XXIX Congress SIPaV
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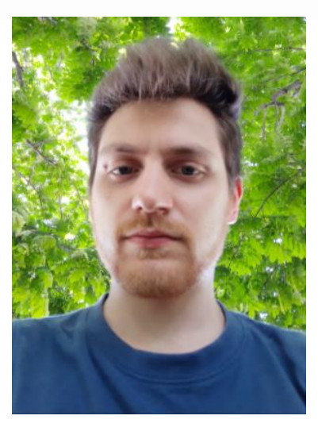
Identification and characterisation of microbial isolates from pear carposphere and their possible role in brown spot disease management



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INTRODUCTION

Brown spot of pear (BSP) caused by *Stemphylium vesicarium* (*Sv*) is one of the most important fungal diseases in Europe, as it may cause more than 90% yield losses. Disease control is based on synthetic pesticide in integrated pest management (IPM) and on copper in organic farming; however, one of the EU Green Deal goals is to reduce synthetic pesticide application by 50% within 2030. To foster the development of sustainable and biological control methods for the IPM of BSP, studies on the carposphere microbiota helped us to identify bacterial and yeast epiphytes suitable to be exploited as microbial biocontrol agents (mBCAs) against *Sv*.

SAMPLING

During summer 2023, pear fruits cv. Abate Fétel were collected from orchards located in five Emilia Romagna provinces (Modena, Bologna, Ferrara, Ravenna, and Forlì-Cesena) under organic management and IPM.

ISOLATION & MORPHOLOGICAL CHARACTERISTICS

From carposphere, a total of 233 yeasts (A) and 359 bacteria (B) were isolate and morphologically characterised. (Fig. 1) to be tested *in vitro* against *Sv* DLS2021 (UniMORE collection)

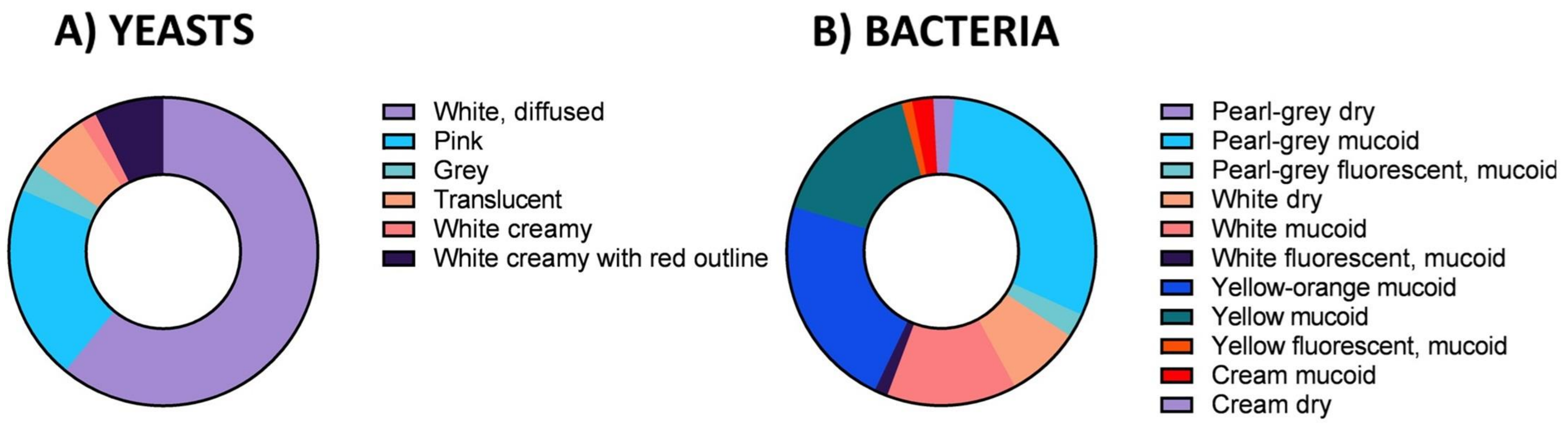

Fig. 1: Morphological features of yeast (A) and bacteria (B) isolated from the carposphere of pear cv. Abate Fétel

In vitro ANTAGONISTIC ACTIVITIES

592 isolates were *in vitro* screened with dual plate assay against *Sv* DLS2021 (UniMORE collection) (Fig. 2); most of them were highly variable in reduction of *Sv* mycelial growth (RMG) and a small number of isolates showed helper activity (Fig. 3). The 18 isolates (9 bacteria and 9 yeasts) showing a RMG higher than 65% were than taxonomically identified.

