



## Identification and molecular characterization of the blueberry stunt phytoplasma in Canada

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### Abstract

Blueberry farms in Quebec, Canada, were recently surveyed for phytoplasma-associated disease presence. Farmers previously reported a number of high bush blueberries showing stunt symptoms in three blueberry farms. Bushes were tested for phytoplasma based on the *16S rRNA* gene. Nested PCR with universal phytoplasma primers resulted in amplification of phytoplasma DNA from blueberry bushes in samples from two of the farms surveyed. The 16S rDNA of the blueberry stunt phytoplasma from Quebec showed a 99% sequence identity with that of the blueberry stunt phytoplasma from Michigan (AY265220) from group 16SrI 'Candidatus Phytoplasma asteris' subgroup 16SrI-E. Phylogeny results confirmed the clustering of the blueberry stunt phytoplasma from Quebec within the same phylogenetic branch of phytoplasmas of group 16SrI, closely related to subgroup 16SrI-E. Preliminary virtual RFLP confirmed the sequence and phylogeny classification results. Further actual and virtual analyses are in progress to verify the presence of other possible phytoplasma strains from the Quebec blueberry farms.

**Keywords:** 16SrI phytoplasma, blueberry, RFLP, 'Candidatus Phytoplasma'

### Introduction

Blueberries have been steadily growing in popularity in Canada and worldwide due to increasing awareness of the health benefits associated with their consumption. Canada is the world's second largest producer of blueberries, second only to the USA (AAFC, 2010). Canadian ministers will invest to the grower representative body to encourage blueberry exports focusing on international export markets including China (www.freshfruitportal.com).

Blueberry plants are susceptible to a range of diseases including blueberry stunt (BBS), which can cause substantial economic damages to their production and quality (Bagadia *et al.*, 2013). BBS disease was first observed in New Jersey in 1928, and became widespread in Arkansas, Maine, Massachusetts, New Hampshire, New York, Michigan, North Carolina, Pennsylvania, Maryland, Virginia and Canada (Ramsdell and Stretch, 1987). BBS symptoms include witches' broom growths, stunting, small and deformed leaves, cupping of the leaves, unseasonal discoloration, and shortened internodes. PCR, sequencing, RFLP and phylogeny analyses were used in the present study to characterize the BBS phytoplasma in Canada from which very little information is available. A possible origin of the disease is suggested and information will be made available to farmers in Quebec to support further studies on the BBS phytoplasma for the development of effective control strategies.

### Materials and Methods

Total DNA was extracted from midribs of randomly collected leaf samples from three farms of highbush blueberry (*Vaccinium corymbosum* L.), located within a 100 kilometers radius from Montreal, which exhibited BBS-like symptoms (Figures 1A and B). Total DNA was used as a template for nested PCR assays with universal primers that target the phytoplasma *16SrRNA* gene R16mF2/R1 (Gundersen and Lee, 1996) for the first PCR reaction, and either R16F2n/R2 or fU5/rU3 (Lorenz *et al.*, 1995) for the nested reaction.

Representatives of R16F2n/R2 PCR amplicons were purified (Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced bi-directionally. Consensus sequences were compared with GenBank reference sequences and aligned using Clustal W. Preliminary RFLP analysis was conducted using iPhyClassifier (Zhao *et al.*, 2013).

A phylogenetic consensus tree was constructed using the neighbour-joining method with MEGA4.0 with default values and 1,000 replicates for bootstrap analysis. Actual and *in silico* restriction analysis pDRAW32 (www.acaclone.com), and PCR-sequencing of non-ribosomal genes are in progress to verify the presence of specific phytoplasma strains in the Quebec blueberry fields.



**Figure 1.** BBS symptoms that include small and deformed leaves with cupping and seasonal discoloration (A) and stunting and short internodes (B) observed on highbush blueberries from southern Quebec blueberry farms.

## Results

Phytoplasma DNA was amplified from highbush blueberries of two Quebec farms which were bearing typical BBS symptoms. No PCR amplicons were obtained from symptomless blueberry bushes. Comparisons with other BBS phytoplasma strains from North America and Europe, and preliminary virtual RFLP patterns of the 16S rDNA sequence of the BBS phytoplasma from Quebec placed it as a member of group 16SrI. BLAST results showed the highest score and 99% of the 16S rDNA sequence identity with a Michigan BBS phytoplasma (AY265220). Phylogeny analysis indicated that the Quebec BBS phytoplasma clustered within the phylogenetic branch of the 16SrI phytoplasmas of subgroups 16SrI-E, 16SrI-F and 16SrI-P, closely related to subgroup 16SrI-E.

## Discussion

Phytoplasmas associated with BBS have been mainly identified as members of the group 16SrI, subgroup E in the USA (Lee *et al.*, 2004; Bagadia *et al.*, 2013). Phytoplasma diseases of blueberry have been also reported in Europe including the Netherlands and Sweden (Valiunas *et al.*, 2004). The group 16SrIII '*Candidatus* Phytoplasma pruni' was reported from wild European blueberry (*Vaccinium*

*myrtillus* L.) exhibiting symptoms of shoot proliferation in Lithuania, and later a '*Ca. P. trifolii*'-related (16SrVI) strain was described in Austria (Borroto Fernández *et al.*, 2007). Recently, Bagadia *et al.*, (2013) identified phytoplasmas of the group 16SrIX '*Ca. P. phoenicium*', subgroup 16SrIX-E in blueberry bushes from New Jersey (USA).

Although the BBS incidence in Michigan is low, and Quebec blueberry growers obtain most of the planting material from New Jersey, the fact that the Quebec BBS phytoplasma was identified as an isolate of the Michigan 16SrI BBS phytoplasma raises suspicions of a possible BBS spread from the United States. The fact that closest strains have been found in different geographical locations in North America suggests that these phytoplasmas may have a complex epidemiology.

## Acknowledgements

We acknowledge the support for field surveys from Ornella Piccolli, Istituto Comprensivo di Fagagna Scuole dell'Infanzia, Elementari e Media, Fagagna, Udine, Italy.

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