



## NEW DISEASE REPORT

# First report of a '*Candidatus Phytoplasma asteris*' strain affecting lingonberry (*Vaccinium vitis-idaea*) and causing lingonberry stunt phytoplasma disease

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## KEYWORDS

16Srl, aster yellows phytoplasma, LbSP

*Vaccinium vitis-idaea* is a red berry-producing woody shrub, native to North America, Europe, and Scandinavia, known commonly as lingonberry (Hendrickson, 1997). During the spring/summer seasons of 2019 and 2020, producers from Quebec, Canada, have noticed symptoms of yellowing leaves and stunted growth, resembling symptoms caused by phytoplasma.

To confirm whether phytoplasmas were indeed associated with these reported symptoms, fifteen lingonberry plants from different producers from the region of Chaudière-Appalaches were analysed (Table 1). Of these, nine had symptoms of stunting, little leaves and yellowing, while the rest showed no visible symptoms when collected (Figure 1). For each plant, total DNA was extracted from leaves and stem tissue using a CTAB-based method. The DNA was used as a template for a *cpn60UT* PCR with primers H279p/H280p (Dumonceaux et al., 2014), and the positive samples were subjected to direct PCR with phytoplasma 16S primers R16F2n/R16R2 as previously described (Perez-Lopez et al., 2019). Amplicons of the expected size (~600 bp for *cpn60UT*) were obtained for 9/15 samples, while those for R16F2n/R2 sequences were obtained for 8/9 samples positives using the *cpn60UT* assay (Table 1). The *cpn60UT* and R16F2n/R2 amplicons obtained

from two symptomatic samples (named LbSP14 and LbSP16 for lingonberry stunt phytoplasma) were purified and sequenced directly with the amplification primers (Centre Hospitalier de l'Université Laval de Quebec, Canada). The R16F2n/R2 sequence from LbSP14 was cloned into the pGEMT-Easy Vector (Promega, USA), and two clones were sequenced. Sequences were deposited in GenBank with Accession Nos OL739234 and OL739235 for LbSP14 and LbSP16 *cpn60UT*, respectively, and OL773369 and OL773370 for LbSP14 and LbSP16 R16F2n/R2 sequences. Accession Nos. OL773367 and OL773368 were used to register the sequences obtained from clone 1 (LbSP14c1) and clone 2 (LbSP14c2), respectively. A group/subgroup classification was performed on the LbSP *cpn60UT* sequences using *CpnClassiPhyR* (Muirhead et al., 2019) and R16F2n/R2 sequences with *iPhyClassifier* (Zhao et al., 2009). Phylogenetic relationships were determined using *cpn60UT* and R16F2n/R2 sequences of the LbSP phytoplasma and those from GenBank with the MEGA program version X (Kumar et al., 2018) and the neighbour-joining method with 1000 bootstraps. The *CpnClassiPhyR* classification suggested that the LbSP phytoplasma strain is a '*Candidatus Phytoplasma asteris*'-related strain and a member of the *cpn60UT* I-I(E/Al)Al subgroup (Figure 2a), with a similarity

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**TABLE 1** Lingonberry samples collected in Quebec, Canada analysed in this study

