Yeasts from pear cv. Abate Fétel carposphere as biocontrol agent candidates against *Stemphylium vesicarium*, the causal agent of the brown spot disease

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1. BACKGROUND

The European **Farm to Fork**, part of the **Green Deal**, aims to reduce chemical pesticides and promote eco-friendly solutions **like microbial biocontrol agents** (BCAs). Microbial biodiversity represents a fundamental resource for sustainable agriculture since plant microbiomes are promising bioreservoir of useful microorganisms for plant disease control. Brown spot disease (BSP), caused by *Stemphylium vesicarium*, leads to major yield losses in pear cv. Abate Fétel, especially in Emilia-Romagna.

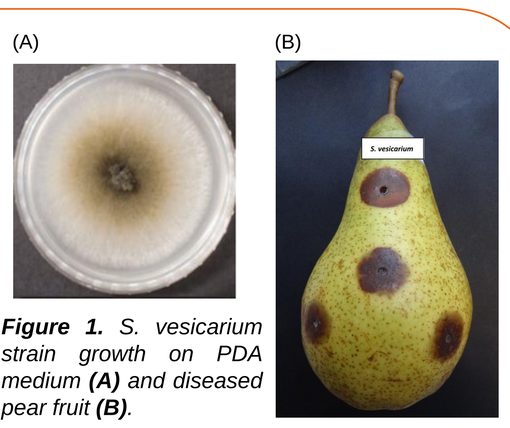


Figure 1. *S. vesicarium strain growth on PDA medium **(A)** and diseased pear fruit **(B)**.*

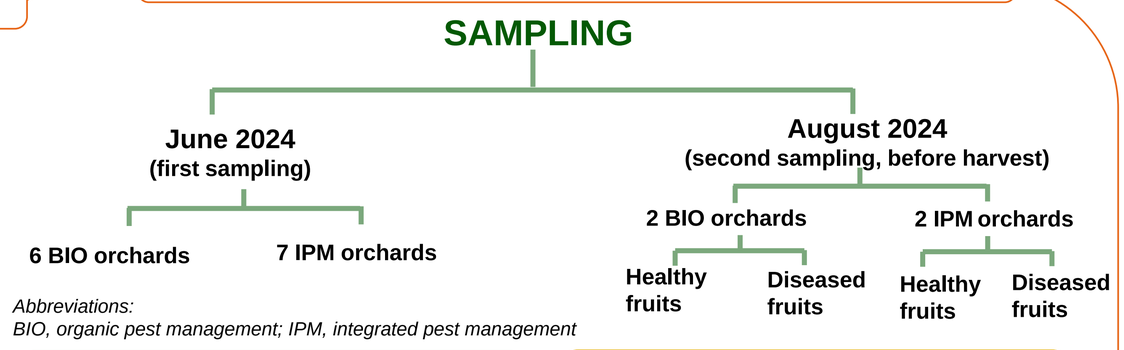
BSP integrated management (*e.g.,* sanitation practices, multiple fungicides sprays) resulted often low effective, calling the attention to the need for sustainable complementary or alternative strategies against *S. vesicarium.*

2. AIMS of the PROJECT

1. Study cultivable fraction of pear yeast carposphere

2. Identify novel yeast BCAs against *Stemphylium vesicarium*

3. SAMPLING and WORKFLOW



4. RESULTS

4.1 YEAST and BACTERIA COUNTS

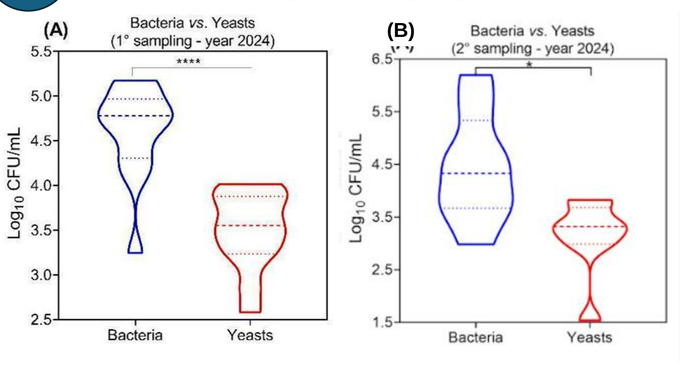


***Figure 2. Bacteria** and **yeast loads** in pear samples collected at the first **(A)** and second **(B)** sampling times during the cropping season 2024.*

Take-home message: In both samplings bacterial loads were significantly higher than yeast loads ($p < 0.05$).

