



Biological and integrated control of plant pathogens

From single microbes to microbiomes targeting One Health

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Characterization of *Stemphylium vesicarium* isolates from pear orchards in Emilia Romagna (Italy) and assessment of potential microbial biocontrol agents

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INTRODUCTION

Stemphylium vesicarium (Sv), the causal agent of brown spot of pear (BSP), is responsible for important economic losses in the main producing areas of Italy, such as in the Emilia Romagna region, on the highly susceptible pear cultivar Abbé Fétel. Currently, the disease pressure and the development of Sv resistance traits to chemical fungicides, call attention to the need for sustainable management strategies. Our study aimed to characterize Sv isolates from Emilia Romagna orchards, at a molecular and pathogenic level, and to search for carposphere epiphytes as possible microbial biocontrol agents (mBCAs).

MATERIALS AND METHODS

In summer 2023 and 2024, fungal isolation were carried out on diseased pear fruits, cv. Abbé Fétel, in four Emilia Romagna orchards. The isolates were morphologically characterized and molecularly identified by means of the partial sequencing of ITS, GAPDH and EF regions and phylogenetic analyses. The BSP severity was assessed for each Sv isolate on detached fruits. The antimicrobial activity of four bacterial isolates from the pear carposphere was tested by: (i) *in vitro* assays against Sv, both in dual culture and double plate assays and (ii) bioassay on detached fruits.

RESULTS AND DISCUSSION

From symptomatic pear fruits, a set of 10 Sv isolates were collected and molecularly identified: BLAST analysis displayed 99–100% identity with the ex-type strain Sv ATCC 18521^{EX-T}. Among them, nine strains clustered together with the Sv ATCC 18521^{EX-T}. Besides, only Sv 2261 clustered separately in a second clade, despite a very low genetic distance (Fig. 1A). Bioassay on detached fruits showed the presence of two Sv groups with “high” (7 strains) and “low” (3 strains) virulence (Fig. 1B). Bacterial isolates from pear carposphere were identified as members of the genera *Bacillus* (DLS188, DLS321 and DLS323) and *Pseudomonas* (DLS329). *Bacillus* sp. DLS321 was the most effective in reducing Sv growth in dual culture assay (Fig. 2 A-C). Meanwhile, *Pseudomonas* sp. DLS329 was the most performing in both double plate assay and bioassay on detached fruits, with a reduction rate up to 29.89% (Fig. 2 D-E), and 80.37%, respectively (Fig. 3; Table 1). Further studies are needed to investigate the mechanisms by which these promising mBCAs might exert their adverse effects against Sv. Future experiments will focus on *in planta* validation to confirm their effectiveness in controlling BSP.