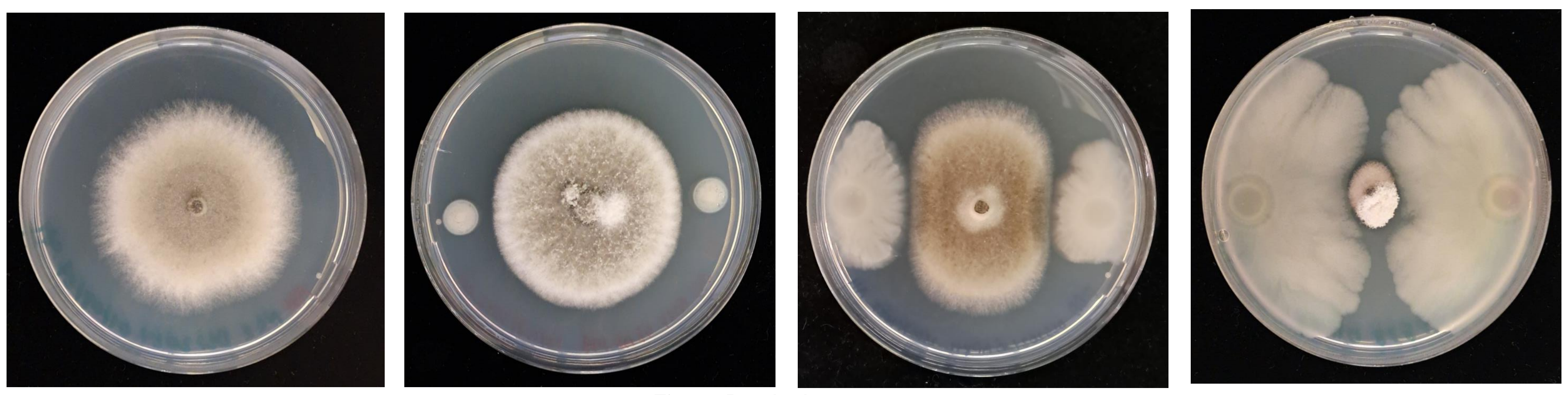

Fig. 2: Dual-plate assay

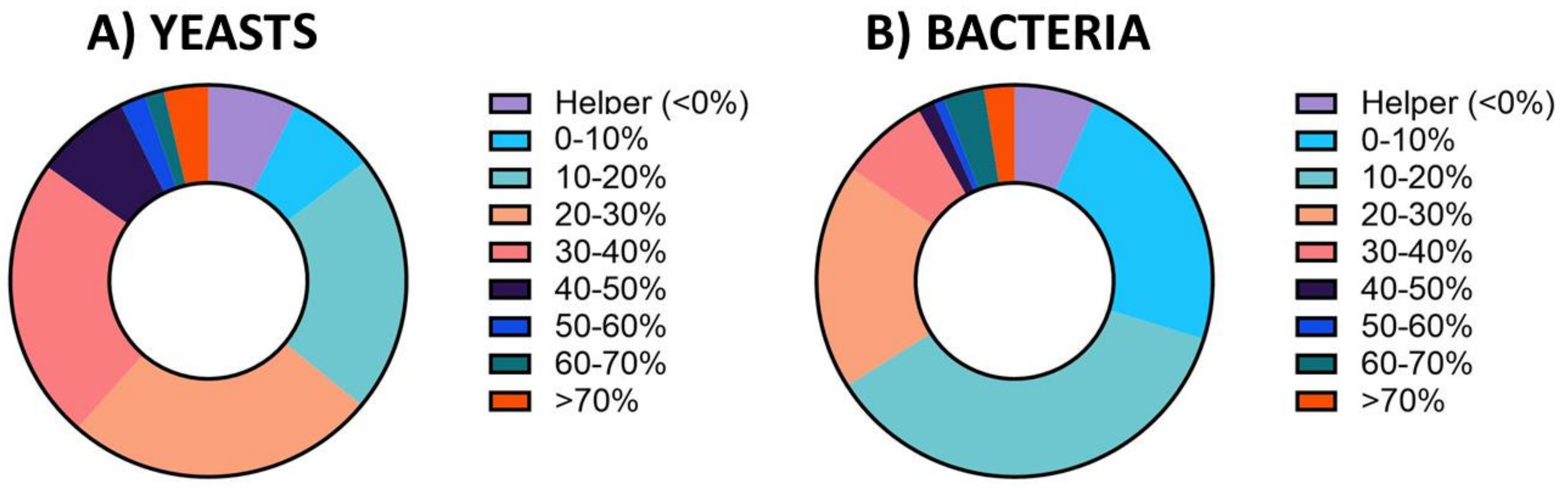

Fig. 3: Antifungal activity of yeasts (A) and bacteria (B) isolated from the pear, cv. Abate Fétel carposphere

