Complete genome sequence of a novel mycovirus from Pleurotus citrinopileatus

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Abstract

The complete genome sequence of a novel single-stranded [+ssRNA] positive-sense (+) RNA mycovirus, designated as "Pleurotus citrinopileatus ourmiavirus 1" (PcOV1), isolated from the *Pleurotus citrinopileatus* strain CCMJ2141, was determined. The complete genome of PcOV1 is composed of 2,535 nucleotides long. It contains a single open reading frame (ORF), which encodes a protein of 657 amino acids (aa) showing conserved domains of RNA-dependent RNA polymerase (RdRp). Phylogenetic analysis based on the RdRp revealed that PcOV1 is a new member of the genus *ourmiavirus* in the family *Botourmiaviridae*. This is the first virus characterized in *P. citrinopileatus*.

Introduction

Pleurotus citrinopileatus, commonly known as golden oyster mushroom, is a basidiomycete fungus of the family *Pleurotaceae* and is native to Japan, northern China, and eastern Russia [1,2]. The genus *Pleurotus* are medicinal, ecologically, and economically critical edible fungi widely cultivated in China [2-4]. *P. citrinopileatus* is well-liked in Asian nations because of its great nutritious content, distinctive flavor, bright yellow color, and anti-cholesterol and anti-diabetic properties [5]. *Pleurotus* species can be affected by many pathogens, including fungi, bacteria, and viruses, which can cause serious diseases [6-8].

All major taxonomic groups of filamentous fungus can be infected by mycoviruses [9-12]. Mycovirus infections are usually latent and do not manifest symptoms in the fungal host. However, several mycoviral infections can induce severe symptoms resulting in economic losses [13,14]. Major symptoms observed include delayed fruiting body formation, shortening in the stipe, thin mushroom caps, abnormal shape, low density, and slow hyphal growth on agar medium [15-17]. Mycoviruses have single-stranded (ss), double-stranded (ds), or DNA-based genomes [18–21]. Mycoviruses with positive-sense single-stranded genomes are classified into thirteen major families. The International Classification Committee on Taxonomy of Viruses (ICTV) recognized the family *Botourmiaviridae* in 2018 and classified it as a new branch of the linear positive-sense (+) single-strand RNA viruses [22]. The family included only plant viruses and was only reclassified after discovering numerous ourmiavirus-like viruses in fungi, and new virus genera were added to it. Mycoviruses in the family *Botourmiaviridae* are currently subdivided into 12 genera [23].

Mycoviruses have been reported to infect *Pleurotus* species. Some viruses reported include oyster mushroom spherical virus (OMSV) [24], Oyster Mushroom Isometric Virus (OMIV) [25,26], Pleurotus ostreatus Virus strain Shin-Nong (PoV-SN) [27], Pleurotus ostreatus Virus 1(PoV1) [28], Pleurotus ostreatus Spherical Virus (PoSV) [29], and Pleurotus eryngii Spherical Virus (PeSV) [30]. However, before this report, no viruses had been described in *P. citrinopileatus*. In this study, we characterized a complete sequence of a novel (+) ssRNA mycovirus isolated from *P. citrinopileatus*. The virus was designated as "Pleurotus citrinopileatus ourmiavirus 1" (PcOV1) and is related to members of the genus *Ourmiavirus* in the family *Botourmiaviridae*.

Provenance And Sequencing Of Strains

Twenty-four *P. citrinopileatus* strains were collected from the Herbarium of the Mycological Institute of the Jilin Agricultural University, China. The strains were maintained on potato dextrose agar (PDA) slants at 4°C. Mycelia for total RNA extraction were inoculated onto PDA plates covered with cellophane sheets and incubated in the darkness at 25°C for 10 days. Total RNAs were extracted from approximately 0.2 g of harvested mycelia using RNA simple Total RNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. The total RNA extracted was quantified using NanoDrop 2000 spectrophotometer (Thermo Scientific, Madison, WI, USA) and separated on a 1% gel electrophoresis in 1x TAE buffer for 20 min at 125 V. The RNA from each strain was pooled into a single tube, and rRNA was depleted using Ribo-ZeroTM rRNA Removal Kit (Epicentre, Madison, WI, USA). The rRNA-depleted total RNA (1200 ng/μL) was used as a template to synthesize cDNA. The cDNA library was constructed using Illumina TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA). The quality of the library was estimated using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). The library was sequenced on an Illumina NovaSeq 6000 (Illumina, USA) paired-end reads 150-bp (PE150) system at the Novogene Bioinformatics Technology Co., Ltd. (Beijing, China).

