

Stemphylium vesicarium affecting pear: exploiting bacterial isolates from carposphere as potential microbial biocontrol agents

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INTRODUCTION

The Farm to Fork Strategy, part of the European Union Green Deal, foster the importance for innovative and sustainable solutions for pathogen management by limiting the use of synthetic pesticides in agriculture. This study explored the potential of bacteria inhabiting the carposphere of pear cv. Abbé Fétel as microbial biocontrol agents (*mBCAs*) against *Stemphylium vesicarium* (*Sv*), the causal agent of brown spot of pear (BSP).

SAMPLING, ISOLATION AND CHARACTERISATION

During summer 2023 and 2024, pear fruits cv. Abbé Fétel were collected from pear orchards located in five provinces of Emilia Romagna (Modena, Bologna, Ferrara, Ravenna, and Forlì-Cesena) under organic (BIO) and integrated pest management (IPM). From carposphere, a total of 639 bacteria were isolated and morphologically characterised (Fig. 1A). The isolates were tested *in vitro* through dual plate assay against *Sv*, showing variable degree in mycelial growth inhibition (MGI). Some isolates exhibited a MGI of more than 60%, while others, referred to as helpers, positively promoted mycelial growth (Fig. 1B).

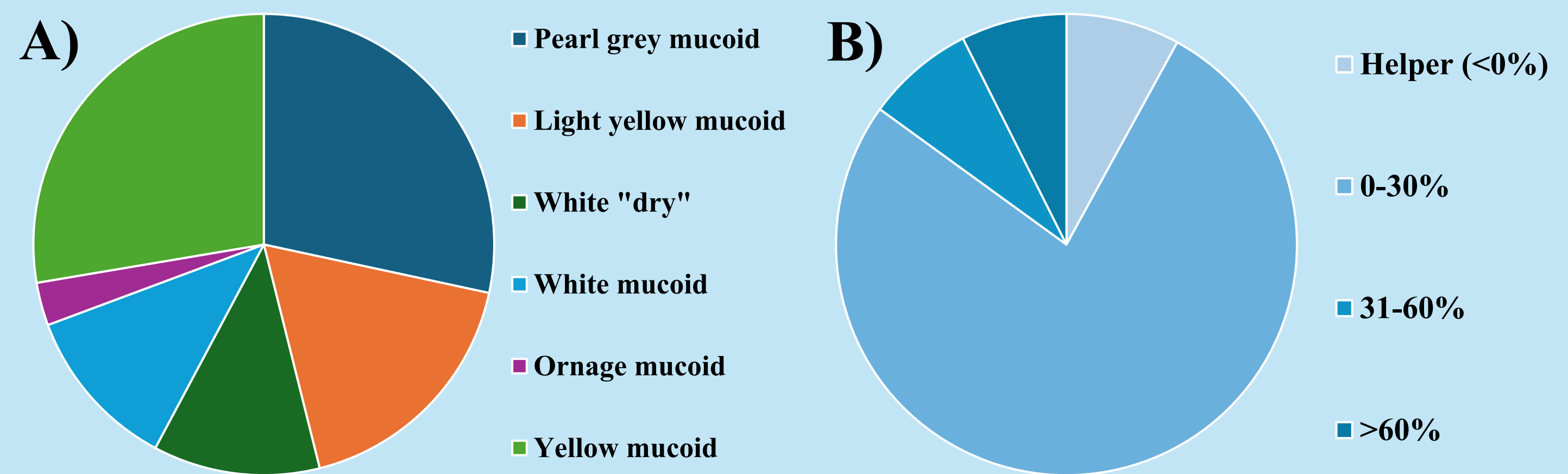


Fig. 1: Collection of bacterial isolates from the pear, cv. Abbé Fétel carposphere: A) morphological features and B) *in vitro* mycelia growth inhibition (MGI; %)