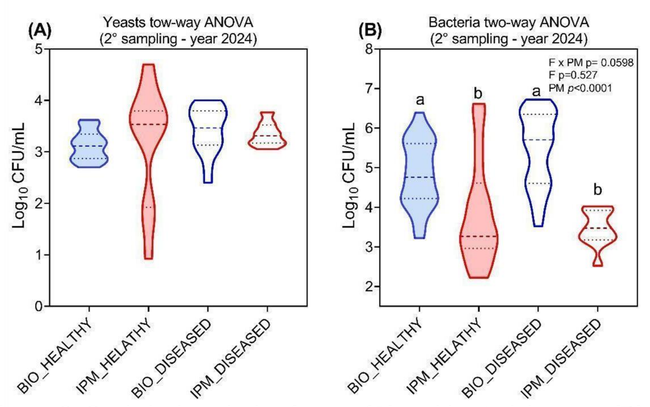


***Figure 3.** Statistical analysis of yeast **(A)** and bacterial counts **(B)** based on farm management (BIO vs. IPM) and healthy vs. diseased fruits.*

Take-home message: Bacterial counts were significantly higher in BIO orchards regardless the status of pear fruits ($p < 0.05$). Pest management and fruits *status* did not affect yeast counts.

4.2 DISTRIBUTION of YEAST MACROMORPHOLOGIES

Yeast isolates with **pink** and **white** macro-morphologies were the most frequently detected in pear carposphere.

Take-home message: A biobank of 260 morphologically characterized yeast isolates from pear cv. Abate Fétel carposphere has been established.

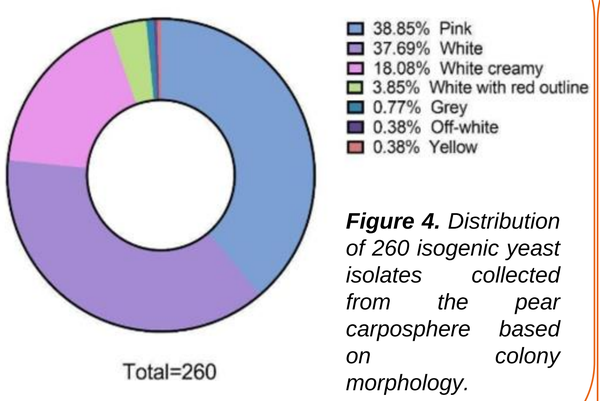


***Figure 4.** Distribution of 260 isogenic yeast isolates collected from the pear carposphere based on colony morphology.*

4.3 SCREENING of ANTAGONISTIC ACTIVITY

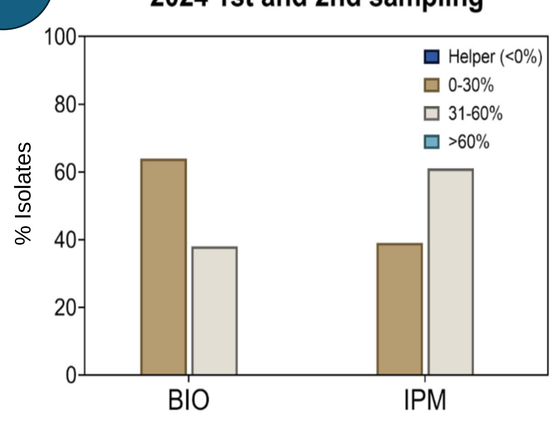


***Figure 5.** Distribution pattern of 144 yeast isolates sampled in 2024 from BIO and IPM orchards and scored through dual-plate assay for activity (MGI%) against S. vesicarium*

Yeast isolates from BIO orchards exhibited low antagonistic activities (MGI 0–30%), while those from IPM orchards showed moderate activity (MGI 30–60%). No highly antagonistic strains (MGI > 60%) or helper phenotypes were detected in either IPM or BIO orchards.

