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ANNOTATED SEQUENCE RECORD

Complete genome sequence of lettuce chordovirus 1 isolated from cultivated lettuce in France

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Abstract

Double-stranded RNAs purified from cultivated (*Lactuca sativa*) or wild (*L. serriola*) lettuce from southwest France were analyzed by high-throughput sequencing. For both samples, BLAST annotation revealed contigs with homology to *Betaflexiviridae* family members. The full genome sequence of the isolate from cultivated lettuce (JG1) was completed (8,536 nucleotides [nt], excluding the poly(A) tail). The sequence of the 3' half of the genome (4,800 nt) of a wild lettuce isolate (P22) was determined and found to share 95.1% nt sequence identity with the JG1 isolate. The JG1 genome contains four open reading frames, encoding a replicase, a movement protein, a capsid protein, and a protein of unknown function, respectively. Based on genome organization and phylogenetic relationships, the lettuce virus is most closely related to the recently described carrot chordoviruses 1 and 2 in the family *Betaflexiviridae*. Considering the species demarcation criteria in this family, the two lettuce viruses represent isolates of a new chordovirus species for which the name "lettuce chordovirus 1" (LeCV1) is proposed.

Members of the family *Betaflexiviridae* have flexuous particles and a single-stranded, polyadenylated RNA genome of 6 to 9.5 kb [2, 3]. The family comprises eleven genera in two subfamilies, *Quinvirinae* and *Trivirinae* [3]. Members have three to six genes, the largest of which encodes a replicase (Rep) at the 5' end of the genome. The Rep gene is followed by a single gene encoding a 30K-like [5] movement protein (MP, *Trivirinae*) or a triple gene-block [6] module (*Quinvirinae*). This is followed by the capsid protein (CP) gene and, in some *Betaflexiviridae* members, an additional gene that encodes a putative nucleic-acid-binding protein [2, 3].

The JG1 isolate was obtained from a lettuce plant (*L. sativa* var. Shanghor) with mosaic symptoms collected in southwest France in November 2014. The P22 isolate was obtained from an asymptomatic wild lettuce plant (*L. serriola*) collected in spring 2010 during a plant virus metagenomic survey. To determine what viruses might be present in the samples, double-stranded RNAs (dsRNAs) were purified as described previously [4]. Purified dsRNAs were amplified [4] and sequenced by 454 (P22) or Illumina HighSeq2000 (JG1, as 2×250 paired reads). Reads were demultiplexed and assembled [4], and contigs were annotated by BLASTn and BLASTx against the GenBank database using CLC Genomics workbench. In both lettuce samples, contigs with similarities to members of the families *Betaflexiviridae* and *Secoviridae* were observed, suggesting mixed infections. The largest *Betaflexiviridae* contig (4.9 kb, coverage 357x) found for isolate JG1, encoded a protein with 54% amino acid (aa) sequence identity with carrot chordovirus 1 (CtCV1, NC025469) [1]. Further assembly efforts yielded a scaffold covering most of the genome. Specific primers and Sanger sequencing of the PCR products were used to obtain the

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The nucleotide sequences reported in this work have been deposited in the GenBank database under accession numbers MG208123-24.

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Fig. 1 Genome organization of lettuce chordovirus 1. REP, replicase gene; MP, movement protein gene; CP, capsid protein gene; $A_{(n)}$, poly(A) 3' tail. The methyltransferase (Met), AlkB, helicase (Hel) and polymerase (RdRp) domains of REP are shaded with dots

genome ends by 5' RACE (Takara) and a polyT-anchored RT-PCR, respectively. Four *Betaflexiviridae* contigs were assembled in a scaffold spanning the 3' genome half of isolate P22. Sanger sequencing of PCR products obtained using pecific primers were used to close internal gaps and to sequence the 3' end of the genome. The complete (JG1)

