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Carposphere microbiome of pear harbours potential microbial biocontrol agents against *Stemphylium vesicarium*

A. Montorsi[1], M. Cortiello[1], F. Bellameche[1], C. Nasuti[1], M. Menghini[2], R. Baroncelli[2], M. Collina[2], L. Solieri[1], E. Stefani[1], D. Giovanardi[1]

[1]Department of Life Sciences, University of Modena and Reggio Emilia, 42122 Reggio Emilia, Italy; [2]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum, University of Bologna, 40127 Bologna, Italy. Email: alessandro.montorsi@unimore.it

Introduction

Stemphylium vesicarium (*S.v.*), causal agent of brown spot of pear (BSP), causes severe losses in Italian pear orchards, especially in Emilia Romagna, where the susceptible cultivar Abbé Fétel is widely cultivated. The pear carposphere microbiome represents a potential source of microbial biocontrol agents (*m*BCAs) for alternative sustainable BSP management. In this study, more than 1,000 yeast and bacterial isolates were collected from the pear carposphere, and putative strains were assessed for antagonistic activity against *S. vesicarium* through *in vitro* (dual culture assays) and *in vivo* (detached leaf assays; DLA).

In vitro dual culture assay

Four yeast and four bacterial strains, selected based on our previous studies, were evaluated for their antagonistic activity against six *S.v.* strains using *in vitro* dual culture assays **(Fig. 1)**. The strains were taxonomically identified through ITS and 16S rRNA gene sequencing and BLAST analysis.

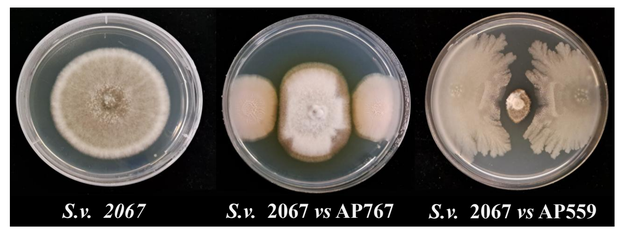

Figure 1: Dual culture assay.

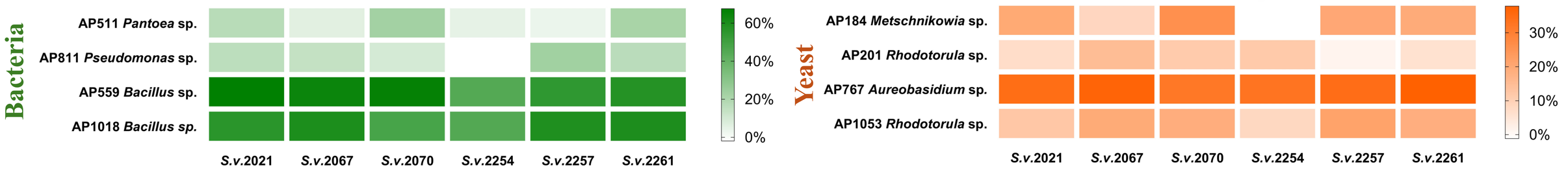

Figure 2 : Heatmap of antagonistic effect of yeast and bacterial isolates against a set of *S. vesicarium* strains.

The antagonistic performance of the strains varied depending on the specific *S.v.* isolates tested, with the highest inhibition rate observed for the *Bacillus* spp. strains (AP559, AP1018) and *Aureobasidium* sp. yeast strain AP767 **(Fig. 2)**.

In vivo detached leaves assay

To evaluate the capacity of the eight strains to control BSP, healthy leaves of pear trees (cv. Abbé Fétel) were collected, dipped in the bacterial or yeast suspension, and spray-inoculated with *S.v.* at 24 hours post treatment. Disease severity was then assessed using a phytopathometric scale **(Fig. 3)**.

Figure 3: Phytopathometric scale for BSP disease severity.

Figure 4: Bioassay evaluation of BSP disease control on detached leaves cv. Abbé Fétel (7 days of incubation).

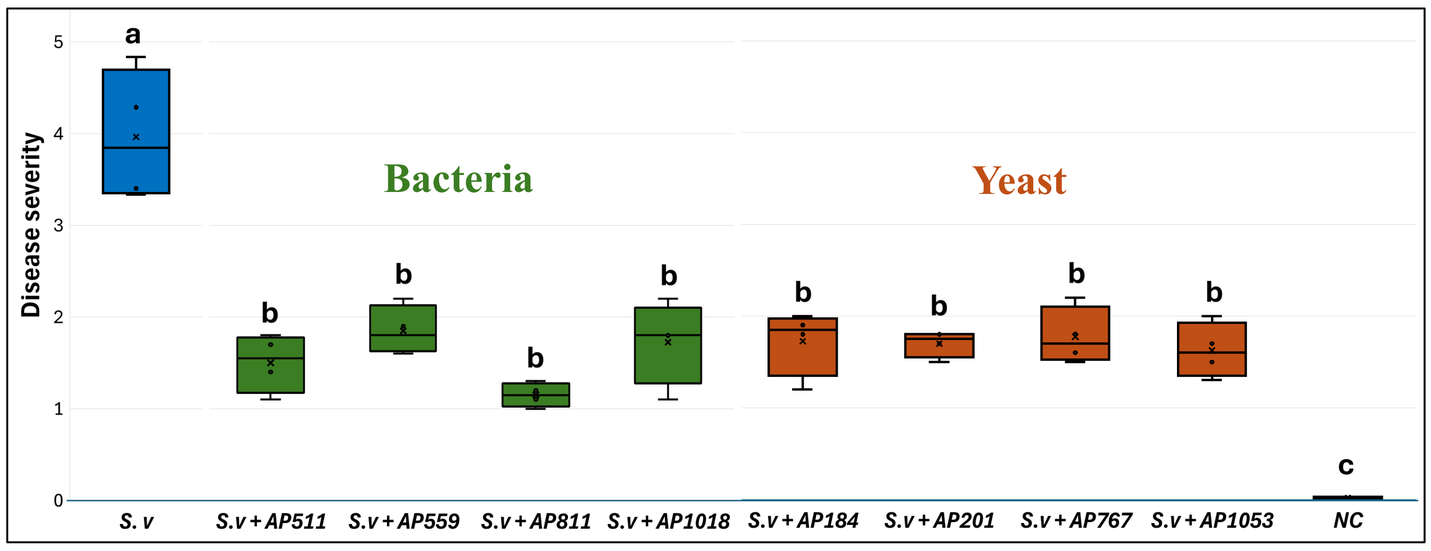

Figure 5: Protective effect of bacterial and yeast strains against *S. v.* on detached leaves (7 days of incubation).

Interestingly, all tested strains significantly reduced BSP severity with a reduction ranged between 52 % to 71 % **(Fig. 5)**. The highest reduction was observed in *Bacillus* sp. strain AP811 **(Fig. 4)**.

Conclusions

Results from *in vitro* assays were not always consistent with those from DLAs, suggesting different biocontrol mechanisms. Further investigations are needed to identify the mechanisms of action of these *m*BCA candidates against *S. vesicarium*. Finally, *in planta* assays are currently ongoing to assess their ecological behaviour and potential for effective BSP control.

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