| Cultivar  | Symptoms                         | Sample | Tissue | Date of sample preparation | Date of DNA extraction | [DNA] ng/μL | A260/A280 | A260/A230 | Cpn60 PCR | 16S PCR |
|-----------|----------------------------------|--------|--------|----------------------------|------------------------|-------------|-----------|-----------|-----------|---------|
| Sussi     | Little leaves and yellowing      | 1      | Leaf   | 9-9-21                     | 12-10-21               | 409.1       | 1.95      | 1.04      | Neg       | Neg     |
|           |                                  | 2      | Stem   | 9-9-21                     | 15-11-21               | 892.4       | 2.10      | 1.78      | Pos       | Pos     |
| Magenta   | Little leaves and yellowing      | 3      | Leaf   | 9-9-21                     | 12-10-21               | 189.1       | 1.85      | 0.89      | Neg       | Neg     |
|           |                                  | 4      | Stem   | 9-9-21                     | 15-11-21               | 981.2       | 2.09      | 1.78      | Pos       | Pos     |
| Koralle   | Little leaves and yellowing      | 5      | Leaf   | 9-9-21                     | 12-10-21               | 433.7       | 1.98      | 1.11      | Neg       | Neg     |
|           |                                  | 6      | Stem   | 9-9-21                     | 15-11-21               | 732.7       | 2.11      | 1.75      | Pos       | Pos     |
| Sussi     | Asymptomatic                     | 7      | Leaf   | 9-9-21                     | 12-10-21               | 186.5       | 1.89      | 0.99      | Neg       | Neg     |
|           |                                  | 8      | Stem   | 9-9-21                     | 15-11-21               | 560         | 2.07      | 1.45      | Pos       | Pos     |
| Magenta   | Asymptomatic                     | 9      | Leaf   | 9-9-21                     | 12-10-21               | 142.7       | 1.75      | 0.74      | Neg       | Neg     |
|           |                                  | 10     | Stem   | 9-9-21                     | 15-11-21               | 211.5       | 1.97      | 1.16      | Neg       | Neg     |
| Koralle   | Asymptomatic                     | 11     | Leaf   | 9-9-21                     | 12-10-21               | 170.2       | 1.91      | 0.93      | Neg       | Neg     |
|           |                                  | 12     | Stem   | 9-9-21                     | 15-11-21               | 673.6       | 2.06      | 1.65      | Pos       | Pos     |
| Koralle   | Little leaves and stunted plants | 13     | Leaf   | 9-9-21                     | 20-10-21               | 546.9       | 1.98      | 1.21      | Neg       | Neg     |
|           |                                  | 14     | Leaf   | 9-9-21                     | 14-9-21                | 1512.5      | 2.01      | 1.8       | Pos       | Pos     |
|           |                                  | 15     | Stem   | 9-9-21                     | 15-11-21               | 2909.5      | 2.12      | 1.72      | Neg       | Neg     |
| Magenta   | Little leaves and yellowing      | 16     | Leaf   | 9-9-21                     | 15-11-21               | 228.3       | 1.51      | 0.69      | Pos       | Pos     |
|           |                                  | 17     | Leaf   | 9-9-21                     | 14-9-21                | 184         | 1.47      | 0.63      | Neg       | Neg     |
|           |                                  | 18     | Stem   | 9-9-21                     | 15-11-21               | 3824.8      | 1.98      | 0.94      | Neg       | Neg     |
| Magenta   | Little leaves and yellowing      | 19     | Leaf   | 9-9-21                     | 12-10-21               | 324.4       | 0.91      | 0.27      | Neg       | Neg     |
|           |                                  | 20     | Stem   | 9-9-21                     | 15-11-21               | 1047.8      | 1.94      | 0.94      | Neg       | Neg     |
| Koralle   | Little leaves and stunted plants | 21     | Leaf   | 9-9-21                     | 12-10-21               | 142.4       | 1.13      | 0.30      | Neg       | Neg     |
|           |                                  | 22     | Stem   | 9-9-21                     | 15-11-21               | 3418.8      | 1.94      | 0.96      | Neg       | Neg     |
| Koralle   | Little leaves and stunted plants | 23     | Leaf   | 9-9-21                     | 12-10-21               | 195.7       | 1.17      | 0.39      | Neg       | Neg     |
|           |                                  | 24     | Stem   | 9-9-21                     | 15-11-21               | 631.2       | 1.92      | 1.06      | Neg       | Neg     |
| Koralle   | Little leaves and stunted plants | 25     | Leaf   | 9-9-21                     | 20-10-21               | 307.6       | 0.92      | 0.25      | Neg       | Neg     |
|           |                                  | 26     | Leaf   | 9-9-21                     | 14-9-21                | 364         | 1.73      | 0.94      | Neg       | Neg     |
|           |                                  | 27     | Stem   | 9-9-21                     | 15-11-21               | 1670.7      | 1.83      | 0.54      | Neg       | Neg     |
| Erntesege | Asymptomatic                     | 28     | Leaf   | 9-9-21                     | 20-10-21               | 101.7       | 1.13      | 0.36      | Neg       | Neg     |
|           |                                  | 29     | Leaf   | 9-9-21                     | 15-11-21               | 389         | 1.68      | 0.94      | Neg       | Neg     |
|           |                                  | 30     | Stem   | 9-9-21                     | 15-11-21               | 1366.8      | 2.03      | 1.3       | Pos       | Neg     |
| Koralle   | Asymptomatic                     | 31     | Leaf   | 9-9-21                     | 20-10-21               | 139.4       | 0.88      | 0.27      | Neg       | Neg     |
|           |                                  | 32     | Leaf   | 9-9-21                     | 15-11-21               | 196.1       | 1.55      | 0.89      | Neg       | Neg     |
|           |                                  | 33     | Stem   | 9-9-21                     | 15-11-21               | 1697        | 2.00      | 1.19      | Neg       | Neg     |
| Koralle   | Asymptomatic                     | 34     | Leaf   | 9-9-21                     | 12-10-21               | 226.8       | 1.76      | 0.87      | Neg       | Neg     |
|           |                                  | 35     | Stem   | 9-9-21                     | 15-11-21               | 704.7       | 2.08      | 1.46      | Pos       | Pos     |

