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Detection and diversity of "flavescence dorée" - related phytoplasmas in alders surrounding infected vineyards in Aquitaine (France)

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Abstract

Alder yellows is a disease caused by phytoplasmas (AlYp) genetically related to the "flavescence dorée" grapevine phytoplasmas (FDp). To better document the genetic diversity of AlYp, alders surrounding infected vineyards were collected in Aquitaine, France. Sequence typing performed on the *map* gene (on 674 bp) revealed variability among AlYp isolates accounting for up to 13 Single Nucleotide Polymorphism. Phylogenetic analyses demonstrated that AlYp do not constitute a homogenous group, but are rather distributed in different clusters which comprise FDp isolates. The *map* gene sequence of some AlYp isolates was found identical to that of the FD1 group while other AlYp isolates clearly belong to the homogenous FD2 group. These data and published knowledge about AlY epidemiology and AlYp ecology are in agreement with existing or past exchanges between the vineyard and the wild compartment.

Key words: alder yellows, grapevine yellows, molecular typing, diversity, *Mollicutes*, *Oncopsis alni*.

Introduction

Alder yellows (AlY) is a frequent disease of *Alnus glutinosa* in Europe caused by phytoplasmas of the 16SrV group (Lederer and Seemüller 1991, Maurer *et al.,* 1993). They are transmitted by the leafhopper *Oncopsis alni* (Schrank) which can occasionally inoculate them to grapevine leading to Palatinate grapevine yellows (PGY) disease (Maixner *et al.,* 1999; 2000). It has been shown that these phytoplasmas are genetically closely related to the FDp, responsible of a quarantine disease of the grapevine epidemically transmitted by the leafhopper *Scaphoideus titanus* Ball (Angelini *et al.,* 2003; Arnaud *et al.,* 2007). To gain ground on the genetic and biological relationships between AlYp and FDp, we studied their diversity by sequence typing of the *map* gene on samplings from the wild and vineyard compartments in FD infected areas of the region Aquitaine in France.

Materials and methods

In 2006, samples from different plant species were collected in wood borders, hedges, riverbanks and vineyards in FD infected areas in Aquitaine. Five sites were sampled along the Dropt and Garonne rivers (figure 1). Collected plants were *A. glutinosa*, *Carpinus betulus*, *Partenocissus quinquefolia, Clematis vitalba*, *Salix viminalis, Robinia pseudoacacia* and *Vitis sp* showing yellowing or reddening. Phytoplasmas of the 16SrV ribosomal group were specifically detected by nested PCR on the *map* gene with *Taq* DNA polymerase (Promega) as described (Arnaud *et al.,* 2007). Molecular typing was performed by sequencing the PCR product on both strands on 674 bp (Arnaud *et al.,* 2007). If sequencing revealed a mix of PCR products, the *map* gene was re-amplified with the high fidelity DNA polymerase "DyNAzyme EXT DNA" (Finnzyme) following the same protocol and PCR products were cloned in the pGEMT-Easy plasmid (Promega). Three clones were then sequenced. Sequence analyses, multiple alignment and phylogenetic analyses by Neighbor-Joining were performed including 17 reference *map* gene sequences of the 16SrV group previously described in Arnaud *et al.,* 2007.

Results

Among the 28 symptomatic plants samples, phytoplasmas of the 16SrV group were detected in *A. glutinosa* (11/13 named A06) and *Vitis sp* (4/6 named V06). None of the other species was infected. For 3 of the 15 positive samples, A06-30-6 A06-22-2 and A06-30-4, *map* gene sequencing of the PCR products revealed polymorphism on 1, 2 and 7 positions respectively, reflecting a mix of phytoplasma isolates in the samples. After cloning, 2 different isolates were revealed for A06-30-6 named a and b, and at least 3 for A06-30-4 named a, b and c. Cloning of A06-22-2 is underway.