MOLECULAR IDENTIFICATION AND IN VITRO ASSAY AGAINST SIX S. VESICARIUM STRAINS

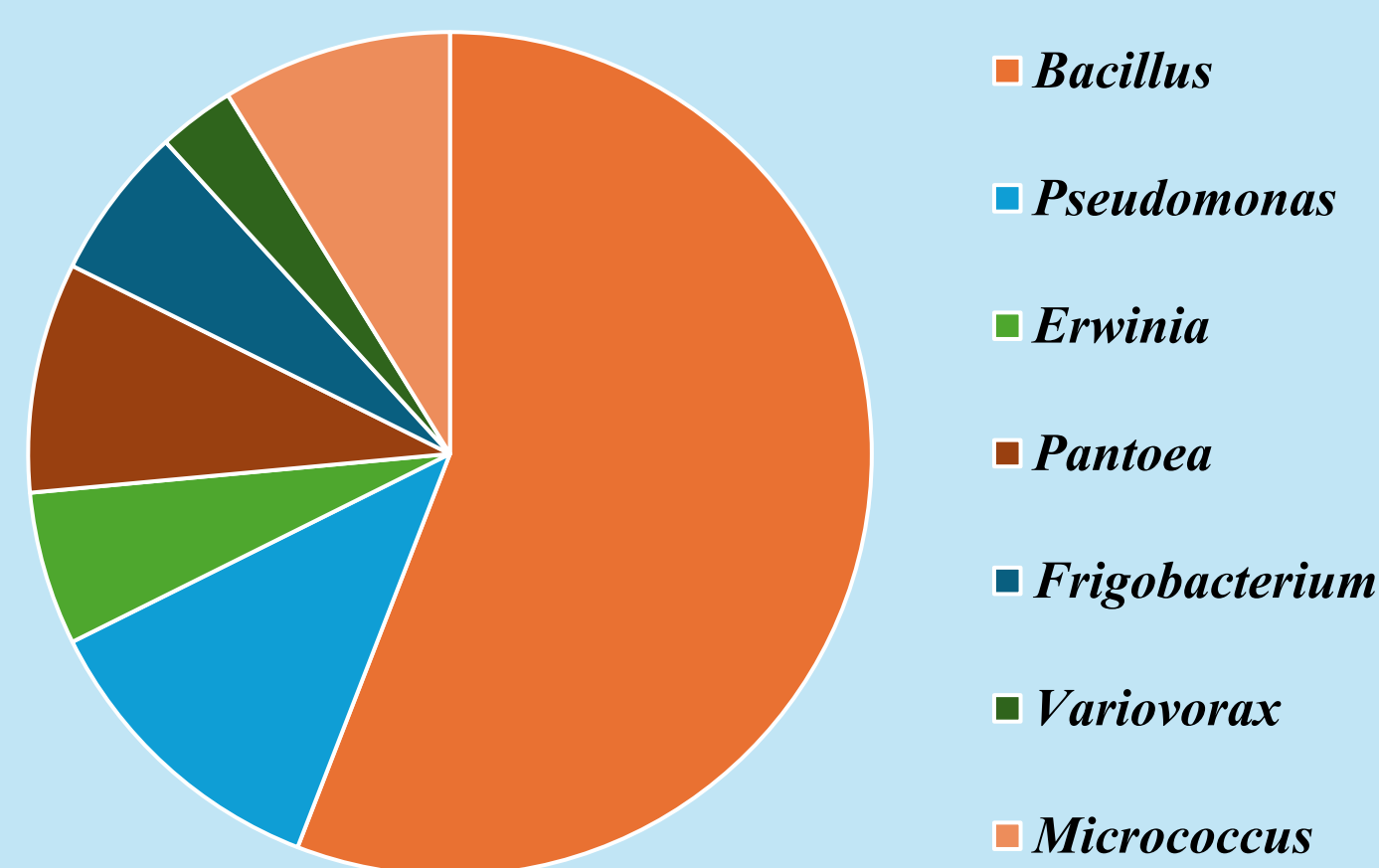


Fig. 2: Taxonomic genera attributed to the bacterial isolates

A selection of 34 isolates, chosen based on their morphology and MGI against *Sv*, were taxonomically identified by partial sequencing of 16S rRNA gene. BLAST analysis confirmed their identity as members of 7 genera (Fig. 2). Eight isolates, representing the identified bacterial genera, were tested *in vitro* against six different *Sv* isolates. Their antagonistic performance slightly varied depending on the specific *Sv* isolate tested (Fig. 3).