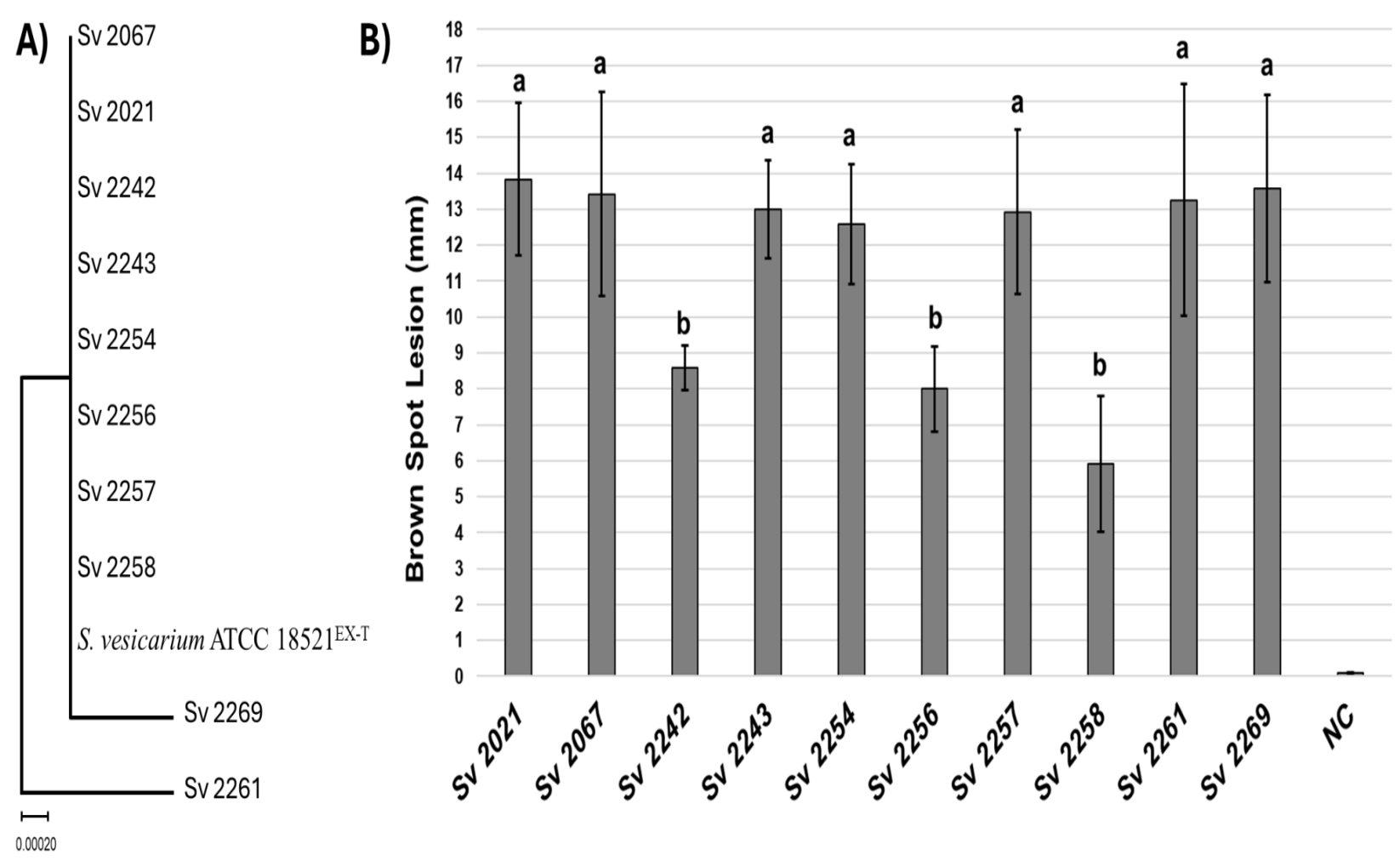


Figure 1. A) Phylogenetic tree based on combined ITS, GAPDH and EF sequences of the 10 Sv strains. B) Evaluation of BSP disease severity on detached pear fruits infected with Sv.

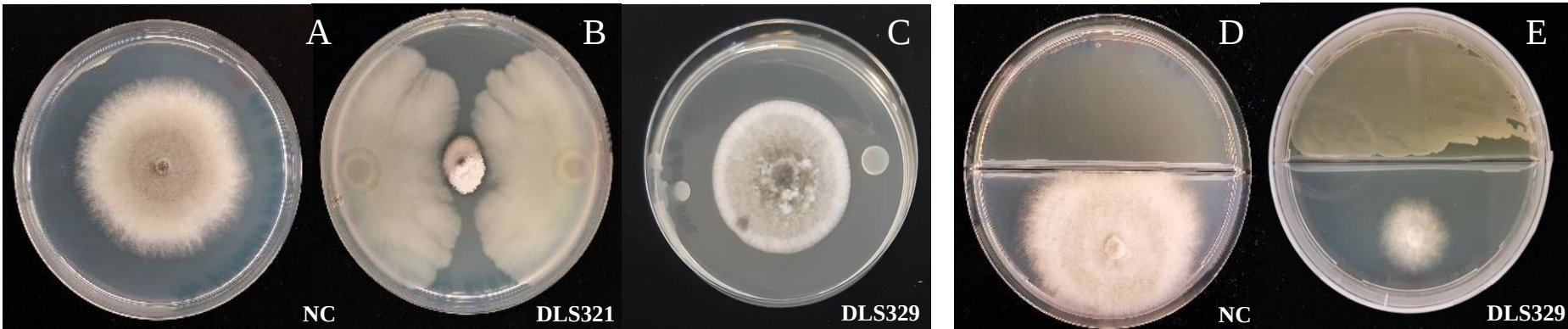


Figure 2. *In vitro* dual culture (A-B-C) and double plate assays (D-E) of representative bacterial isolates against Sv.



Figure 3. Evaluation of BSP disease control on detached pear fruits.

Table 1. Identity and effect of 4 bacterial isolates on Sv 2021 mycelial growth inhibition (MGI) *in vitro* (dual and double plate assays) and BSP disease severity reduction on detached pear fruits.

Strain	Identity (BLASTn 16S rRNA)	Dual plate MGI (%)	Double plate MGI (%)	BSP severity reduction on detached fruits (%)
DLS188	<i>Bacillus</i> sp.	72.41 ^a	16.67 ^c	26.30 ^c
DLS321	<i>Bacillus</i> sp.	74.29 ^a	24.46 ^b	11.48 ^c
DLS329	<i>Pseudomonas</i> sp.	21.00 ^b	29.89 ^a	80.37 ^a
DLS323	<i>Bacillus</i> sp.	70.11 ^a	22.96 ^b	33.33 ^b

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