* [8, 9]. The potential interference of viruses on fungal development, sporulation, and virulence against arthropod-hosts are the main factors explored in infected strains [10, 11]. Although a high prevalence of viral sequences has been found in *Metarhizium* species, only three viral genomes are available to date. Notably, all available genomes belong to Partiviridae (dsRNA) family [12–14].

The family Totiviridae comprises five genera: Totivirus, Victorivirus, Leishmaniavirus, Trichomonasvirus, and Giardiavirus. The Victorivirus genus, which currently comprises 14 species, harbors filamentous-fungi-infecting viruses (International Committee on Taxonomy of Viruses - ICTV: https://ictv.global/taxonomy/ release 2021). The genome of Victorivirus members is characterized by one linear dsRNA segment (4 to 6 kbp), encoding a single protein RNA-dependent RNA polymerase (RdRp) and a coat protein (CP). Here we describe the genome of a new member of the family Totiviridae, from Victorivirus genus, found in *M. anisopliae* strain M5. This new victorivirus has been named *Metarhizium anisopliae* victorivirus 1 (MaVV1).

Metarhizium anisopliae strain M5 was isolated from an insect cadaver (*Deois* sp. - Homoptera, Cercopidae) in Pernambuco, Brazil and belongs to the Escola Superior de Agricultura Luiz de Queiroz (ESALQ-USP/Brazil) collection of entomopathogenic fungi [15]. The fungus was maintained in Cove's Complete Medium (MCc) agar plates for routine uses [16]. For dsRNA extraction, mycelium was cultivated in 50 mL liquid MCc (180 rpm; 28 °C; 72 hours) and cells were grinded to powder in mortar and pestle with liquid nitrogen. Nucleic acid extraction was performed employing the phenol/chloroform method [17] and dsRNA segments were precipitated with LiCl [18]. The samples were treated with DNAse and S1 Nuclease (Promega) for 1 hour at 37 ºC. To check dsRNA integrity, electrophoresis was performed in a 1.2% TAE agarose gel containing 0.5 µg/mL ethidium bromide (Figure 1A). The dsRNA samples were lyophilized and then sequenced on an Illumina NovaSeq Platform (Macrogen, Republic of Korea). Contigs were obtained by assembling using Q30-filtered raw data and SPAdes and were subsequently subjected to BLASTx platform against the non-redundant protein database at NCBI GenBank. A potential virus genome was identified as belonging to the Totiviridae family and considered for full description.

To determine both 5' and 3' untranslated regions (UTR), the RNA Ligase Mediated – Rapid Amplification of cDNA Ends (RLM-RACE) protocol was performed. Total dsRNAs were extracted from the mycelia as described above. Approximately 250 nanograms of dsRNA were used as template for synthesis of first-strand cDNA using an adapter and a complementary primer as described by Xie et al. with minor modifications [19]. DNA amplicons corresponding to the 5' and 3' terminus sequences were cloned into the pUC18 plasmid, transformed into 10β *Escherichia coli* cells, and sequenced using M13 primers, regions which are designed to flank the cDNA insert.

The full MaVV1 genome is 5,353 bp in length with 58.45% GC content. A motif search on the NCBI Conserved Domain Database (CDD) revealed that MaVV1 has a coat domain as well as a RdRp domain in the same single dsRNA segment (cl25797 – Totivirus Coat protein, and pfam02123 – viral RNA-directed RNA-polymerase) (Figure 1B). The ORFs were identified using the NCBI ORFfinder web software and were subjected to BLASTx (NCBI) to detect sequence similarities. ORF1, which encodes a coat protein (2,274 bp) has 68,23% identity to a coat protein from *Ustilaginoidea virens* RNA virus L. ORF2, which encodes a RdRp (2,520 bp) has 58.11% identity to a RdRp protein from *Ustilaginoidea virens* RNA virus 1. Overlapping the two ORFs an unusual octanucleotide (***AUG***AG**UAA**) containing both the ORF2 start codon (***AUG***) and ORF1 stop codon (**UAA**) was observed (Figure 1C). The genome is completed by the 5' and 3' UTR regions corresponding to 507 and 60 bp, respectively. MaVV1 complete genome sequence was deposited in the GenBank database (accession number OP959068 - temporary).