MOLECULAR IDENTIFICATION

Sequencing and phylogenetic analysis of ITS regions (spanning ITS1, 5.8S rRNA and ITS2) showed that the 9 most antagonistic yeasts (RMG > 65%) belonged to the species *Aureobasidium pullulan* (AP198; AP203; AP351), *Metschnikowia pulcherrima* (AP184), *Rhodotorula babjevae* (AP196; AP201), *Rhodotorula glutinis* (AP24; AP202), and *Sporobolomyces patagonicus* (AP86), respectively. For the 9 bacterial isolates (RMG > 65%), the partial sequencing and NCBI blast of the *16s rRNA* gene confirmed their identity as members of the genera: *Bacillus* (AP 27; AP105; AP180; AP367; AP559), *Pseudomonas* (AP114; AP566), *Pantoea* (AP511), and *Erwinia* (AP506).

In vivo ANTAGONISTIC ACTIVITIES

A selection of 12 putative mBCAs with the highest antagonistic activity *in vitro* and representing the 5 yeast species and the 4 bacterial genera (Fig. 4), were checked for their biocontrol activity *in vivo* against *Sv* DLS2021 on detached fruits, under laboratory conditions (Fig. 5). The bacterial strain AP559 (*Bacillus* sp.) and the yeast strain AP184 (*Metschnikowia pulcherrima*) resulted the most active mBCAs, reducing the BSP severity on fruits up to 55%.

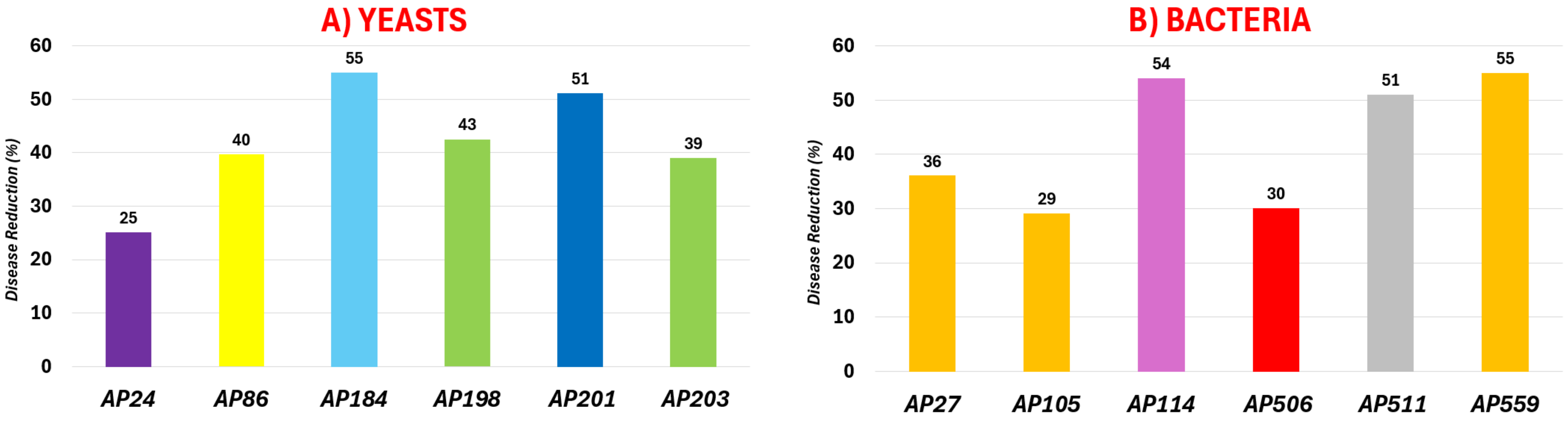

Fig. 4: Disease severity reduction by yeasts (A) and bacteria (B)

Fig. 5: Pathogenicity test on pear, cv. Abate Fétel

CONCLUSIONS

Our study may help to understand the epidemiological importance of culturable microbiota residing on the pear carposphere and the best-performing mBCAs will represent a sound basis for the development of a microbial biopesticide for implementing a sustainable management of BSP. In 2024, a second-year of research activities is aiming at isolating new microbes from pear cv. Abate Fétel carposphere and at identifying novel promising mBCAs: such search is currently ongoing. Further steps of the study will validate the most promising mBCAs against additional *Sv* strains isolated from pear cv. Abate Fétel in different growing seasons and Emilia Romagna provinces.

Acknowledgements

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