coefficient of 1.00 compared with blueberry stunt phytoplasma (BbSP; MH279496). The phylogenetic tree showed that LbSP strains branches with *cpn60*OUT I, closely related to BbSP isolates from Quebec, Nova Scotia and New Brunswick (Figure 3). The 16S classification linked to the *cpn60*OUT identified is 16SrI- (E/AI)AI (Perez-Lopez et al., 2019). We also found that LbSP displays 16S rRNA-encoding gene sequence heterogeneity with two distinct *rrn* operons, *rrnE* (OL773367) and

*rrnAI* (OL773368), as previously reported for BbSP (Figure 2b and Figure 4).

To our knowledge, this is the first report of lingonberry as a possible phytoplasma host. Other *Vaccinium* species, for example blueberry (*Vaccinium corymbosum*), are known to be affected by phytoplasmas in the same 16SrI- (E/AI)AI subgroup in Quebec (Perez-Lopez et al., 2019), and by 16SrI-E phytoplasmas in Canada and the USA (Lee et al., 2004;



**FIGURE 1** Lingonberry plants analysed in this study: (a) asymptomatic lingonberry plant with normal growth and development; (b) lingonberry plant showing typical symptoms of lingonberry stunt phytoplasma disease including stunted growth, little leaves, and yellowing

