



Complete Genome Sequence of a Little Cherry Virus-2 Isolate from Sweet Cherry in China

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ABSTRACT The first complete genome sequence of a little cherry virus-2 (LChV-2-TA) isolate from China was determined using small RNA deep sequencing combined with overlapping reverse transcriptase PCR (RT-PCR). Phylogenetic analysis revealed that LChV-2-TA grouped in a well-supported cluster with members of the genus *Ampelovirus* with close relationships to previously reported LChV-2 isolates.

The most important sweet cherry (*Prunus avium* L.) production area in China is in the Shandong Province, and the trees are susceptible to several diseases. One of these diseases, little cherry disease (LChD), is caused by two members of the family *Closteroviridae*, little cherry virus-1 (genus *Velarivirus*) and little cherry virus-2 (LChV-2; genus *Ampelovirus*); both affect the yield and quality of sweet cherry crops worldwide (1–11).

LChV-2 was reported from flowering cherry in China in 2011 (8); however, only a partial nucleotide sequence was obtained. In this study, the complete nucleotide sequence of LChV-2-TA, isolated from an infected sweet cherry tree exhibiting small fruit symptoms (*P. avium* L. cv. Hongdeng) in Taian, Shandong Province, China, was determined. A small RNA deep-sequencing experiment was performed by the CapitalBio Corporation (Beijing, China) (5). Total RNAs were isolated from young leaves of the infected tree using the PureLink plant RNA reagent (Ambion, Austin, TX, USA). A small RNA library was constructed using a TruSeq small RNA sample prep kit (Illumina, San Diego, CA, USA). The library was size selected using 6% polyacrylamide gels (Invitrogen, Carlsbad, CA, USA) and sequenced on an Illumina HiSeq 2000 instrument. The raw data generated 20,648,778 reads with an average read length of 50 nucleotides (nt). Trimming of adapters and removal of low-quality reads using the CLC Genomics Workbench 7.5 software (CLC Bio, Aarhus, Denmark) resulted in 20,157,991 reads (average length, 23.4 nt) for nucleotide sequence assembly; 204 contigs longer than 100 nt were assembled. The screening conditions were very strict (length fraction, 0.94; similarity fraction, 1.0). BLASTn searches of the assembled contigs resulted in detection of cherry virus A (4 contigs), *Prunus necrotic ringspot virus* (9 contigs), and LChV-2 (1 contig). Overlapping reverse transcriptase PCR (RT-PCR) and rapid amplification of cDNA ends (RACE) PCR using the SMARTer RACE cDNA amplification kit (Clontech, Mountain View, CA) were conducted to amplify the full genomic sequence of LChV-2-TA. Briefly, sequences of the 5'- and 3'-terminal regions were amplified using a RACE kit (TaKaRa, Dalian, China), primers LChV2-5' R-O (5'-AACTTATGAGACAACACATCTTCCTT-3') and LChV2-5' R-I (5'-GGGAAGCAAAGAAATTCGCCAGA-3') were used for 5' RACE, and LChV2-3' R-O (5'-CGAGATACCGAGTCTAACGAGGAGAA-3') and LChV2-3' R-I (5'-GTCAAAGTATGGAAGCAGTAGACGGG-3') were used for 3' RACE. All amplified sequences were cloned into the pEASY-T1 vector (Transgen Biotech, Beijing, China) and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). The sequences were then assembled using the software Vector NTI Advance 10.3 (Invitrogen).

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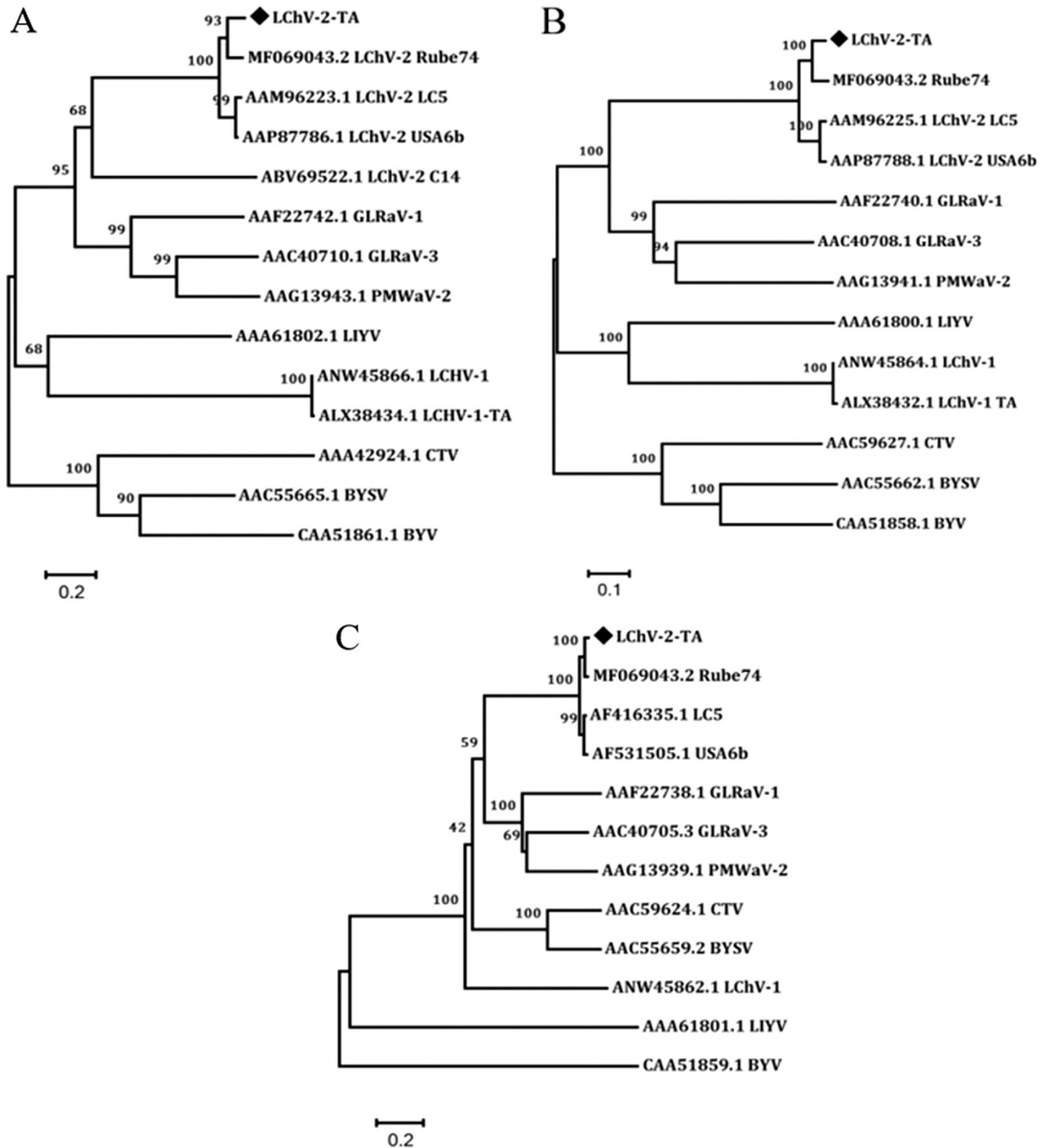