Phylogenetic analyses were performed to clarify the taxonomic placement of MaVV1. Sequences employed by Khalifa and MacDiarmid as well as sequences from the GenBank/NCBI database were included [20]. Multiple sequence alignments of RdRp amino acid sequences were generated using THE GUIDANCE2 SERVER employing MAFFT algorithm [21]. The phylogenetic tree (Figure 2) was constructed using the PhyML (Maximum likelihood) platform [22] and employing the best substitution model (Q.pfam +R+F) predicted by SMS [23]. Branches lower than 0.8 SH-like branch support were collapsed using TreeGraph 2 software.

These results support previous work in which the taxonomy of viral particles present in the *Metarhizium anisopliae* M5 strain was inferred from protein and structural characteristics [3]. Therefore, we propose MaVV1 to be a new member of the genus Victorivirus (Gharbivirales: Totiviridae) based on phylogenetic analysis and RdRp sequence comparisons. This Victorivirus was designated as "*Metarhizium anisopliae* victorivirus 1".

Declarations

Data availability. The genomic sequence determined in this study was submitted to the GenBank under the accession number OP959068 (temporary).

Funding. This work was supported by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) protocol no 41172.541.29362.15072019. And the scholarship support by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Conflict of interest. The authors have no conflict of interest.

References

1. Iwanicki NS, Pereira AA, Botelho ABRZ, et al (2019) Monitoring of the field application of Metarhizium anisopliae in Brazil revealed high molecular diversity of Metarhizium spp in insects, soil and sugarcane roots. Sci Rep 9:4443. https://doi.org/10.1038/s41598-019-38594-8
2. Stone LBL, Bidochka MJ (2020) The multifunctional lifestyles of Metarhizium: evolution and applications. Appl. Microbiol. Biotechnol.
3. De la Paz Giménez-Pecci M, Bogo MR, Santi L, et al (2002) Characterization of mycoviruses and analyses of chitinase secretion in the biocontrol fungus Metarhizium anisopliae. Curr Microbiol. https://doi.org/10.1007/s00284-002-3678-9
4. Santos V, Mascarin GM, da Silva Lopes M, et al (2017) Identification of double-stranded RNA viruses in Brazilian strains of Metarhizium anisopliae and their effects on fungal biology and virulence. Plant Gene. https://doi.org/10.1016/j.plgene.2017.01.001
5. Martins MK, Furlaneto MC, Sosa-Gomez DR, et al (1999) Double-stranded RNA in the entomopathogenic fungus Metarhizium flavoviride. Curr Genet. https://doi.org/10.1007/s002940050477
6. Sasaki A, Nakamura H, Suzuki N, Kanematsu S (2016) Characterization of a new megabirnavirus that confers hypovirulence with the aid of a co-infecting partitivirus to the host fungus, Rosellinia necatrix. Virus Res 219:73–82. https://doi.org/10.1016/j.virusres.2015.12.009
7. Zhong J, Chen D, Zhu HJ, et al (2016) Hypovirulence of Sclerotium rolfsii caused by associated RNA mycovirus. Front Microbiol. https://doi.org/10.3389/fmicb.2016.01798