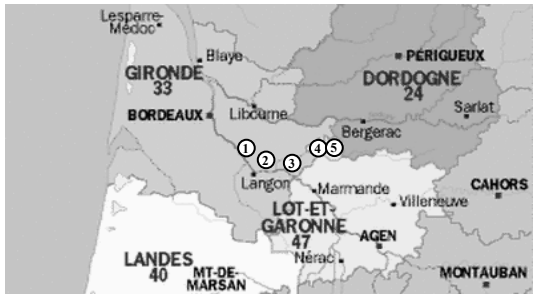

Figure 1. Localisation of the sampling sites in Aquitaine. 1: Cadillac, 2: Gironde sur Dropt, 3: Roquebrune, 4: Allemans du Dropt, 5: Sauvetat du Dropt.

Phylogenetic analysis including reference strains of the 16SrV group showed the existence of six clusters (figure 2). Each one was identified by using the name of the included reference strains: FD1, 2, 3, PGY-A, B and C. the sampled AlYp isolates presented a certain degree of diversity with up to 13 substitutions on 674 bp. They did not form a homogenous phylogenetic group but were distributed in every cluster, except FD3. The FD1 cluster comprised four AlYp isolates of which three exactly had the same *map* gene sequence as the FD1 reference strain originating from Gironde as well as a grapevine isolate from Cadillac. FD2 comprised three AlYp isolates presenting 3 to 5 mutations with the sequence of the FD2 reference strain from Gironde, two FDp isolates with no variability and one grapevine isolate with 2 mutations. The FD3 Italian cluster did not comprise any French isolates. One of the two AlYp isolates present in the PGY-A cluster had a sequence identical to that of the reference strain found in Germany. One alder isolate was identical to the German PGY-B strain cluster. Finally, the PGY-C cluster comprised three identical AlYp isolates presenting 3 mutations with the reference strain. Clustering of isolates was not related to their geographical origin because isolates from one site could be distributed in four different clusters and isolates from different countries could be found in one cluster. Moreover, different isolates from one plant could be distributed in two different clusters.

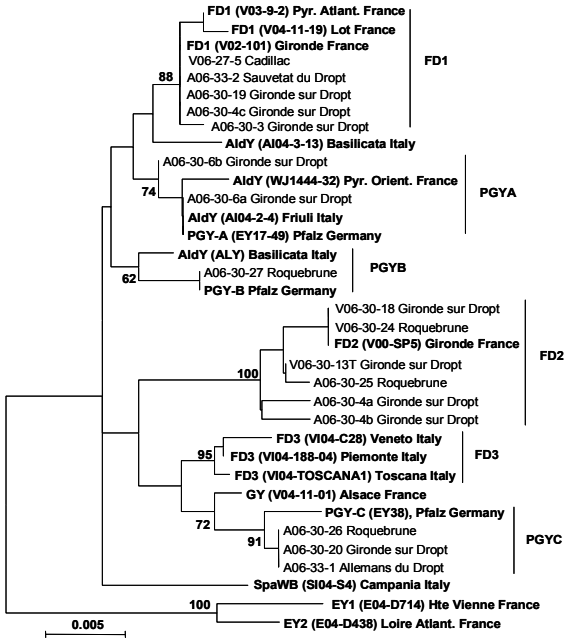

Figure 2. Neighbor-Joining phylogenetic tree constructed by *map* gene sequence analysis of the 16SrV phytoplasma isolates collected in Aquitaine. A06: AlYp isolates, V06: FDp isolates. Reference strains are in bold. GY: grapevine yellows, EY: elm yellows.

Discussion

In this study, we've found for the first time a FDp variant in the FD2 cluster (2 SNPs) which, until now, was found to be clonal (Arnaud *et al.,* 2007). It is furthermore confirmed that AlYp do not form a homogenous group but strains are distributed in clusters associated with FDp or PGYp as already presented in Arnaud *et al.,* 2007. Finally, we have shown that some AlYp isolates have exactly the same *map* gene sequence than FDp. These results confirm that phytoplasma exchanges occur between vineyards and wild alder compartment as it was already shown by Maixner *et al.,* 2000 and Arnaud *et al.,* 2007. It strengthens the hypothesis that the firsts FD outbreaks observed in the 50's in Aquitaine after the accidental introduction of *S. titanus* could have originated from alder by the intermediary of *O. alni* transmission (Arnaud *et al.,* 2007). The capacity of *S. titanus* to transmit the different AlYp strains remains to be studied, especially the ones which show no variability by comparison to FDp, in order to investigate the role of alder as a FD reservoir.

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