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The inhibitory activity of the biocontrol agent *Lysobacter capsici* AZ78 is negatively modulated by *Bacillus* spp. isolated from grapevine leaves

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Extended abstract: The bacterial genus *Lysobacter* includes different species that can produce molecules and lytic enzymes with activity against fungi and oomycetes (Panthee *et al*., 2016). For example, *L. capsici* AZ78 (AZ78) produces antibiotics that have a toxic effect on *P. viticola* sporangia (Puopolo *et al*., 2014 a) and effectively controls *P. viticola*, both if used alone and in combination with copper (Puopolo *et al*., 2014 b).

Since bacterial communities may modulate the antibiotic activity of biocontrol agents (De Boer, 2017), our aim was to determine how the interactions with phyllosphere-dwelling bacteria can influence AZ78 biocontrol activity in a simplified model system.

We recovered 47 bacterial isolates from leaves of *Vitis vinifera* L. cv. Pinot gris and Goldtraminer and identified them at species level by 16S rDNA phylogenetic analysis. To assess the impact of the 47 bacterial isolates on the *in vitro* inhibitory activity of AZ78, we designed experiments where the bacterial isolates and AZ78 were coinoculated on Luria Bertani Agar (LBA). Briefly, 5 µl of bacterial cell suspension (≈ 10^9 cells/ml) of AZ78 and bacterial isolates were spot-inoculated on LBA plates at 1 cm of distance. Once dried under laminar flow, LBA plates were incubated at 25 °C. After 48 h incubation, the plates were inoculated with 5 mm plugs of *Pythium ultimum* at 2.5 cm from AZ78 developed macrocolony. The controls consisted of LBA plates inoculated with *P. ultimum* alone, as well as *P. ultimum* inoculated with only AZ78 (2.5 cm of distance) and *P. ultimum* inoculated with only one bacterial isolate (3.5 cm of distance). After seven days incubation at 25 °C, the growth area of *P. ultimum* was measured. To determine whether modulation of AZ78 inhibitory activity was due to changes in its viability, AZ78 viable cells were counted after 48 h of interaction with the bacterial isolate that had the most negative effect.

Most of the bacteria isolated from grapevine leaves were Gram-negative belonging to the γ-Proteobacteria, while the Gram-positive bacterial isolates belonged to Actinobacteria and Firmicutes. The interactions tests carried out *in vitro* revealed that most of the bacterial strains evaluated had a positive effect on AZ78 ability to inhibit *P. ultimum* growth. On the contrary, bacterial strains belonging to *Bacillus* spp. showed a negative effect on AZ78 inhibitory activity. In particular, the bacterial strain that had the most negative effect was *Bacillus* sp. L30 that reduced of the 18.8 ± 0.7% the ability of AZ78 to inhibit *P. ultimum* growth.

Although there was a significant decrease of viable cells when AZ78 was co-inoculated with L30 (8.90 ± 0.07 $\log_{10}$ cells/macrocolony) compared to the control (9.46 ± 0.04 $\log_{10}$ cells/macrocolony), this reduction was not enough to fully explain the drop in *P. ultimum* growth inhibition. Conversely, it is likely that AZ78 invested more energy in the protection

against toxic secondary metabolites produced by *Bacillus* rather than the release of secondary metabolites active against *P. ultimum*.

These results show that the interactions among biocontrol agents and the natural microbiome are an important factor to be considered in evaluating their efficacy, since they can modify the inhibitory activity either in a positive or in a negative way. In light of that, more studies are required to consider the variation in AZ78 gene expression, taking into account the plant response as well.

Key words: *Lysobacter*, *Bacillus*, inhibitory activity, biocontrol agents, microbial interactions

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