Take-home message: BIO and IPM orchards exhibited a mirror distribution of MGI percentage values.

Abbreviations: MGI %: percentage of mycelium growth inhibition through in vitro dual plate assay.

4.4 SELECTION of the BEST BCA CANDIDATES

Isolates with a white (lilac) and red outline (dark purple) morphologies showed the highest antagonistic activity against *S. vesicarium*, followed by isolates with a pink morphology (dark pink), indicating a possible association between colony appearance and biocontrol potential.

Selection criteria:
- Antagonistic activity
- Morphological diversity
- No redundant clones

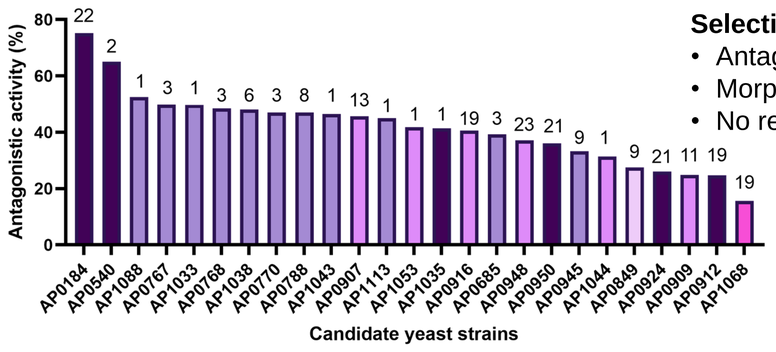


***Figure 6.** Selection of 25 best yeast BCA isolates. The pool Includes 23 new candidates and 2 reference strains from 2023 sampling (AP0184 and AP0540). Antagonistic activity was measured as the percentage of mycelium growth inhibition (MGI %) .*

4.5 SPECIES ATTRIBUTION THROUGH ITS SEQUENCING

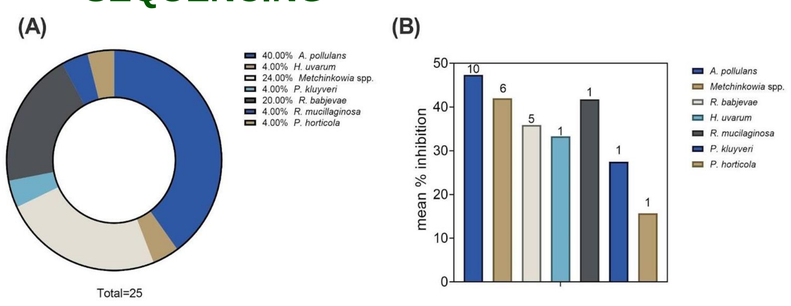


***Figure 7.** Pie-chart of candidate BCA species **(A)** and distribution of mean antagonistic activity **(B)** against S. vesicarium for each yeast species.*

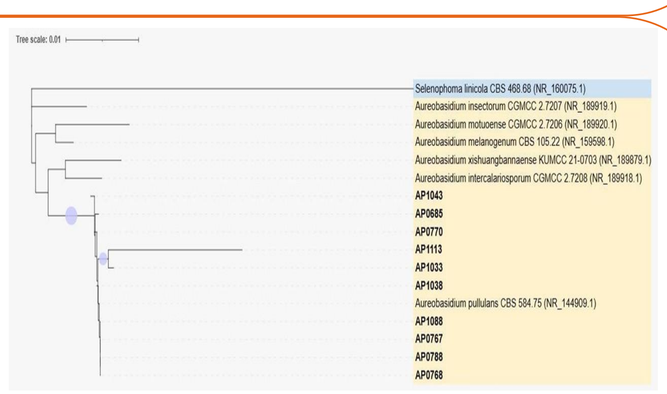
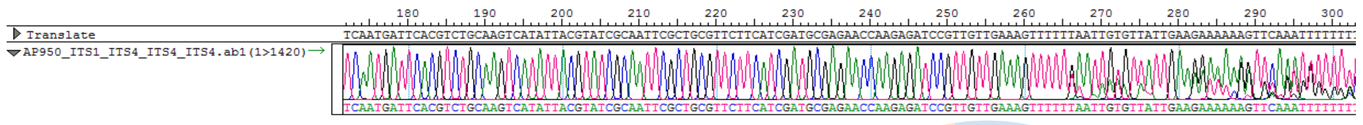