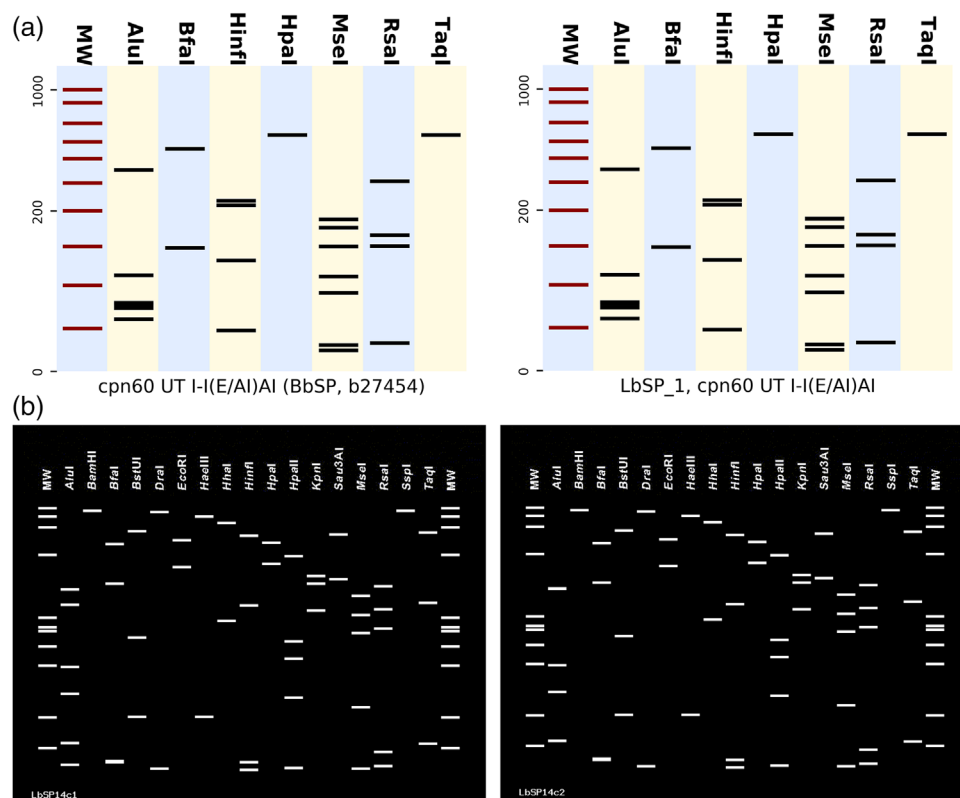


**FIGURE 2** Virtual RFLP pattern obtained for blueberry stunt phytoplasma (panel (a) left) and lingonberry stunt phytoplasma (panel (a) right) phytoplasma strains using CpnClassiPhyR. Virtual RFLP profiles generated with iPhyClassifier for lingonberry phytoplasma *rrnE* operon (panel (b) left) and *rrnAl* operon (panel (b) right)

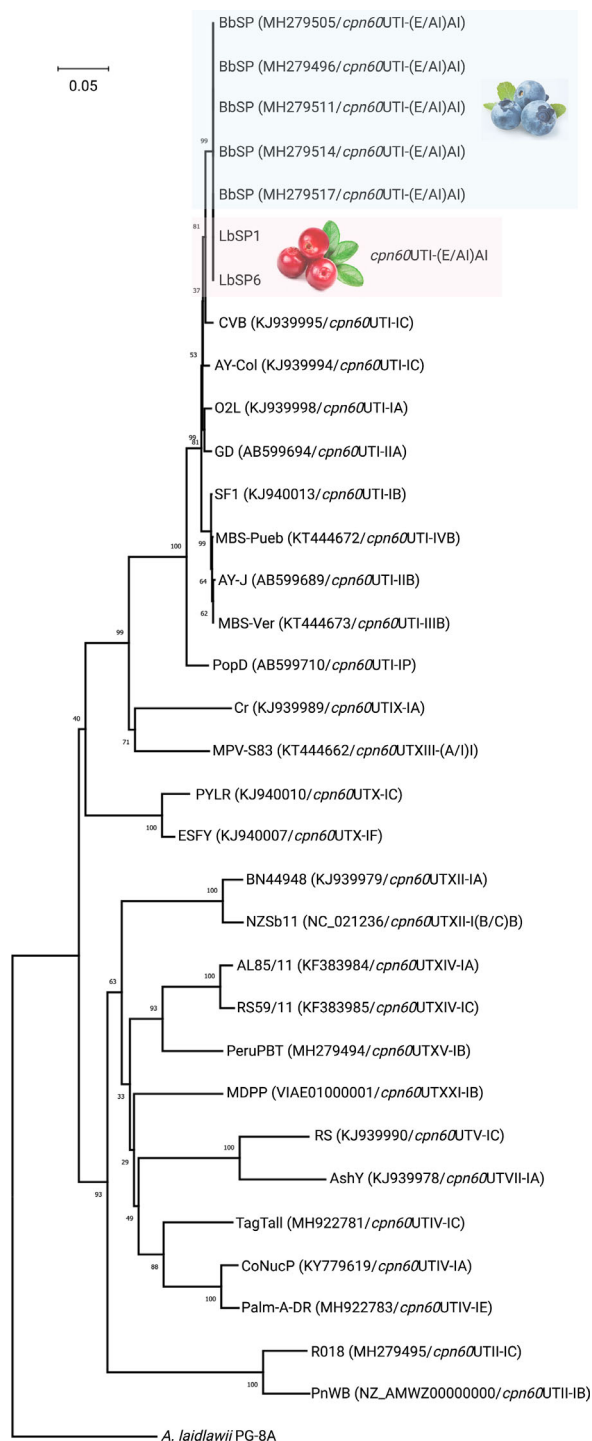


**FIGURE 3** Phylogenetic tree based on the *cpn6OUT* sequences of the lingonberry stunt phytoplasma (LbSP) strains identified in this study and '*Ca. Phytoplasma*' reference strains. GenBank accession numbers and group and subgroup classifications are provided. Bars represent 5 nucleotide substitutions in 100 positions

