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Completion of the genome sequence of *Lettuce necrotic yellows virus*, type species of the genus *Cytorhabdovirus*☆

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Abstract

We completed the genome sequence of *Lettuce necrotic yellows virus* (LNYV) by determining the nucleotide sequences of the 4a (putative phosphoprotein), 4b, M (matrix protein), G (glycoprotein) and L (polymerase) genes. The genome consists of 12,807 nucleotides and encodes six genes in the order 3′ leader-N-4a(P)-4b-M-G-L-5′ trailer. Sequences were derived from clones of a cDNA library from LNYV genomic RNA and from fragments amplified using reverse transcription-polymerase chain reaction. The 4a protein has a low isoelectric point characteristic for rhabdovirus phosphoproteins. The 4b protein has significant sequence similarities with the movement proteins of capillo- and trichoviruses and may be involved in cell-to-cell movement. The putative G protein sequence contains a predicted 25 amino acids signal peptide and endopeptidase cleavage site, three predicted glycosylation sites and a putative transmembrane domain. The deduced L protein sequence shows similarities with the L proteins of other plant rhabdoviruses and contains polymerase module motifs characteristic for RNA-dependent RNA polymerases of negative-strand RNA viruses. Phylogenetic analysis of this motif among rhabdoviruses placed LNYV in a group with other sequenced cytorhabdoviruses, most closely related to *Strawberry crinkle virus*.

Keywords: Plant rhabdovirus; LNYV; Phylogeny

1. Introduction

Rhabdoviruses can infect vertebrates, invertebrates and plants and represent a large virus family of importance to agriculture and human health. They have a single-stranded, negative-sense RNA genome of ca. 12–15 kb, which encodes five functionally conserved proteins. Axillary proteins are encoded by some species. Conserved intergenic regions are located at the gene junctions and partially complementary untranslated regions, termed 3′ leader and 5′ trailer are located at either end of the genome. In rhabdovirus-infected cells, the viral RNA polymerase facilitates the transcription of distinct mRNA species from each gene and genome replication (Tordo et al., 2005; Jackson et al., 2005).

Lettuce necrotic yellows virus (LNYV) is the type species of the genus *Cytorhabdovirus* (Tordo et al., 2005), members of which are characterised by accumulation of enveloped virions in the cytoplasm of infected cells (Dietzgen, 1995; Jackson et al., 2005). LNYV causes a serious disease of lettuce in Australia and is transmitted in a persistent, propagative manner by the aphid *Hyperomyzus lactucae* (Francki et al., 1989). The LNYV genome consists of a monopartite, negative-sense, single-stranded RNA of about 13,000 nucleotides (Wetzel et al., 1994a). The physical map of the LNYV genome is 3′ leader-N-P-4b-M-G-L-5′ trailer, where N is the nucleocapsid gene, P is the putative phosphoprotein gene, 4b encodes a protein of unknown function, M is the matrix protein gene, G is the glycoprotein gene and L is the polymerase gene (Wetzel et al., 1994a). The sequences of the 3′ leader, N gene, 5′ trailer and all intergenic sequences have been reported previously (Wetzel et

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al., 1994a,b). In this paper, we present the completed nucleotide sequence of LNYV and compare it to the genomes of other plant rhabdoviruses.

2. Materials and methods

2.1. Virus propagation, purification and isolation of viral RNAs

The previously described garlic isolate (Sward, 1990) was used throughout. LNYV was propagated in *Nicotiana glutinosa* and leaves were collected 10–12 days post inoculation (dpi). Virus was purified and the genomic RNA extracted as described previously (Francki et al., 1989; Dietzgen et al., 1989). Total RNA from LNYV-infected *N. glutinosa* was extracted as described by Rezaian et al. (1983) or Higgins and Dietzgen (2000), or using RNeasy® Plant Mini kits (Qiagen, Hilden, Germany) following the manufacturer's instructions. Poly(A)$^+$ RNA was fractionated on oligo(dT)-cellulose using a mRNA purification kit (Pharmacia Biotech, Freiburg, Germany).