FIG 1 Unrooted phylogenetic trees showing relationships among the coat protein (CP) (A), heat shock protein 70 (HSP70)-like (B), and RNA-dependent RNA polymerase (RdRp) (C) nucleotide sequences of several members of the *Closteroviridae*. Phylogenetic trees were generated using MEGA 6.0 software using the neighbor-joining method with strict nucleotide identity distances, and the statistical significance of branches was evaluated using bootstrap analysis (1,000 replicates). Only bootstrap values of >40% are indicated. Branch lengths on the phylogenetic trees represent genetic distance. The LChV-2-TA isolate is indicated by a black diamond. The accession numbers of the sequences retrieved from GenBank are indicated, together with the isolate names. LIYV, lettuce infectious yellows virus; LChV-1, little cherry virus-1; GLRaV-1, grapevine leafroll associated virus-1; LChV-2, little cherry virus-2; BYSV, beet yellow stunt virus; BYV, beet yellows virus; CTV, citrus tristeza virus; PMWaV-2, pineapple mealybug wilt-associated virus.

The complete genome of LChV-2 is 15,031 nt long and encodes 11 predicted proteins, similar to other annotated LChV-2 isolates. The first open reading frame (ORF), ORF0 at the 5' end, is in the negative sense (nucleotides 4 to 257) and encodes a predicted 18.1-kDa protein. This is followed by a 182-kDa protein (ORF1a; nucleotides 539 to 5461) that contains amino acid (aa) motifs characteristic of methyltransferase and helicase domains (3) and a replicase (RdRp; ORF1b; 54.3 kDa) with a predicted GUG start codon at nucleotide 5565, located 103 nt downstream of the ORF1a UAA stop

codon. The predicted RdRP ORF is truncated compared to the LChV-2-USA6b (GenBank accession no. [AF531505](#)) and LChV-2-LC5 (GenBank accession no. [AF416335](#)) isolates, which suggests that it is translated via a +1 frameshift from a region upstream in the genome.

ORF2 encodes a protein of 9.26 kDa with a putative GUG initiation codon immediately following the ORF1b UAA stop codon. ORF3 encodes a 55.4-kDa coat protein (CP) duplicate. The remaining ORFs, 4 through 9, encode a putative 6.2-kDa membrane protein, a protein containing a nucleotide-binding domain characteristic of the HSP70 family, a viral HSP90, a 21.6-kDa hypothetical protein, a 39.0-kDa CP that showed the highest identity to the corresponding protein in the other isolates, and a putative 226-aa polypeptide, respectively.

Comparison to the nearly full-length genomes of three previously reported LChV-2 isolates conducted using BLAST (<https://blast.ncbi.nlm.nih.gov>) revealed that LChV-2-TA shares nucleotide sequence identities of 84%, 77.61%, and 63.62%, respectively, with LChV-2 isolates Rube 74 (Czech Republic; GenBank accession no. [MF069043](#)), USA6b (United States; GenBank accession no. [AF531505](#)), and LC5 (Canada; GenBank accession no. [AF416335](#)). Phylogenetic trees based on pairwise comparisons of CP, HSP70, and RdRP amino acids between LChV-2 and members of the *Closteroviridae* revealed that LChV-2-TA formed a distinct branch with the Rube 74, USA6b, and LC5 isolates (Fig. 1). Interestingly, LChV-2-TA and the Rube74 isolate from the Czech Republic are in a branch separate from that of the North American isolates LC5 and USA6b, suggesting a common evolutionary history, a recombination event, or movement of germplasm. To our knowledge, this is the first report of the complete nucleotide sequence of LChV-2 infecting sweet cherry in China.

In conclusion, our results warrant investigations of the incidence of the LChV2-TA isolate in the sweet cherry-growing regions of China and further studies of the molecular implications of predicted genomic differences for the expression of viral proteins.

Data availability. The complete genome sequence of the LChV-2 isolate was deposited in GenBank under accession no. [MG881767](#). Raw sequencing reads were deposited in NCBI under accession no. [PRJNA559006](#).

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