Identification and characterization of a new virus in the genus *Potyvirus* from wild populations of *Angelica lucida* L. and *A. genuflexa* Nutt., family *Apiaceae*

N. L. Robertson

USDA, ARS, Subarctic Agricultural Research Service, Arctic Plant Germplasm Research and Introduction, Palmer, AK, U.S.A.

Received 21 December 2006; Accepted 4 May 2007; Published online 11 June 2007
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Summary

A novel potyvirus was discovered in *Angelica lucida* L. (wild celery) and *A. genuflexa* Nutt. (kneeling Angelica) (family *Apiaceae*) in the Matanuska-Susitna Valley, Alaska. The experimental plant host range of the virus included species in three families: *Chenopodiaceae* (*Chenopodium amaranticolor* Coste et Reyn and *Chenopodium quinoa* Willd.), *Solanaceae* (*Nicotiana benthamiana* Domin), and *Apiaceae* (*Anethum graveolens* L., *Apium graveolens* L. var. *dulce* (Miller), *Daucus carota* L. subspecies *sativus* (Hoffm.) Arcang., and *Petroselinum crispum* (Miller) Nyman ex A. W. Hilland). The virus contained flexuous rods with an ssRNA genome ~9.5–10 k nts and a CP (~35 kDa) that reacted to a universal potyvirus monoclonal antibody in Western blot analysis. The sequenced genomic 3'-end (~1850 nt) contained a potyvirus genomic arrangement that included the 3'-terminus of the NiB (nuclear inclusion) gene, the CP (coat protein) gene, and a 3'-UTR (untranslated region) attached to a poly(A) tail. The CP amino acids had between 54 and 70% identity with 12 selected members from the genus *Potyvirus*. Phylogenetically, the Alaskan potyvirus clustered with three other apiaceous potyviruses from Australia. The novel Alaskan virus was confined to *A. lucida* L. and *A. genuflexa* Nutt. in nature, and was classified in the genus *Potyvirus*, family *Potyviridae*, and in part named after its natural plant hosts, angelica virus Y (AnVY).

Introduction

Native plant species that contribute to the natural landscape in remote regions of Alaska are also represented in remnant areas intermixed with agricultural and residential lands in the Matanuska–Susitna Valley of south central Alaska. They grow along the roadside among invasive weeds, in residential gardens, on the edge of crops, and on undeveloped wooded lots. Like most regions throughout the world, plant viral disease studies in Alaska have been mainly confined to economically important crops, with few observations and surveys of wild
plants growing in their natural environments [4, 6, 7, 13, 14, 17, 20, 23]. A novel carmovirus confined to wild *Lupinus nootkatensis* Donn ex Simm near the headwaters of the Little Susitna River in the Talkeetna Mountains [19], and a unique carlavirus and potyvirus complex that is distributed throughout south central Alaska in twisted stalk, *Streptopus amplexifolius* (L.) DC. [20], are examples of two recent efforts to expose the existence of plant viruses in Alaskan natural landscapes.

In 2004, a wild celery plant, *Angelica lucida* L., growing on the edge of a road in Palmer, Alaska, had distinctly mottled leaves that resembled viral symptoms. Western blots of protein extracts from partially purified virus particles derived from the leaves of the diseased wild celery plant suggested that the plant was infected by a potyvirus. The next year, the same plant and several other *A. lucida* plants appeared with similar symptoms and also were potyvirus positive by Western blot.

Species in the genus *Angelica* are classified in the carrot family Apiaceae (formerly Umbelliferae) and are mostly represented in the northern hemisphere. They are biennials or perennials with deep tap roots and contain diagnostic umbrellalike compound inflorescences called umbels [8, 16]. The two native species in Alaska, *A. lucida* L. and *A. genuflexa* Nutt. (kneeling Angelica), inhabit moist environments along streambanks, with the former growing on beaches and inland meadows, and the later in moist thickets, forest openings and edges [24]. *Angelica lucida* L. is widely distributed along the coastal regions and in the Alaskan Range, and *A. genuflexa* Nutt. occurs from the Aleutians to the coastal zone of southeastern through central Alaska [8, 24].

In 2005–06, *A. lucida* L. and *A. genuflexa* were surveyed in south central Alaska for viruses, with an emphasis on identifying the tentative potyvirus and studying its biology. The potyvirus was named in part after its natural host and will be referred to as angelica virus y (AnVY).

**Materials and methods**

**Virus source**

In 2005–06, individual plants of *A. lucida* L. were surveyed on ~5 km of roadsides in a residential/farming community (61°37'N, 149°13'W) near Palmer, Alaska. Leaves from plants with and without symptoms were collected and processed within a week or stored at ~80 °C. Similar collections from *A. genuflexa* Nutt. plants took place at a remote site (61°57'N, 150°58'W) near Skwentna. These plants provided the virus for characterization and transmission studies discussed below.

**Virion extraction and characterization**

Virions were extracted from leaf tissue and partially purified as described by Lane [10, 11]. Collection and detection assays used 1 g leaf tissue/plant that was homogenized with 20 ml 0.4 M sodium citrate buffer (pH 6.7) containing 0.15 ml of 0.5 M sodium diethyldithiocarbamate; the protocol was also scaled up with ~25 g tissue/500 ml buffer to obtain greater virion concentrations. The sap was expressed through muslin and centrifuged for 10 min at 111,000 g in a Beckman 50.2 Ti rotor. The supernatant was filtered through Miracloth (CalBiochem, La Jolla, CA, USA), and 0.3 ml 10% Triton X-100 was added before another centrifugation at 111,000 g for 45 min. The final virus pellet in each tube was resuspended in 100–150 μl sterile water and stored at ~80 °C.

Formvar-carbon coated grids (Ted Pella Inc., Redding, CA) were placed on drops of the partially purified particles and stained with 2% uranyl acetate. The virus particles were examined and digitally photographed with a JEM-1200EX electron microscope (JEOL USA Inc., Peabody, MA).

The virion RNA was removed from partially purified virus samples as previously described [18] and visualized on ethidium-bromide-stained non-denaturing 1% agarose gels with RNA markers (Invitrogen, Life Technologies, Carlsbad, CA, USA).

Protein extracts from the virion preparations were processed with Precision Plus Protein™ Standards (Bio-Rad, Hercules, CA, USA) and visualized on 12% SDS-PAGE gels with Coomassie Blue R-250 [9] or by Western blots. The separated proteins were blotted onto nitrocellulose membranes and screened for potyvirus with universal potyvirus antiserum (Agdia Inc., Elkhart, Indiana, USA) diluted 1:2000 and goat anti-mouse Ig-G-conjugated alkaline phosphatase (1:3000) using the protocol for the Immuno-Blot Colorimetric assay kit (Bio-Rad, Hercules, CA, USA).

**Natural plant host range**

Native plant species, *A. lucida* L., *Heracleum lanatum* Michx. (cow parsnip), and *Cicuta mackenzieana* Raup (Mackenzie water hemlock), were surveyed from disturbed ecosystems near agricultural and residential lands on the Palmer site (61°37'N, 149°13'W) in 2005 and/or 06. While the majority of angelica and cow parsnip were sampled along the roadside, the water hemlock samples were collected in a marsh next to Walby Lake. On the Skwentna site, *A. genuflexa* Nutt. plants were examined from a remote natural ecosystem.