***Figure 8.** Neighbor-Joining phylogenetic tree of ITS sequences of BCA candidates belonging to Aureobasidium pullulans.*

Take-home message: ***Aureobasidium pullulans*** was the most represented and effective BCA species, followed by ***Metschnikowia*** *spp.*, ***Rhodotorula babjevae***, ***Pichia kluyveri***, ***Hanseniaspora uvarum, R. mucilaginosa,*** and ***Papiliotrema horticola.***

4.6 rDNA HETEROGENEITY in *Metschnikowia spp.*



Cloning of rDNA amplicons was necessary to resolve direct sequencing failure of ITS region in strains with red-outlined morphotypes. Phylogenetic analysis of cloned sequences revealed intragenomic ITS diversity within the *Metschnikowia pulcherrima* complex. AP0184 clone 2 clustered with *M. pulcherrima* and *M. andauensis*, while clone 13 grouped with *M. chrysoperlae*. AP0540 clones 31 and 33 formed a distinct branch, suggesting a novel ITS variant.

Take-home message: rDNA heterogeneity hampers species identification using the rDNA barcode in *Metschnikowia* spp. and suggests potential hybridization events.

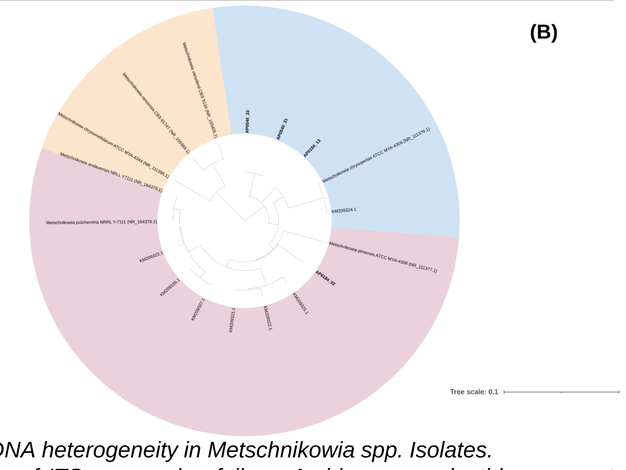


***Figure 9.** rDNA heterogeneity in Metschnikowia spp. Isolates.*
***(A)**Examples of ITS sequencing failure. Ambiguous nucleotides suggest intragenomic rDNA heterogeneity.*
***B)**Neighbor-Joining phylogenetic tree of ITS amplicons cloned in E. coli.*

5. CONCLUSIONS

- ❑ Culturomics-based survey of the pear carposphere microbiome revealed high yeast diversity. Dominant species included *Aureobasidium pullulans, Metschnikowia* spp*., Rhodotorula spp.,* and *Pichia kluyveri.*
- ❑ Notably, *Papiliotrema horticola* was identified for the first time in the pear carposphere.
- ❑ rDNA heterogeneity is frequent in *Metschnikowia pulcherrima* complex.
- ❑ *A. pullulans* is a high antagonistic species, whereas *Metschnikowia* spp. generally displays a middle antagonistic phenotype against *S. vesicarium*.
- ❑ For the most performing BCA candidates, *in planta* experiments are ongoing to investigate their ecological role and potential in controlling BSP.

Acknowledgements

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