8. Filippou C, Garrido-Jurado I, Meyling N V., et al (2018) Mycoviral population dynamics in Spanish isolates of the entomopathogenic fungus beauveria bassiana. Viruses. https://doi.org/10.3390/v10120665
9. Kotta-Loizou I, Coutts RHA (2017) Studies on the Virome of the Entomopathogenic Fungus Beauveria bassiana Reveal Novel dsRNA Elements and Mild Hypervirulence. PLoS Pathog. https://doi.org/10.1371/journal.ppat.1006183
10. Filippou C, Diss RM, Daudu JO, et al (2021) The Polymycovirus-Mediated Growth Enhancement of the Entomopathogenic Fungus Beauveria bassiana Is Dependent on Carbon and Nitrogen Metabolism. Front Microbiol. https://doi.org/10.3389/fmicb.2021.606366
11. Shah UA, Kotta-Loizou I, Fitt BDL, Coutts RHA (2019) Identification, molecular characterization, and biology of a novel quadrivirus infecting the phytopathogenic fungus leptosphaeria biglobosa. Viruses. https://doi.org/10.3390/v11010009
12. Wang P, Yang G, Shi N, Huang B (2020) Molecular characterization of a new partitivirus, MbPV1, isolated from the entomopathogenic fungus Metarhizium brunneum in China. Arch Virol. https://doi.org/10.1007/s00705-019-04517-1
13. Wang P, Yang G, Shi N, Huang B (2021) A novel gammapartitivirus from the entomopathogenic fungus Metarhizium brunneum. Arch Virol. https://doi.org/10.1007/s00705-020-04939-2
14. Wang P, Yang G, Shi N, Huang B (2022) Molecular characterization of a novel double-stranded RNA virus infecting the entomopathogenic fungus Metarhizium brunneum. Arch Microbiol 204:606. https://doi.org/10.1007/s00203-022-03224-x
15. Yanaka-Schäfer FY, Dall'Onder LP, Panichi MC, et al (2008) Sequence analysis of the rDNA intergenic spacer of Metarhizium strains isolated in Brazil. Genet Mol Biol 31:116–121. https://doi.org/10.1590/S1415-47572008000100020
16. Sbaraini N, Bellini R, Penteriche AB, et al (2019) Genome-wide DNA methylation analysis of Metarhizium anisopliae during tick mimicked infection condition. BMC Genomics 20:836. https://doi.org/10.1186/s12864-019-6220-1
17. Evans GA (1990) Molecular cloning: A laboratory manual. Second edition. Volumes 1, 2, and 3. Current protocols in molecular biology. Volumes 1 and 2. Cell. https://doi.org/10.1016/0092-8674(90)90210-6
18. Diaz-Ruiz JR, Kaper JM (1978) Isolation Of Viral Double-Stranded Rnas Using Alicl Fractionation Procedure. Prep Biochem. https://doi.org/10.1080/00327487808068215
19. Xie J, Xiao X, Fu Y, et al (2011) A novel mycovirus closely related to hypoviruses that infects the plant pathogenic fungus Sclerotinia sclerotiorum. Virology 418:49–56. https://doi.org/10.1016/j.virol.2011.07.008
20. Khalifa ME, MacDiarmid RM (2019) A Novel Totivirus Naturally Occurring in Two Different Fungal Genera. Front Microbiol 10:. https://doi.org/10.3389/fmicb.2019.02318
21. Katoh K (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436

22. Guindon S, Dufayard JF, Lefort V, et al (2010) New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Syst Biol 59:307–321. https://doi.org/10.1093/sysbio/syq010
23. Lefort V, Longueville J-E, Gascuel O (2017) SMS: Smart Model Selection in PhyML. Mol Biol Evol 34:2422–2424. https://doi.org/10.1093/molbev/msx149