2.2. Oligonucleotide primers

Oligonucleotide primers for determination of the P, 4b and M gene sequences were synthesized using a PCR-MATE DNA synthesizer (Applied Biosystems). Primers for amplification and sequencing of the G and L genes were synthesized by Bresatec Pty Ltd. (Adelaide, South Australia) and Sigma-Genosys (Melbourne, Australia). Primer names and sequences are listed in Table 1.

2.3. PCR amplification and RACE

Poly(A)$^+$ RNA preparations from LNYV-infected *N. glutinosa*, and purified LNYV genomic RNA were used for cDNA synthesis and PCR amplification as described previously (Wetzel et al., 1994a). For the P, 4b and M genes, the reverse transcription mixture was denatured for 5 min at 94 °C prior to PCR amplification for 40 cycles of 94 °C for 20 s, 42 °C for 20 s and 72 °C for 30 s.

For amplification of the G and L genes, primers G1 and G3, respectively were heat-denatured prior to cDNA synthesis and *Pfu* Turbo DNA polymerase (Stratagene, La Jolla, CA) was used for PCR. Primers G1 and G2 were used to amplify the complete G gene between the G-M and the G-L intergenic regions (PCR-1; Fig. 1) using 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min. Primers L11 and L12 were used in PCR for 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 6 min to amplify the majority of the L gene (PCR-3; Fig. 1). L gene cDNA was also used to amplify additional DNA fragments, which overlapped each of the two internal *Eco*RI sites of the PCR-3 DNA fragment (L4/L5 and L6/L7) or linked PCR-3 to the G gene (G3/L3), yielding clones PCR-4, PCR-5 and PCR-2, respectively (Fig. 1).

For 3′ RACE (rapid amplification of cDNA ends), an oligo(dT)$_{12–18}$ primer was used for cDNA synthesis. The subsequent PCR amplification was done with oligo(dT)$_{12–18}$ and the LNYV-specific primers m4a, m4b1 or m4b2, and mM (Table 1) for 3′ RACE of the LNYV 4a, 4b, and M mRNAs, respectively. For 5′ RACE, cDNA was synthesized, purified, tailed with dCTP or dGTP and PCR-amplified using the 5′ RACE system (Life Technologies), according to protocols provided by the supplier. For dG-tailing, an oligo-dC$_{15}$ primer was used in

Table 1
LNYV-specific oligonucleotide primers used in this study

Clone	Primer name	Nucleotide sequence 5′ → 3′	Remark
	g4a	TAGAGCTATCATCAGAAGTTTTAGG	5′ RACE
	m4a	GATTGTTGACTTCCTTGTCC	3′ RACE
	g4b	CGCTCTTCATAGCATCCAGATCCTC	5′ RACE
	m4b1	GAGGGATGATTGCGTGATCACGTTG	3′ RACE
	m4b2	AGTGCTGACATTGATGAGTTAGGGG	3′ RACE
	gM	ACGGAAACAAGAACTCACATCG	5′ RACE
	mM	TGTTTCCGTCTGAAGTGTTC	3′ RACE
	gG	ACCTTGTTGCATCTAGTCAC	5′ RACE
PCR-1	G1	GGTCTAGAGATTCACATATAGACGAGTTATATCCG	*Xba*I
	G2	CGGAATTCTTTTCTTAAATCACACATGCCACTTGG	*Eco*RI
PCR-2	G3	GTCAAGCGACATGGATCTGA	
	L3	TATCATAGAGAGATTGAAGG	
PCR-3	L11	GCGAATTCTTGGAGGTTGTTGAGATTTGAC	*Eco*RI
	L12	GCGAATTCTTGGTTCTAATTGCATACACC	*Eco*RI
PCR-4	L4	CTTTAACTTGTATGGACTC	
	L5	CTCTTCATCACTCATCAACC	
PCR-5	L6	CGATATGCCAAAGGAGCCC	
	L7	GCTTCCGTGGTTGGTGTC	

"g" corresponds to the genomic sense, "m" to the messenger RNA sense and "4a", "4b", "M", "G" and "L" correspond to the respective genes; non-viral sequences are underlined.