New Disease Reports

The first detection of a phytoplasma from the 16SrV (Elm yellows) group in the mosaic leafhopper *Orientus ishidae*

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In 2009 in Slovenia, an uncultivable cell-wall-less pathogenic bacterium of the class *Mollicutes* - a phytoplasma belonging to the 16SrV (Elm yellows) group was found in the mosaic leafhopper (*Orientus ishidae*) [Hemiptera, Cicadomorpha: Cicadellidae]. Molecular analyses showed that the phytoplasma isolates from *O. ishidae* resembled those of flavescence dorée (FD) phytoplasma strains, which are present in European grapevines. FD phytoplasmas in Europe are classified as quarantine organisms and are associated with the most important grapevine yellows disease flavescence dorée. FD's only known natural vector is the leafhopper *Scaphoideus titanus* [Homoptera: Cicadellidae] (Mori *et al*., 2002), although, *Dictyophara europaea* [Hemiptera: Dictyopharidae] has been shown to transmit FD phytoplasmas among grapevine plants in greenhouse conditions (Filippin*et al*., 2009). *O. ishidae* is a polyphagous leafhopper species which originates from the eastern Palaearctic region, and was reported for the first time in Europe in Switzerland in 2002 (Günthart & Mühlethaler,2002). Since its first detection in Slovenia in 2004 (Seljak, 2004), it has spread countrywide.

In July 2009, adults of *O. ishidae* were captured on bushy vegetation by sweep net sampling in two locations (both in South West Slovenia) away from vineyards and were then stored in 96% ethanol until DNA extraction. Sixty two specimens from one location were divided into four subsamples, and fourteen specimens from the other location were divided into two subsamples. The presence of the 16SrV-group phytoplasma in all *O. ishidae* subsamples was first determined with a TaqMan real-time PCR assay using FDgen set of primers and probe, which amplified the *secY* gene (Hren *et al*., 2007). Additional amplifications with conventional nested PCR assays (Filippin*et al*., 2009) were carried out to obtain amplicons of greater length (about 1200 bp with rp(V)F1A/R1A and about 1150 bp with FD9f3b/r2 primers) suitable for the subsequent characterization of the phytoplasma isolates by RFLP. The amplicons included genes coding for the L22 and S3 ribosomal proteins, and the L15 and SecY proteins, respectively. Amplicon digestion with the restriction enzymes *Hpa*II and *Taq*I revealed that all phytoplasmas detected in *O. ishidae* had RFLP patterns identical to those of reference strains FD70 and FD92 and FD strains found in the Slovenian grapevines (Figs. 1, 2). Additional digestion of the amplicon FD9f3b/r2 with *Alu*I suggested that both FD70 and FD92 strains were present in the samples of *O. ishidae* (Fig. 3). The nested FD9f3b/r2 PCR products were cloned into pGEM-T vector and three clones from subsamples were sequenced. The sequences (1112-1113 bp) from two subsamples (GenBank Accession No. HM367596) showed the highest identity (99.7%) with the strain FD70 (AM238512.1), but the sequences of the other (HM367597) showed the highest identity (99.7%) with the strain FD92 (AY197685.1). Further research is needed to shed light on the role of *O. ishidae* in the possible transmission of 16SrV-group phytoplasmas, including FD, from either reservoir host plants, which have not yet been found, or even from grapevine to grapevine.

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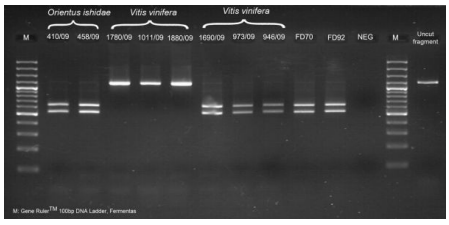

Figure 1

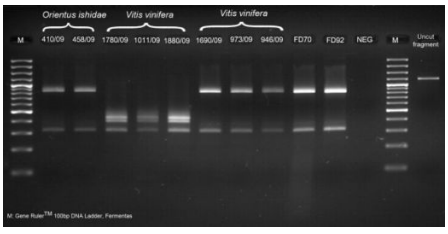

Figure 2

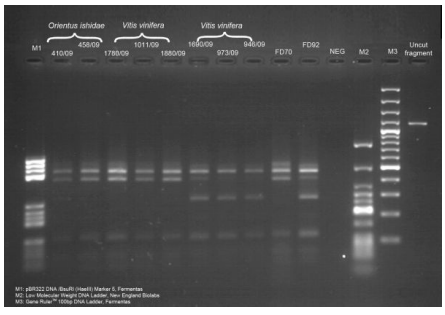

Figure 3

To cite this report: Mehle N, Seljak G, Rupar M, Ravnikar M, Dermastia M, 2010. The first detection of a phytoplasma from the 16SrV (Elm yellows) group in the mosaic leafhopper *Orientus ishidae*. *New Disease Reports* **22**, 11. [doi:10.5197/j.2044-0588.2010.022.011]

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