Figures

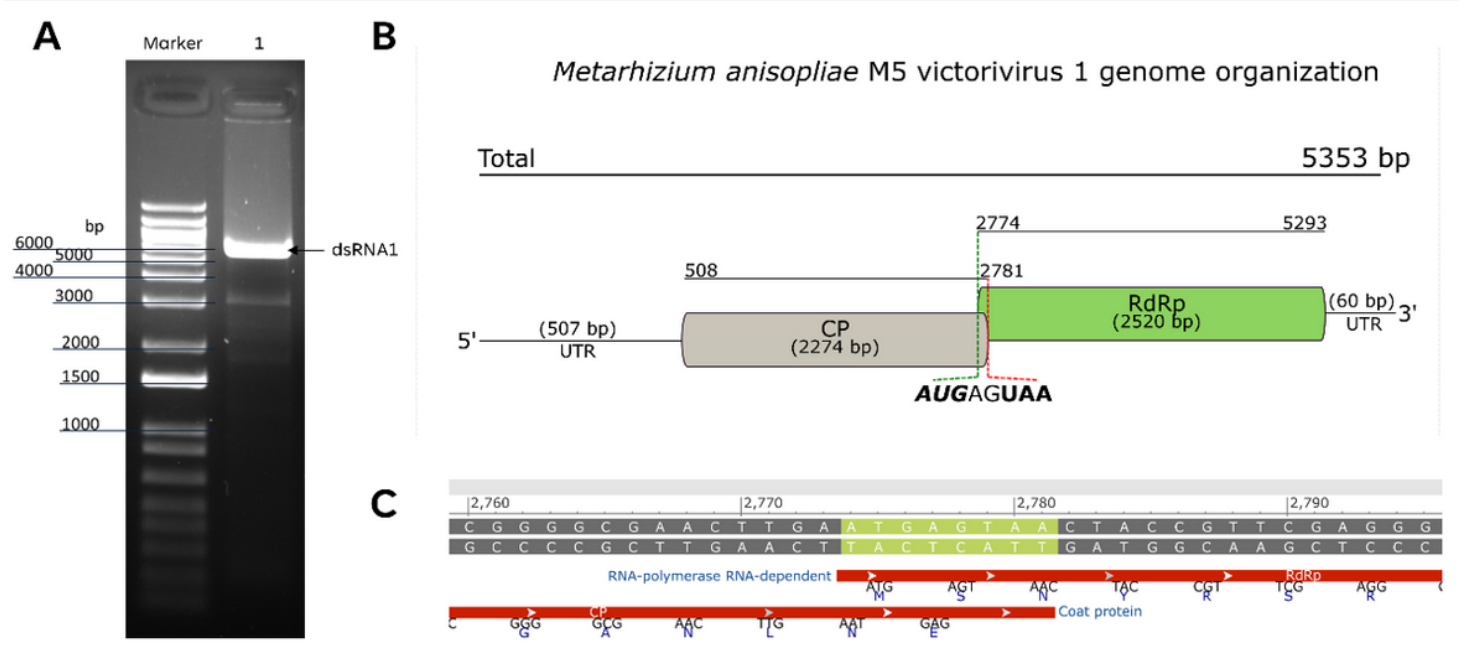

Figure 1

Schematic representation of the genomic organization of MaVV1. A) Pattern of the *M. anisopliae* strain M5 dsRNAs in agarose gel (Marker - Invitrogen 1 kb Plus DNA molecular weight; 1 - DNAse and S1 nuclease treated dsRNAs). B) MaVV1 genome representation, the genome is 5,353 bp in length and contains two ORFs which encodes a coat protein (CP) and RNA-dependent RNA polymerase (RdRp), respectively. The black lines indicate the 5' and 3'-UTR regions determined by RLM-RACE. C) An octanucleotide overlap between the two ORFs was highlighted in green.