A total of 78,860,122 raw reads were generated, and 77,919,412 (97.57%) clean reads were obtained after quality filtering. The clean reads were de novo assembled into 38745 contigs using Trinity [31] and subjected to BlastX analyses against the NCBI GenBank non-redundant protein database (https://www.ncbi.nlm.nih.gov). One contig (contig_17942: 1902 nt length) showed the highest sequence similarity (40.23% identity with 66% query coverage) to Heterobasidion ourmia-like virus 1 (UHK02571.1). The total RNA of each strain was subjected to reverse transcription (Moloney murine leukemia virus (M-MLV) reverse transcriptase; TaKaRa, Dalian, China) and cDNA synthesis using random hexanucleotide primers (dN6 primers: 5'-CGATCGATCATGATGCAATGCNNNNNNN-3'). Specific primers (Table S1) were designed based on contig_17942 to identify the putative viral sequence to obtain the target strain. Strain CCMJ2141 was confirmed to harbor the putative viral sequence.

To determine the complete viral genome, cDNA amplification of the 5' and 3' ends were performed using the rapid amplification of cDNA ends (RACE) method with multiple sets of overlapping primers in nested PCR according to target contig 17942 (Table S1) [32] using the rapid amplification of cDNA ends (RACE) using a high-efficiency DNA gel recovery kit (DingGuo, Beijing, China), cloned into the pMD18-T vector (TaKaRa, Dalian, China), and transformed in *Escherichia coli* strain DH5α. Recombinant plasmids were extracted, amplified with M13 universal primers from individual colonies, and sequenced in both directions by the Sanger method at Sangon Biotech (Shanghai) Co., Ltd. for sequencing. At least three independent clonal inserts of each fragment were sequenced to reduce experimental error. The viral fragment sequences were assembled into contiguous sequences based on common regions that overlap using DNAMAN 9.0 software (version 8.0; Lynnon Biosoft, Quebec, Canada).

A BLAST search was conducted using the full-length cDNA sequence against the NCBI GenBank database. Open reading frames (ORFs) were predicted using the NCBI ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). Putative conserved protein domains were searched using the

NCBI Conserved Domain tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and the GenomeNet tool MOTIF (https://www.genome.jp/tools/motif/). The protein domain structure was constructed using IBS1.0.3. software.

The ClustalX program was used to make multiple sequence alignments of the RdRp amino acid sequences [33]. Phylogenetic analysis was carried out using the maximum-likelihood (ML) method using PhyML3.0 software [34, 35]. MEGA-X software [36] was used to visualize and edited the phylogenetic tree using. To confirm the presence of assembled viral contig, primer pairs Ple1900F/Ple1900S were designed to cover the genomic region. RT-PCR was performed, and PCR products were assessed using gel electrophoresis and sequenced at Sangon Biotech (Shanghai) Co., Ltd.

Sequence Properties

Metatranscriptome and RACE-PCR analysis results showed that *P. citrinopileatus* strain CCMJ2141 was infected with a novel mycovirus named "Pleurotus citrinopileatus ourmiavirus 1" (PcOV1). The complete genome sequence of PcOV1 is 2535 base pairs (bp) long. The GC content is 51%, with a base composition of 24.1% A, 20.9% C, 30.1% G, and 24.9% U, respectively. The 5' and 3'-untranslated regions (UTRs) of the mycovirus were 233 and 35 nt in length, respectively. PcOV1 genome contains one ORF of 1974 nt in length, starting at 223 nt and terminating at 2500 nt. The ORF potentially encodes a protein of 657 amino acids (aa) in length with a predicted molecular mass of 7.54 kDa (isoelectric point of 4.67). It has conserved motifs characteristic of an RNA-dependent RNA polymerase (RdRp) (Fig. 1). The full-length nucleotide sequence of PcOV1 was deposited in the GenBank database and assigned the accession number MW772941.

A Blastp search of PcOV1 RdRp protein showed 42.42% identity to Armillaria mellea ourmia-like virus 2 (66% query coverage) and 41.04% identity to Heterobasidion ourmia-like virus 1 (57% query coverage). Multiple sequence alignments and MOTIF search of PcOV1 RdRp amino acid sequences with representative viruses of the family *Botourmiaviridae* (Table S2) revealed six highly conserved motifs (Fig. S1). A maximum-likelihood phylogenetic tree was constructed by aligning the RdRP amino acid sequences of PcOV1 and some representative viruses in the five genera of the family *Botourmiaviridae*, as well as some viruses from the family *Narnaviridae* [35, 36] (Fig. 2). The phylogram showed that PcOV1 clustered together with members of the genus *ourmiavirus* and separate from other genera of the family *Botourmiaviridae*. The findings suggest that the + ssRNA mycovirus (PcOV1) isolated from *P. citrinopileatus* is a new member of the genus *ourmiavirus* in the family *Botourmiaviridae*.