Arocha-Rosete et al. 2019). The fact that blueberries and lingonberries can be affected by phytoplasma strains in the same phytoplasma subgroup may suggest a common insect vector with variable feeding habits. Our findings are the basis for the understanding of the colonisation of a new plant host in the family Ericaceae by phytoplasmas.

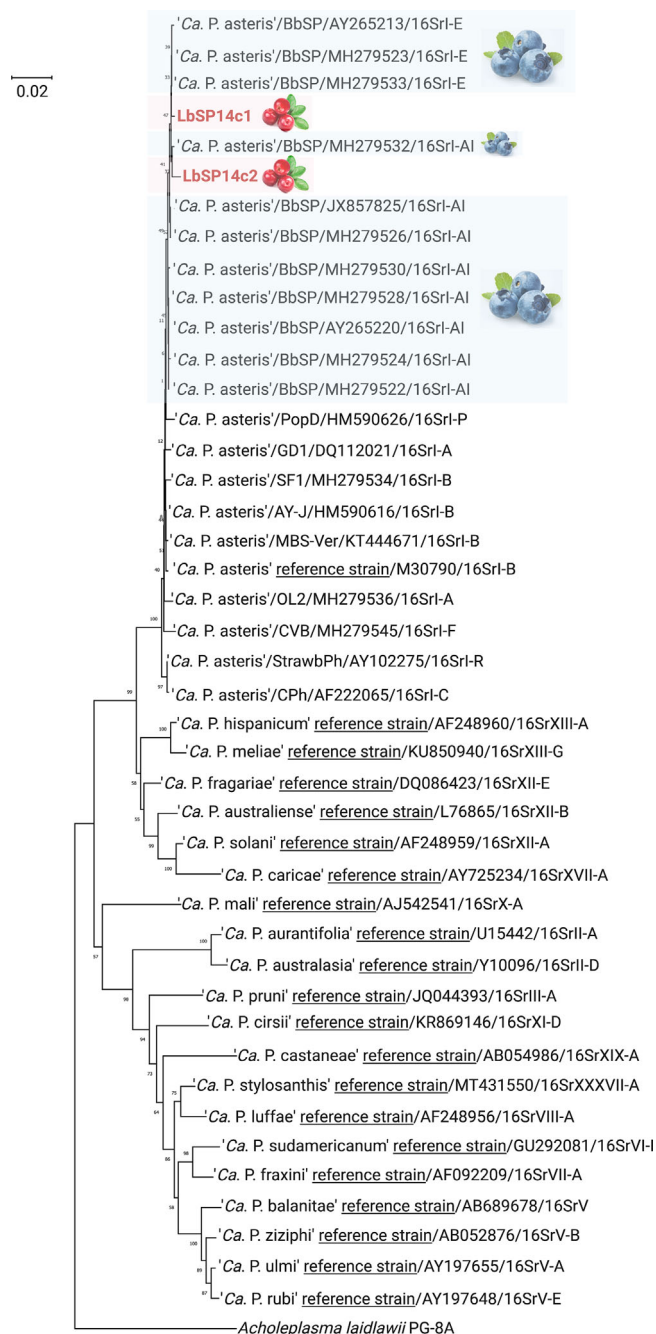


**FIGURE 4** Phylogenetic tree based on the 16S rRNA gene sequences of the lingonberry stunt phytoplasma (LbSP) strains identified in this study and '*Ca. Phytoplasma*' reference strains. GenBank accession numbers and group and subgroup classifications are provided. Bars represent 2 nucleotide substitutions in 100 positions

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