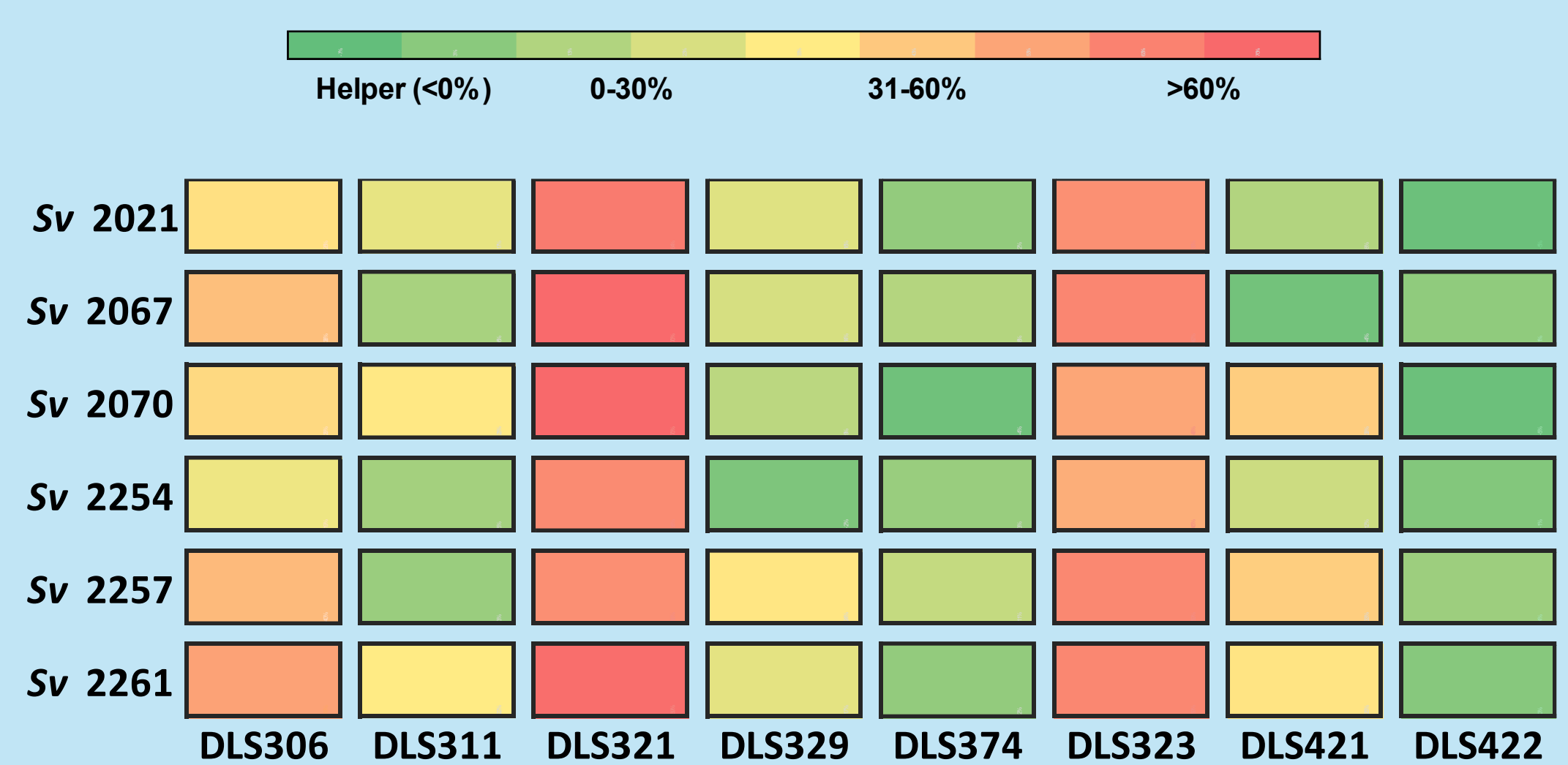


Fig. 3: Antifungal activity (MGI%) of 8 bacteria vs. 6 *Sv* strains isolated from symptomatic pear cv. Abbé Fétel during the 2023 and 2024 cropping season

BIOASSAY ON BSP DISEASE CONTROL

A set of 8 putative *mBCAs* with different *in vitro* antagonistic activity and representing the 7 genera were tested for their biocontrol activity against *Sv* on detached fruits (Fig. 4). The bacterial strains DLS311 (*Pantoea* spp.), DLS321 and DLS323 (*Bacillus* spp.), and DLS329 (*Pseudomonas* spp.) were the most effective *mBCAs* and significantly reduced brown spot of pear (BSP) severity on fruits by up to 80% (Fig. 5). Interestingly, some isolates like DLS329, that showed a slight *in vitro* inhibition of *Sv* growth, exhibited a strong protective effect against BSP in fruits.