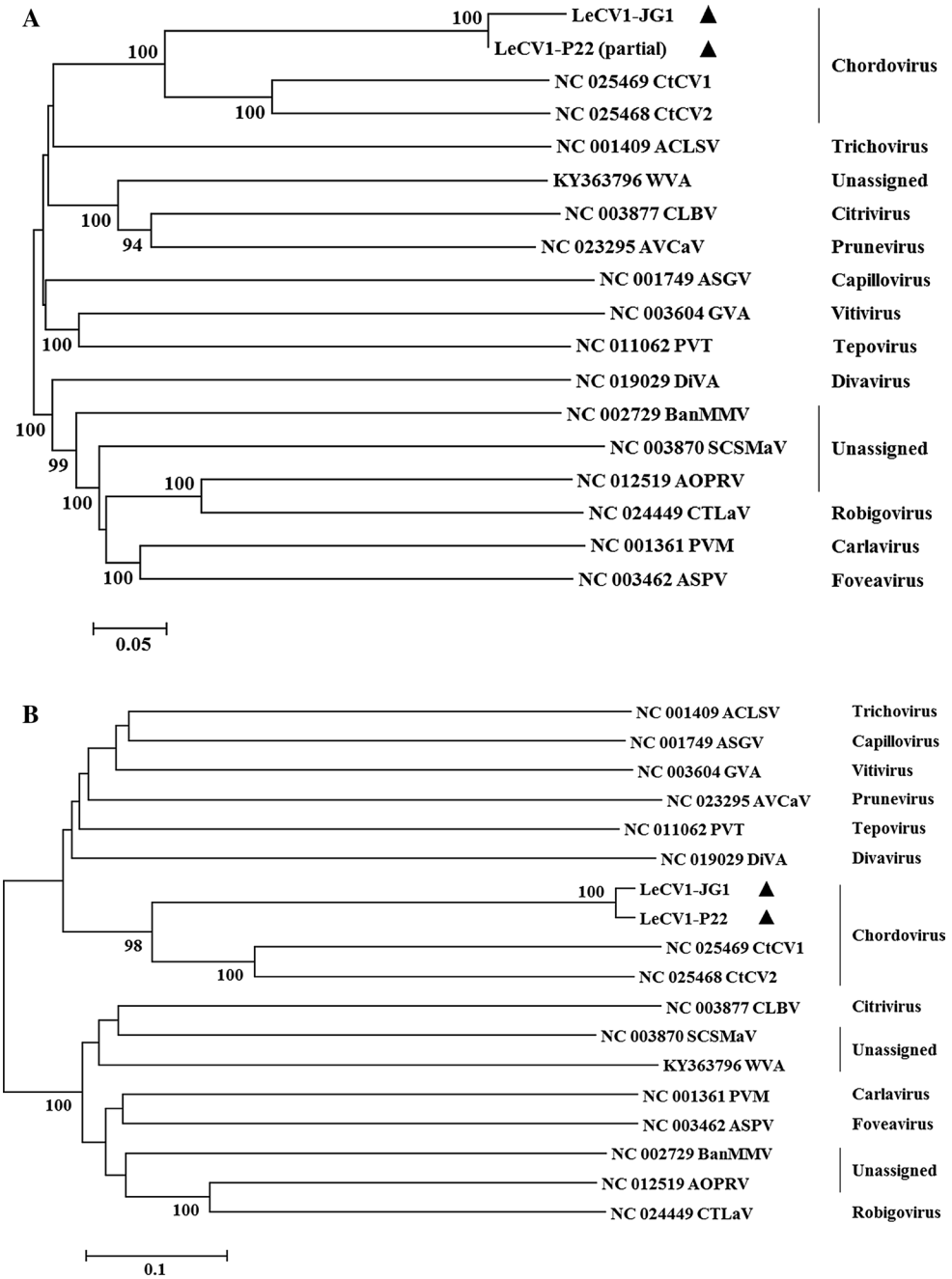

Fig. 2 Neighbor-joining phylogenetic trees constructed based on the replicase proteins (A) or capsid proteins (B) of members of the family *Betaflexiviridae*. Strict amino acid sequence identity distances were used to construct the trees. Statistical branch significance was tested by bootstrapping (1,000 replicates). Scale bars represent 5% (2A) or 10% (2B) aa sequence divergence. Lettuce chordovirus 1 (LeCV1) proteins are indicated by a black triangle. ACLSV, apple chlorotic leaf spot virus; AOPRV, African oil palm ringspot virus; ASGV, apple stem grooving virus; ASPV, apple stem pitting virus; AVCaV, apricot vein clearing-associated virus; BanMMV, banana mild mosaic virus; CLBV, citrus leaf blotch virus; CtCV1, carrot chordovirus 1; CtCV2, carrot chordovirus 2; CTLaV, cherry twisted leaf-associated virus; DiVA, diuris virus A; GVA, grapevine virus A; PVM, potato virus M; PVT, potato virus T; SCSMaV, sugarcane striate mosaic-associated virus; WVA, watermelon virus A

or partial (P22, 4.8 kb) sequences have been deposited in the GenBank database (MG208123-24, respectively). These two sequences share 95.1% nucleotide (nt) sequence identity, have the same genomic organization, and encode highly similar proteins (97.2% identity for the CP and 97.9% for the partial Rep), showing that they represent isolates of the same virus species.

Excluding the poly(A) tail, the JG1 genome is 8,536 nt long and contains four genes encoding a replicase (Rep, 1992 aa, 225.9 kDa), a 30K-family movement protein (MP, 355 aa, 40.2 kDa), a capsid protein (CP, 254 aa, 29 kDa) and a protein of unknown function (121 aa, 14.4 kDa) (Fig. 1). The 5' and 3' non-coding regions (NCR) are 205 and 198 nt long, respectively. This genomic organization is seen in members of several *Trivirinae* subfamily genera, including tricho-, prune- and chordoviruses [3], since the ORF in the 3' terminal region of CtCV1 and carrot chordovirus 2 (CtCV2, NC025468) was