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Characterization of the microflora associated to *Scaphoideus titanus*, the insect vector of the "flavescence dorée"

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Abstract

Several wine production areas in Europe are afflicted by "flavescence dorée" (FD), a grape yellows caused by '*Candidatus* Phytoplasma vitis', transmitted by the phloem-feeder leafhopper *Scaphoideus titanus* Ball (Hemiptera Cicadellidae). Symbiotic control of disease transmission, exploiting natural symbionts of the vector, could represent an innovative strategy against FD diffusion. A study was conducted to characterize the microflora associated to *S. titanus* specimens, collected from FD-contaminated vineyards in the Italian region of Piedmont. By length heterogeneity PCR we identified a major peak associated with almost all the individuals examined. Characterization by denaturing gradient gel electrophoresis confirmed the presence of a major band corresponding to sex-ratio-distorting endosymbionts of the '*Candidatus* Cardinium hertigii' group; electron microscopy of tissues of *S. titanus* showed bacterial cells with the typical morphology of '*Ca.* Cardinium hertigii' together with cell morphologies resembling those of '*Ca.* Phytoplasma vitis'. Our findings suggest that this endosymbiont, named ST1-C, described for the first time in Cicadellidae, is transovarially transmitted and could have a complex life cycle in the insect body; in addition it colocalizes with '*Ca.* Phytoplasma vitis' several host tissues. These results make ST1-C an interesting candidate for the symbiotic control of FD diffusion.

Key words: Flavescence dorée Phytoplasma, *Scaphoideus titanus*, symbiotic control, Cardinium.

Introduction

"Flavescence dorée" (FD) is a grapevine disease that afflicts several wine production areas in Europe (Angelini *et al.*, 2001), caused by a bacterium, '*Candidatus* Phytoplasma vitis'. Its vector is the leafhopper *Scaphoideus titanus* Ball (Hemiptera Cicadellidae) (Bianco *et al.*, 2001). Commonly the control of the diffusion of the insect and the disease is obtained through pesticide treatment on vineyards. An innovative strategy is the 'symbiotic control' approach (Bextine *et al.*, 2004), in which microorganisms symbionts of the insect host could exploit mechanisms for reducing vector competence or interfering with the pathogen itself. Such a technique is based on the identification of the right microorganisms that can act as vectors of anti-pathogen molecules. Despite the economical impact of FD, a few is known on the bacterial community associated with its vector *S. titanus*. A study was conducted to characterize the microflora associated to *S. titanus* specimens from FD-contaminated vineyards in the Piedmont region of Italy.

Materials and methods

A total of 118 *S. titanus* individuals (69 females, 34 males, and 15 nymphs) were collected between 2002 and 2004 in vineyards with heavy symptoms of FD from seven different areas in the Piedmont region. 20 insects to be studied by transmission electron microscopy (TEM) were dissected; salivary glands, gut, fat bodies, and ovaries were isolated. DNA extraction was performed as previously described by Doyle and Doyle (1990). Molecular characterization of the microflora associated with *S. titanus* was performed by using length heterogeneity PCR (LH-PCR) (Ritchie *et al.*, 2000) with universal bacterial primers and by denaturing gradient gel electrophoresis (DGGE) analysis as previously described (Sass *et al.*, 2001) on a polyacrylamide gels (7%) with a denaturant gradient of 40 to 60%. Most prominent DGGE bands were excised from the gel and sequenced as reported in Marzorati *et al.* (2006). Primers Endo F1 and Endo R3, deriving from the alignment of ST1-C 16S rRNA sequence with related bacterial sequences, were used to determine the prevalence of this endosymbiont in 103 *S. titanus* individuals (Marzorati *et al.*, 2006). For searching '*Ca.* Phytoplasma vitis', the nested PCR previously described was used (Lee *et al.*, 1993; 1994)

Results and discussion

Using length heterogeneity PCR with universal primers for bacteria we identified a major peak associated with almost all of the males and females individuals examined. Characterization by denaturing gradient gel electrophoresis confirmed the presence of a major band that, after sequencing, showed a 97 to 99% identity with Bacteroidetes symbionts of the '*Candidatus* Cardinium hertigii' group (sex-ratio-distorting endosymbiotic bacteria) (Zchori-Fein *et al.*, 2001; 2004). In addition, electron microscopy of tissues of *S. titanus* showed bacterial cells

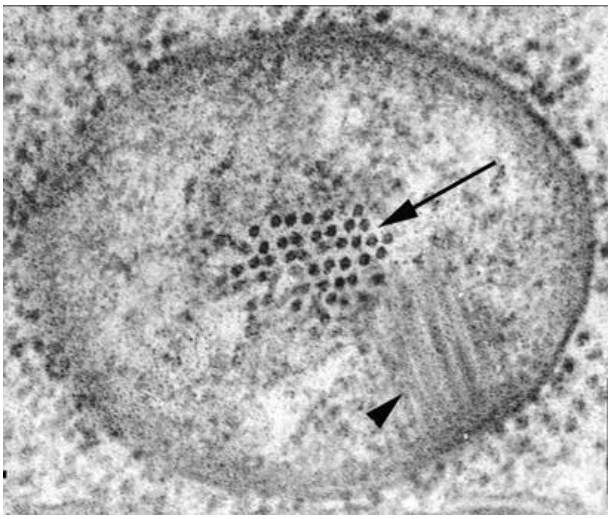

Figure 1. Micrographs of *Cardinium* sp. symbiont in *S. titanus* ovaries showing both transverse (arrow) and longitudinal (arrow head) sections of the microtubule-like elements (from Bigliardi *et al.*, 2006).

with the typical brush-like structure morphology of '*Ca.* Cardinium hertigii' (figure1). This endosymbiont, named ST1-C, was found in the cytoplasm of previtellogenic and vitellogenic ovarian cells, in the follicle cells, and in the fat body and salivary glands. Besides, cell morphologies resembling those of '*Ca.* Phytoplasma vitis' were detected in the midgut, and specific PCR assays indicated the presence of the phytoplasma in the gut, fat body and salivary glands. ST1-C endosymbiont was found in 97 of the 103 field-collected individuals (minimal field infection rate, 94.2%), while '*Ca.* Phytoplasma vitis' was identified in 22 individuals (minimal field infection rate, 21.4%) by a nested PCR assay on the 16S rRNA gene.

Conclusion

In this study on the bacterial diversity associated with *S. titanus* microorganisms philogenetically and morphologically correlated with '*Ca.* Cardinium hertigii' have been described for the first time as endosymbionts in Cicadellideae. Our findings suggest that ST1-C is transovarially transmitted to the insect progeny and that it could have a complex life cycle in the insect body, colonizing different and rather separate tissues.

The colocalization with '*Ca.* Phytoplasma vitis' in the same host tissues makes it possible to study the potential interactions between these bacteria in the insect body and makes ST1-C an interesting candidate for the symbiotic control of the FD agent, e.g., through a paratransgenesis approach (Beard *et al.*, 1998; Bextine *et al.*, 2004; Rio *et al.*, 2004).

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