Fig. 4: Bioassay evaluation of BSP disease control on detached pear cv. Abbé Fétel

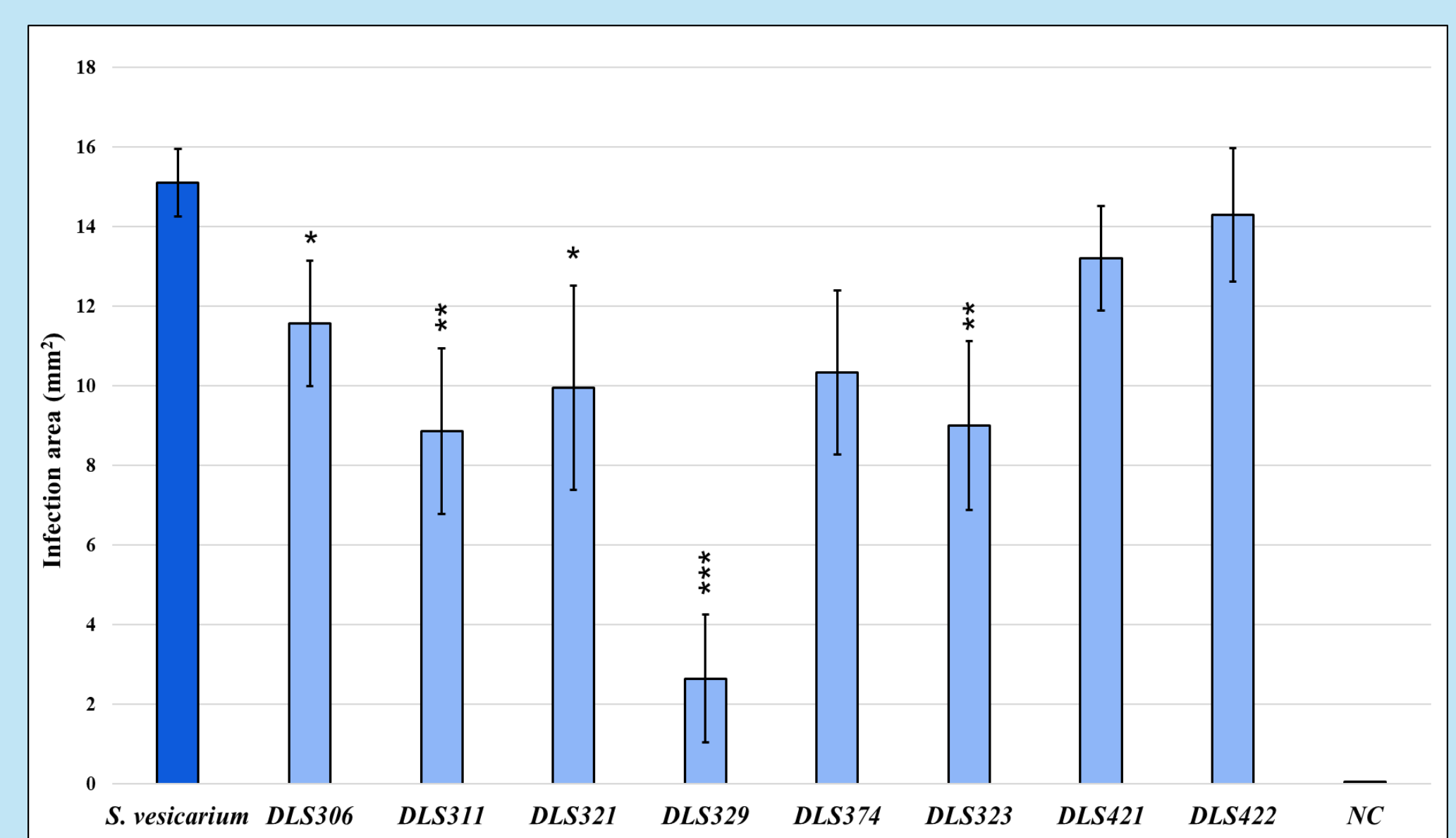


Fig. 5: BSP disease control on detached pear cv. Abbé Fétel. Student's t test (*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001)

CONCLUSIONS

To control BSP, our findings highlight a set of promising *mBCAs*, belonging to the genera *Pseudomonas*, *Pantoea* and *Bacillus*. Intriguingly, the results of *in vitro* and pear fruit bioassay were not always correlated. Further studies are needed to investigate the mechanisms by which these *mBCAs* might exert their protective effects against *Sv* and the possible role of "helpers" in fostering directly or indirectly the *Sv* ability to infect pear.

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