Declarations

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Figures

Figure 1

Schematic diagram of the genomic structure of the PcOVL1 virus. The gray box represents an open reading frame (ORF) in which the conserved sequence of RdRp is located. The conserved sequences of the RdRp domain in ORF are shown.
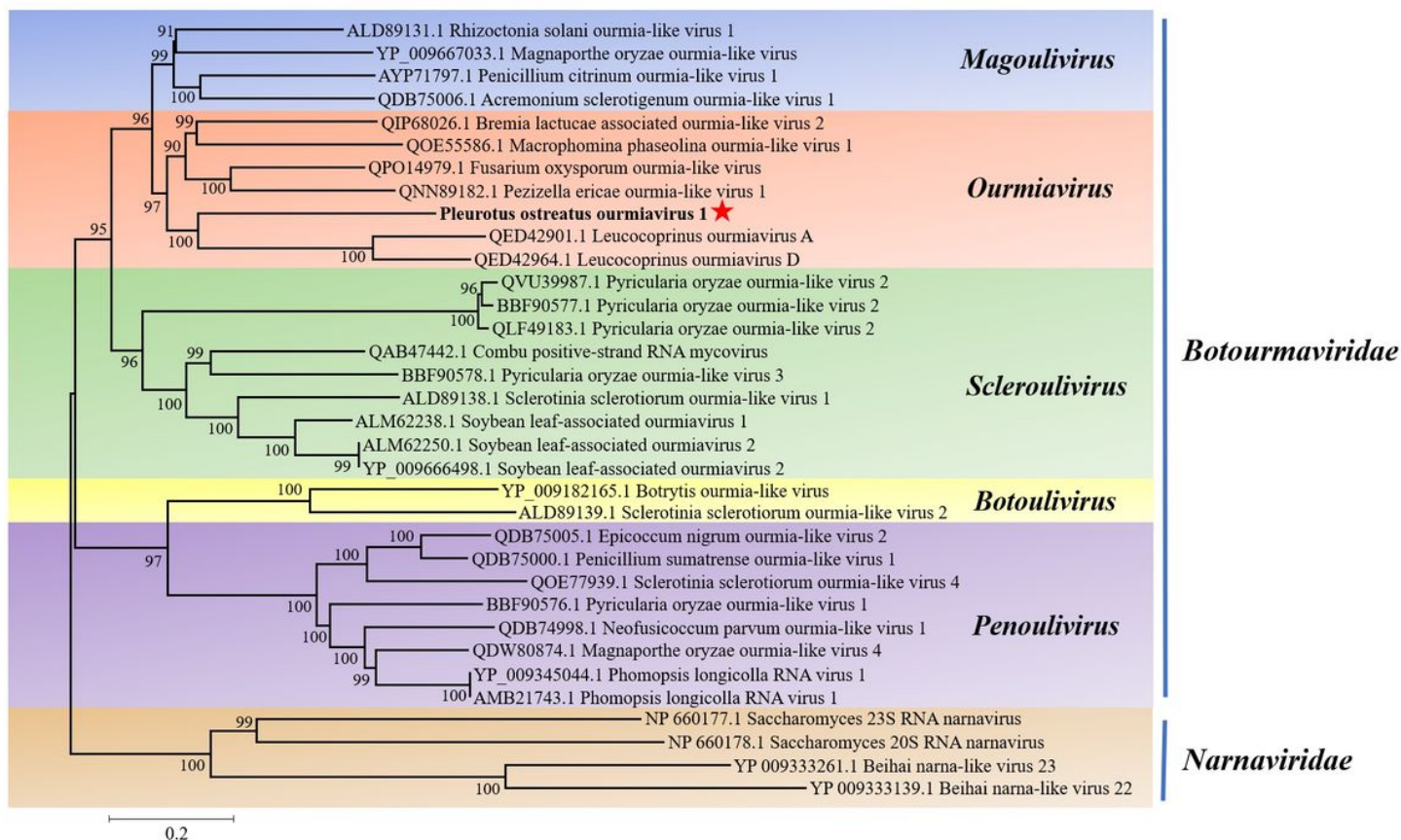
Figure 2

A phylogenetic tree was constructed using the maximum likelihood method based on the conserved RdRp amino acid motif of PcOV1 and the conserved RdRp amino acid sequences from some viruses in five genera of the family *Botourmiaviridae*. Based on PhyML 3.0, the phylogenetic tree was constructed using the maximum likelihood method of 1000 bootstraps. Bootstrap support value > 60% is displayed next to the branch, and the branch length ratio represents the amino acid change number of each node. The scale bar indicated the genetic distance of branch length.

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