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Preliminary results on endophytic bacterial community fluctuation during phytoplasma infection

Daniela BULGARI, Fabio QUAGLINO, Piero Attilio BIANCO, Paola CASATI
Department of Crop Production- Plant Pathology, State University of Milan, 20133 Milan, Italy

Abstract

In this study we investigated the influence of 'flavescence dorée' 16SrV-C/-D phytoplasmas on endophytic bacterial community by studying the seasonal fluctuation of bacterial species associated with healthy, 'flavescence dorée'-diseased and recovered plants during phytoplasma infection process. Preliminary results showed that, before phytoplasma titre inside diseased plant tissues becomes detectable, endophytic bacterial community is similar to that associated with healthy plants and differs from that associated with recovered plants.

Key words: Correspondence analysis, length heterogeneity-PCR, 'flavescence dorée'.

Introduction

Grapevine yellows (GY), a complex of phytoplasma-associated diseases, cause severe crop losses and damaged the quality of the grapes in Italy (Maixner, 2006). Recent researches, focused on fungal and bacterial endophytes associated with grapevines, suggested a possible involvement of endophytic species in the recovery phenomenon, the spontaneous remission of symptoms observed in phytoplasma-infected grapevine and fruit tree plants (Bulgari *et al*., 2009; Musetti *et al*., 2011). In particular, *Pantoea agglomerans*, *Curtobacterium*, *Bacillus pumilus* and *Burkholderia*, identified in association with grapevine tissues, are biocontrol agents against a broad spectrum of plant pathogens in other plants. Studies of grapevine associated bacterial endophytes and of their possible role as biocontrol agents against phytoplasmas could open new perspectives for developing novel strategies for the control of GY epidemics.

In previous work, we evidenced that endophytic bacterial richness decreases in GY-diseased and recovered grapevine plants (Bulgari *et al*., 2010). Furthermore, we speculated that phytoplasmas can directly or indirectly determine a reorganisation of endophytic bacterial community.

In this study we investigated the influence of 'flavescence dorée' 16SrV-C/-D phytoplasmas (FD) on endophytic bacterial community by studying the seasonal fluctuation of bacterial species associated with healthy, FD-diseased and recovered plants during phytoplasma infection process.

Materials and methods

Four healthy, four FD-diseased and four recovered Barbera plants were selected and sampled each month from June to October. Leaf tissues preparation and total DNA extraction from 20 g of grapevine leaf was carried out as described (Bulgari *et al*., 2009).

Molecular identification and FD quantification was carried out by real-time PCR on target *rplN* gene sequence (Durante *et al*., 2009). To study endophytic bacterial structure and diversity from June to October in healthy, FD-diseased and recovered grapevine plants, total DNA was analysed by length heterogeneity-PCR (LH-PCR) (Bulgari *et al*., 2009).

For all grapevine samples PCR amplification was run three times and three separate PCRs were also run to confirm the LH-PCR peak sizing through different PCR reactions. Statistical analyses were carried out to study the variation of endophytic bacterial composition in association to phytoplasma infection. Profiles obtained by LH-PCR analysis of healthy, diseased and recovered grapevines sampled in June were processed by correspondence analysis (CA).

The same analysis was repeated only on diseased grapevine plants collected from June to October. This analysis allowed to evaluate the influence of phytoplasmas on endophytic bacterial community composition. The statistics were performed with JMP software (JMP, version 7, SAS Institute Inc., Cary, NC, 1989-2007).

Results

No phytoplasma was detected in all grapevine plants sampled in June. On the contrary, symptomatic plants from July to October were characterized by the presence of phytoplasmas, whose concentration increased during the season reaching the highest titre in October, when the symptoms are severe. LH-PCR profiles were processed by correspondence analysis (CA), evidencing that endophytic bacterial community living in diseased plants in June was comparable to that of the healthy ones.

On the contrary the higher diversity was detected in recovered plants in comparison to diseased and healthy grapevines. Furthermore, bacterial community associated with diseased plants in June was different in comparison to the same plants sampled in the other months.

Discussion

Phytoplasma detection and quantification by real-time PCR revealed that phytoplasma titre generally increased during the season reaching the highest concentration in October. Statistical analysis of LH-PCR profiles indicated that, before phytoplasma titre inside diseased plant tissues becomes detectable, endophytic bacterial community is similar to that associated with healthy plants and differs from that associated with recovered plants. Consequently, it seems that a change in microbial composition could be determined when phytoplasmas start to replicate.

On the basis of our preliminary findings, we can speculate that endophytic bacterial community associated with grapevine plants is altered after phytoplasma infection (increase of phytoplasma titre by replication). This reorganization could be mediated by a direct competition between phytoplasmas and endophytic bacteria for colonizing a favorable niche. On the other hand, phytoplasmas can induce plant defense response leading to select some strains that are able to adapt to the new niche. In fact, plant defense responses are not activated directly, but are accelerated upon pathogen or insect attack (Frost *et al*., 2008).

Pathogen infection can also modify endophytic bacteria quantitatively determining a change in the relative proportion of some bacterial groups (Trivedi *et al*., 2010). Our preliminary analyses do not allow to verify this kind of influence but it could be interesting investigate them by quantitative real-time PCR.

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Corresponding author: Paola CASATI (e-mail: paola.casati@unimi.it), Department of Crop Production- Plant Pathology, State University of Milan, 20133 Milan, Italy.