Complete genome sequences of two isolates of spiraea yellow leafspot virus (genus Badnavirus) from Spiraea x bumalda ‘Anthony Waterer’

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Abstract
The complete genome sequences of two isolates of spiraea yellow leafspot virus (SYLSV) were determined. Spiraea (Spiraea x bumalda) ‘Anthony Waterer’ plants showing virus-like symptoms including yellow spotting and leaf deformation were used for sequencing. The viral genome of SYLSV-MN (Minnesota) and SYLSV-MD (Maryland) is 8,017bp in length. The sequences share 95% identity at the nucleotide level. Both isolates have the same genome organization containing three open reading frames (ORFs), with ORF3 being the largest, encoding a putative polyprotein of 232 kDa with conserved domains including a zinc finger, pepsin-like aspartate protease, reverse transcriptase (RT), and RNase H. Pairwise comparisons between members of the genus Badnavirus showed that gooseberry vein banding associated virus GB1 (HQ852248) and rubus yellow net virus isolate Baumforth’s Seedling A (KM078034) were the closest related virus sequences to SYLSV, sharing 73% identity at the nucleotide level. Bacilliform virions with dimensions of 150 nm x 30 nm were observed in virus preparations from symptomatic, but not asymptomatic, plants.

Spiraeas (Spiraea spp.) (Fam. Rosaceae) are deciduous shrubs widely cultivated as landscape ornamentals in northern zones because of their winter hardiness. In the late 1990s, through a combination of electron microscopy and molecular techniques, a badnavirus was identified in Minnesota from Spiraea x bumalda plants displaying yellow leafspot symptoms. The virus, tentatively named after the associated symptoms (spiraea yellow leafspot virus, SYLSV), was found to be aphid-transmitted, and its prevalence reached up to 100% in commercial nurseries, public plantings, and home gardens [1]. Despite the prevalence of SYLSV, its complete genomic sequence has not yet been determined. Here, we report the complete sequence and genome organization of two SYLSV isolates, and the data support its classification as a member of the genus Badnavirus in the family Caulimoviridae.

Between 2018 and 2020, an increase in the occurrence of yellow spot disease of spiraea was observed in commercial nurseries and in landscape ornamentals in the Midwestern region of the USA. A total of 52 samples from several cultivars and species of spiraea collected in Minnesota and Maryland were surveyed for the presence of viral diseases. Virus indexing was done by transmission electron microscopy (TEM), polymerase chain reaction (PCR), and reverse transcription (RT)-PCR. Samples were obtained from commercial nurseries, landscape ornamentals, the Charlotte Partridge Ordway Japanese Garden, and the Minnesota Landscape Arboretum. One SYLSV isolate from Spiraea x bumalda ‘Anthony Waterer’ was selected for genome sequencing from each location (Minnesota and Maryland). Symptomatic plants showed severe virus-like disease symptoms including yellow leaf spots, leaf distortion, and stunting (Fig. 1A). TEM visualization confirmed the presence of bacilliform virus-like particles in symptomatic plants.

Virus-like particles were partially purified by extracting 20 grams of leaves in 500 mM sodium phosphate (pH 7.5)
containing 1 M urea, 4% (w/v) polyvinylpyrrolidone (average molecular weight, 40,000), and 0.5% (v/v) 2-mercaptoethanol. The extract was filtered through cheesecloth and centrifuged at 20,000 \(g\) for 20 min. After centrifugation, the supernatant was recovered and layered over a 5-mL cushion of 30% (w/v) sucrose in 100 mM sodium phosphate (pH 7.0) and centrifuged at 109,000 \(g\) for 2 hours. The pellet was resuspended in 300 µL of 100 mM sodium phosphate buffer (pH 7.0), and this suspension was clarified by shaking with an equal volume of chloroform and centrifugation at 10,000 \(g\) for 15 min. The supernatant constituted the partially purified extract from which samples were applied to carbon-coated formvar (1%) grids, negatively stained with 2% phosphotungstic acid (pH 7.0), and examined using a JEOL JEM-1400Plus transmission electron microscope at the University of Minnesota Imaging Center. Bacilliform virions characteristic of members of the genus *Badnavirus* were observed in extracts from symptomatic, but not asymptomatic, plants (Fig. 1B). Virus particles were, on average, 150 nm in length and 30 nm in width. No other virus-like particles were observed in any of the partially purified extracts.

The complete genome sequences of two isolates of SYLSV were determined using two different approaches. Sanger sequencing was used to characterize the virus isolate from Minnesota (SYLSV-MN), and high-throughput sequencing (HTS) was applied for the isolate from Maryland (SYLSV-MD).

SYLSV-MN was sequenced from virion-associated DNA as described previously [2]. PCR was done using the outward-facing primers SYLSV-Det-R1 (5'- CCATCGACA GCTATCAGATCTGC-3') and SYLSV-Right-1 (5'- AGA ATTTCAGGAATTTTAGGAGGG-3'), designed based on a partial genome sequence of SYLSV (AF299074) corresponding to the region of the viral genome encoding RT-RNase H [1]. PCR amplicons of approximately 8 kb were generated and cloned using a TOPO™ XL-2 Complete PCR Cloning Kit (Thermo Fisher) according to manufacturer’s instructions. Five clones were sequenced in both directions by primer walking using Sanger technology. An additional PCR product was amplified to confirm a 218-bp region not covered by the outward-facing primers. Three clones of each PCR product (~1 kb) were cloned and sequenced in both directions. The genome sequence was assembled using Geneious Prime 2021.