not reported [1]. The expected domains were identified in the JG1 Rep (Fig. 1), *i.e*,. the methyltransferase (Met, pfam01660, aa 43-354), helicase (Hel, pfam01443, aa 1194-1442) and RNA-dependent RNA polymerase (RdRp, pfam00978, aa 1596-1900). An AlkB domain, found in the Rep proteins of some *Betaflexiviridae* members, was also present (pfam13532, aa 856-964). Amino acid sequence comparisons and phylogenetic analysis of the replicase and coat protein showed that JG1 and P22 are most closely related to and cluster with 100% bootstrap support with viruses of the genus *Chordovirus* (Fig. 2A and B). The identity values, however, indicate distant relationship with the chordoviruses, with aa sequence identities of only 44.9%, 32.2%, 33.2 and 23.8%, respectively, between the Rep, MP, CP and ORF4 proteins of JG1 and CtCV2 (the corresponding nt identities are 52.4%, 45%, 46.9% and 44.2%, respectively). Given the genus and species demarcation criteria within the family *Betaflexiviridae* (45% nt and 40% aa and 72% nt and 80% Rep and CP aa identities for genus and species thresholds, respectively [2]), the JG1 and P22 sequences represent a novel virus within the genus Chordovirus. The name "lettuce chordovirus 1" (LeCV1) is therefore proposed for this new agent. Despite extensive efforts, it was not possible to mechanically transmit LeCV1, while a novel *Secoviridae* member present in coinfection in the JG1 plant was readily transmitted and detected by RT-PCR and reproduced the mosaic symptoms initially observed (data not shown). Attempts to transmit the P22 isolate similarly failed. The prevalence, mechanism(s) of spread and potential pathogenicity of LeCV1 remain to be analyzed in more detail.

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Compliance with ethical standards

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval This article does not contain any studies with humans or animals.

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