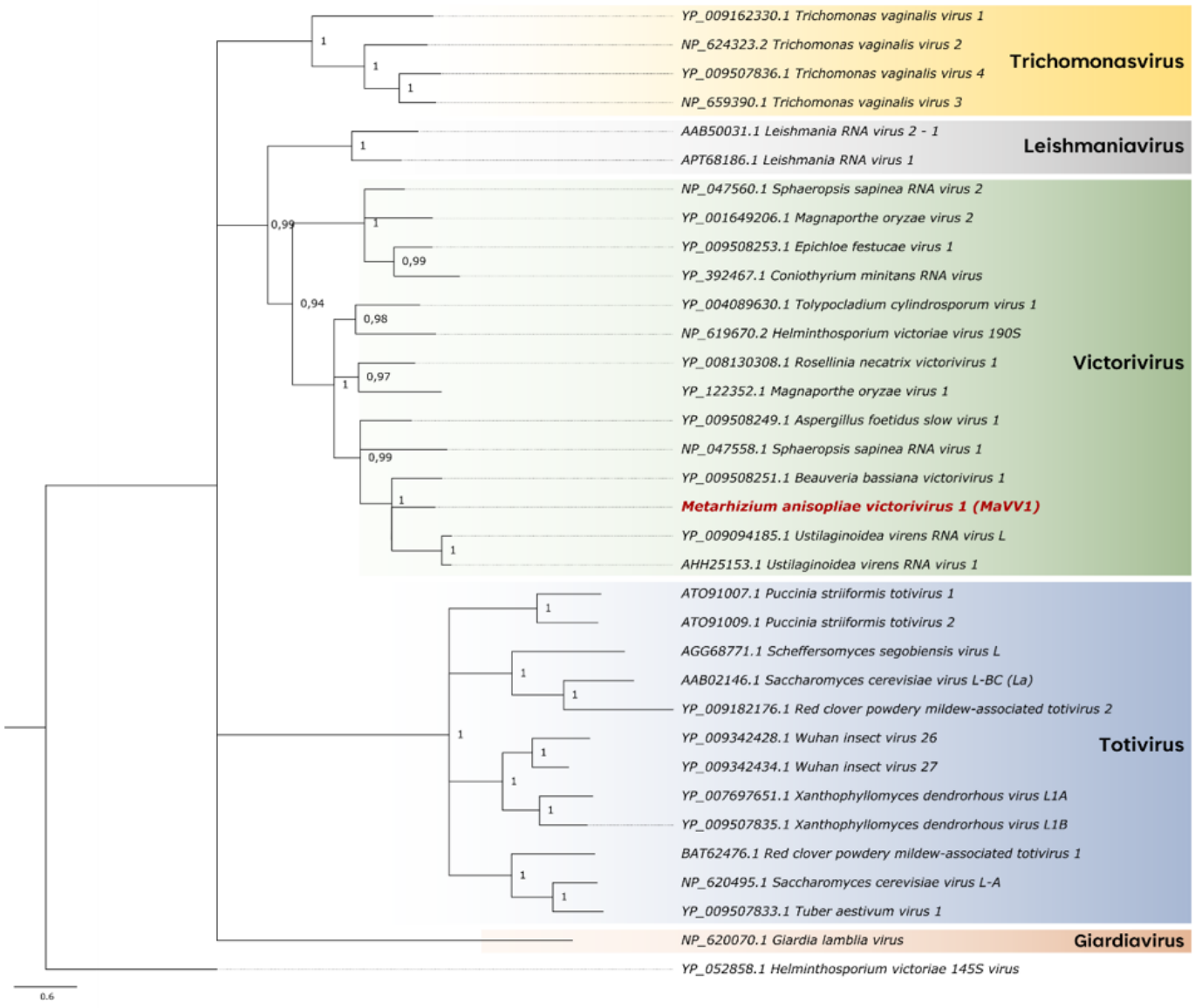

Figure 2

Phylogenetic placement of *Metarhizium anisopliae* victorivirus 1 (MaVV1 – in bold/red) among the five genera comprised into the Totiviridae family. RdRp sequences were aligned using MAFFT algorithm and the phylogenetic reconstruction was conducted with PhyML (Maximum Likelihood, Q.pfam +R+F as the substitution model) with aLRT SH-like branch support. *Helminthosporium victoriae* 145S virus (Chrysoviridae: Chrysovirus) was used as the outgroup.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- MaVV1MetarhiziumanisopliaeM5Victorivirus.fasta