The complete genome sequence of SYLSV-MD was obtained by RNAseq from a symptomatic spiraea plant (*Spiraea x bumalda* 'Anthony Waterer'). Total RNA was extracted using an RNaseq Plant Mini Kit (QIAGEN), followed by DNase treatment and ribosomal RNA depletion. A complementary DNA (cDNA) library was generated by TrueSeq and sequenced on a HiSeq 4000 Illumina platform (100-bp paired-end reads). Raw data (~21M reads) were processed and assembled using CLC Genomics Workbench 11 (QIAGEN). A total of 44,564 contigs were assembled, one of which was confirmed to be SYLSV by BLAST analysis.

The viral genome of both isolates consisted of a circular DNA molecule comprising 8,017 bp with 48% GC content (Fig. 1C). Sequences were deposited in the NCBI GenBank database under accession nos. MW080369 and MW080370 for SYLSV-MN and SYLSV-MD, respectively. A genome sequence comparison of the two isolates revealed a 95% identity at the nucleotide level, with an identical nucleotide sequence motif complementary to the 3′ end of the methionine tRNA (5′- TGG TAT CAG AGC TTC GGC -3′). This motif was set as the starting point of the assembled sequences. Prediction of putative open reading frames (ORFs) was done using ORFinder (NCBI). The NCBI Conserved Domain search tool was used to identify putative functional domains in the translated protein sequences. The predicted genome organization consisted of three ORFs with an arrangement typical of badnaviruses [3, 4]. The positions of the ORF in the annotated genomic sequences were the same for both virus isolates, except for ORF3. ORF1 (nt...
Two isolates of spiraea yellow leafspot virus position 399-1055) encodes a putative protein of approximately 24.83 kDa, and no conserved domains were identified in this putative protein. The protein encoded by ORF1 shares 61% identity at the amino acid level with gooseberry vein banding associated virus (AEE39274). The ORF1 sequences of SYLSV-MN and SYLSV-MD are 100% identical at the amino acid level.

ORF2 (nt position 1059-1520) codes for a hypothetical protein of 14.13 kDa with no conserved domains detected. The sequence identity between SYLSV-MN and SYLSV-MD in ORF2 is 92% at the amino acid level. The ORF2-encoded protein shows the highest level of sequence identity (48%) to gooseberry vein banding associated virus (AEE39278). The ORF2 sequences of SYLSV-MN and SYLSV-MD are 100% identical at the amino acid level.

ORF3 encodes a putative polyprotein of 232.5 kDa with conserved domains for a zinc finger, pepsin aspartate-like protein, reverse transcriptase (RT), and RNase H. ORF3 extends from nt 1,459 to nt 7,596 in SYLSV-MD and from nt 1,457 to nt 7,594 in SYLSV-MN. The sequence identity of ORF3 between the two virus isolates is 97% at the amino acid level. Pairwise comparisons of the predicted amino acid sequence of the protein encoded by ORF3 with those of other members of the genus Badnavirus showed the highest similarity to gooseberry vein banding virus (UCJ00521; 67% aa sequence identity) and rubus yellow net virus (AHB61260; 61%, aa sequence identity). Additional pairwise alignments using the RT+RNase H region (~900 bp), which is used for species demarcation, showed the highest nt sequence identity (74%) to gooseberry vein banding associated virus isolate BC (HQ52250).

The phylogenetic relationships between SYLSV and members of the genus Badnavirus were examined using selected badnavirus sequences from the NCBI database. Phylogenetic inference was done using the polyprotein (ORF3) amino acid sequence (Fig. 2). Sequence alignment was done using the MAFFT algorithm implemented in Geneious Prime. A maximum-likelihood tree was generated using the RaxML algorithm in Geneious Prime and showed that SYLSV-MN and SYLSV-MD grouped in a clade that shares a common ancestor with gooseberry vein banding associated virus and rubus yellow net virus.

Fig. 2 Maximum-likelihood (ML) phylogenetic tree estimated using the RaxML algorithm implemented in Geneious Prime 2021. The bootstrap value (1000 replicates) is shown at each node. The tree is drawn to scale, with branch lengths corresponding to the number of substitutions per site. The arrow indicates the position of SYLSV. Rice tungro bacilliform virus (genus Tungrovirus) was used as an outgroup.
In this study, the complete genomic sequences of two isolates of SYLSV were determined. Both the genome organization of these isolates and the results of sequence comparisons support the conclusions of a previous study demonstrating the presence of a novel badnavirus in coinfections with a spherical dsRNA virus in symptomatic spiraea plants [1]. During this work, however, TEM and molecular analysis failed to detect the dsRNA virus in samples collected from symptomatic spiraea plants in Minnesota and Maryland, while SYLSV was easily detectable by both approaches. This finding suggests that SYLSV can induce symptoms without the presence of the previously reported coinfecting spherical dsRNA virus. Unlike typical badnaviruses, which are transmitted by mealybugs, SYLSV is transmitted by the aphid *Aphis spiraecola* through an unknown mechanism [1]. Large populations of *A. spiraecola* were observed during the sampling process in this study.

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**Declarations**

**Conflict of interest** All of the authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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