



Future IPM 3.0 towards a sustainable agriculture

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Future IPM 3.0

BOOK OF ABSTRACTS



First insights on the ability of a *Lysobacter capsici* member to induce resistance mechanisms in grapevine plants

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Highlights

- Viable and heat-killed cells of the biocontrol agent *Lysobacter capsici* AZ78 are as effective as copper hydroxide in controlling grapevine downy mildew
- Application of viable and heat-killed *L. capsici* AZ78 cells induces the deposition of callose in grapevine leaves

Introduction

The bacterial genus *Lysobacter* is regarded as a valuable source of novel biocontrol agents against phytopathogenic oomycetes (Hayward et al., 2010). Application of *L. capsici* AZ78 (AZ78) cells on grapevine plants drastically reduced attacks of *Plasmopara viticola*, the causal agent of grapevine downy mildew, and antibiotics produced by AZ78 were shown to be toxic against *P. viticola* sporangia (Puopolo et al., 2014). However, the high level of disease reduction suggested that more than one AZ78 mechanism could be involved in the plant protection. Since biocontrol *Lysobacter* strains can also induce plant resistance (Kilic-Ekici and Yuen 2004), our aim was to assess the ability of AZ78 to trigger in grapevine molecular mechanisms related to plant resistance. We tested the expression pattern of plant pathogenesis-related genes and the deposition of callose in grapevine leaves treated with viable (vAZ78) and heat-killed (hkAZ78) AZ78 cells.

Material and methods

Lysobacter capsici AZ78 was routinely grown at 27°C onto Luria-Bertani agar. The efficacy of AZ78 against *P. viticola* was evaluated on susceptible plants of *Vitis vinifera* cv. Pinot Noir, grown under controlled greenhouse conditions (25 ± 1°C, 60 ± 10% RH) for two months. Plants were treated with suspensions of 10⁸ CFU/ml of vAZ78 and hkAZ78 (90°C for 10 min) cells 24 h and 6 h before *P. viticola* inoculum. Control plants were treated 6 h before the *P. viticola* inoculum with distilled water (DW) and copper [Cu(OH)₂; 2.5 g/l; Kocide 3000, Du Pont de Nemours, USA]. *Plasmopara viticola* was propagated onto Pinot Noir plants; the inoculum suspension (5 × 10⁵ sporangia/ml) was prepared according to Puopolo et al. (2014). One hour before the *P. viticola* inoculation, AZ78 populations residing onto leaves treated with vAZ78, hkAZ78, DW and Cu(OH)₂ were quantified through dilution plating method. The percentage of leaf area covered by sporulating lesions (disease severity) was evaluated 120 h after *P. viticola* inoculum. Five plants were used for each treatment; the experiments were repeated three times.

Deposition of callose in leaves treated with DW, vAZ78 and hkAZ78 cells was evaluated 1 h before *P. viticola* inoculum and 24 h after *P. viticola* inoculum according to Diez-Navajas et al.



(2007). At the same time points, modulation of *PR-1* and *PR-4* genes in leaves treated with DW, DW, vAZ78 and hkAZ78 cells was assessed through quantitative Real Time-PCR (qRT-PCR) according to Nesler et al. (2015).

Results and discussion

Understanding how the biocontrol agent AZ78 can effectively control *P. viticola* is an important step for its development as a novel biofungicide. In this study, we evaluated the ability of this biocontrol agent to reduce *P. viticola* infections through the activation of plant resistance mechanisms.

Greenhouse trials clearly showed that the application of both vAZ78 and hkAZ78 cells was effective in reducing *P. viticola* attacks similarly to $\text{Cu}(\text{OH})_2$. Indeed, DW treated plants showed a disease severity of $51 \pm 12\%$, whereas disease severity reached not significantly different values of $11 \pm 12\%$, $8 \pm 9\%$ and $11 \pm 15\%$ on plants treated with $\text{Cu}(\text{OH})_2$, vAZ78 and hkAZ78 cells, respectively. No AZ78 cells were recovered from plants treated with $\text{Cu}(\text{OH})_2$, DW and hkAZ78 cells, demonstrating that the heat-treatment was effective in killing AZ78 cells. On the other hand, an AZ78 population of $4.30 \pm 0.16 \log_{10}$ CFU/g of leaf was recovered from plants treated with vAZ78 cells, confirming the AZ78 ability to persist on grapevine leaves (Puopolo et al., 2014).

The deposition of callose is one of the quickest responses of the plant to the invasion of pathogens. Before *P. viticola* inoculum, callose deposition was observed in leaves treated with vAZ78 cells whereas no reaction was observed in DW- and hkAZ78-treated leaves. However, 24 h after the *P. viticola* inoculum, callose deposition was observed in the stomata of both vAZ78- and hkAZ78-treated leaves, while different zoospores nearby the stomata were registered in DW-treated leaves. Interestingly, callose deposition increased during the incubation period: an extended and intense callose deposition around the stomata was observed 120 h after *P. viticola* inoculation both in vAZ78- and hkAZ78-treated leaves. On the other hand, many sporangiophores emerged from stomata in DW treated leaves. The increase in callose deposition in vAZ78- and hkAZ78-treated leaves was not associated with the modulation of genes related to plant resistance. In fact, qRT-PCR analysis revealed no differences in relative expression level of *PR-1* and *PR-4* between DW-treated leaves and leaves treated with vAZ78 and hkAZ78 cell at 6 h and 24 h after *P. viticola* inoculation.

Overall our results confirmed the ability of AZ78 to effectively control *P. viticola* on grapevine plants under controlled conditions. Since hkAZ78 cells were effective in reducing the disease similarly to vAZ78 cells and $\text{Cu}(\text{OH})_2$, it is conceivable that AZ78 may induce some defence strategy in grapevine plants. Although *PR-1* and *PR-4* genes were not induced by the application of vAZ78 and hkAZ78 cells, we showed that AZ78 cells trigger the deposition of callose in correspondence of the stomata, reducing the grapevine downy mildew severity.

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