



Bioprospecting of native *Metschnikowia pulcherrima* strains for biocontrol and aroma enhancement in the wine production chain

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Background

Metschnikowia pulcherrima is a well-characterized non-conventional yeast valued in oenology for its dual role as a bioprotective agent against spoilage microorganisms, including *Botrytis cinerea*, and for its contribution to wine aroma enhancement through extracellular enzymatic activities such as β -glucosidase and β -lyase [1] [2] [3].

Aim: This study aimed to screen the VUCC-DBT collection of native *M. pulcherrima* strains to identify candidates for sustainable application in viticulture and winemaking. In detail, high pulcherrimin production, low H₂S release, and strong β -glucosidase and β -lyase activities were evaluated by spot assay. Furthermore, their biocontrol potential against *B. cinerea* was assessed through *in vitro* and *in vivo* trials.

Enzymatic Evaluation:

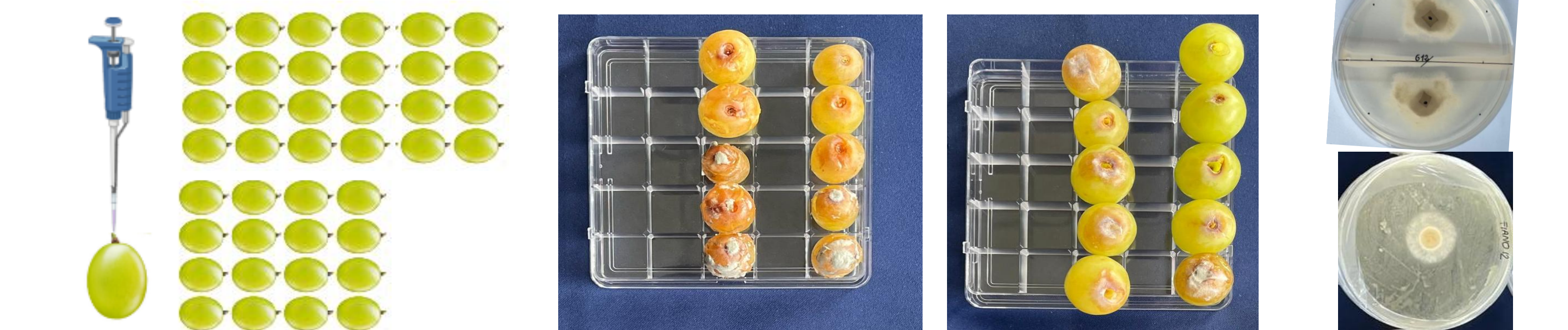
A total of 139 *Metschnikowia pulcherrima* strains from diverse origins were screened alongside three commercial strains (Level² Flavia, Level² Initia, Excellence B-Nature) and two *S. cerevisiae* strains (EC1118, Zymaflore X5).

- β -Glucosidase: Detected on esculin agar by dark halo formation after 72 h at 28 °C.
- β -Lyase: Assessed via growth on YCB-SMC medium at pH 3.5.
- H₂S production: Evaluated on BIGGY agar, colony color ranging from white (negative) to dark brown (positive).
- Pulcherrimin production: Scored on FeCl₃-supplemented YG medium after 72 h at 27 °C.

Strains selection

Biocontrol Assays:

- In vitro* dual culture: Co-inoculation of yeast and *B. cinerea* (TOB62, B.C.) on PDA; inhibition zones measured.
- VOC-mediated inhibition: Double-plate assay to assess yeast volatile effects on fungal growth.
- In vivo* test: Wounded grape berries inoculated with *B. cinerea* and treated with selected yeasts; infection severity evaluated after 5 days at 25 °C.



Conclusions

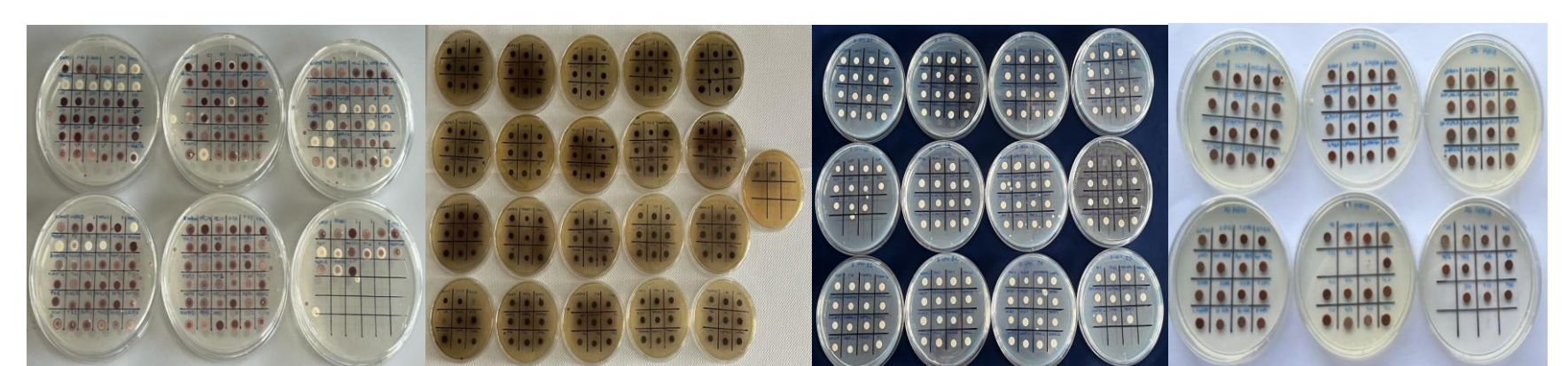
The enzymatic evaluation revealed substantial **phenotypic diversity** among *M. pulcherrima* strains. **Antagonistic activity** against *B. cinerea* was **highly strain-dependent**, with native strains that performed comparably to commercial strains: NLSFS4 emerged as the most consistent performer across conditions, while FM15 and MONL18 were effective *in vitro*, but showed reduced efficacy *in vivo*.

Take-home Message

Native strains of *M. pulcherrima* could play a dual role in the wine production chain showing **biocontrol** efficacy on grapes and potential capacity to **enhance** the **aroma** of wine.

Results

Enzymatic Evaluation:



- β -Glucosidase: 63% of strains.
- β -Lyase: 98% of strains.
- H₂S: Low producers (score ≤ 2.5): 41%, High producers (score ≥ 4.5): 13%.

The strains are grouped into 53 clusters and 25 have unique profile.

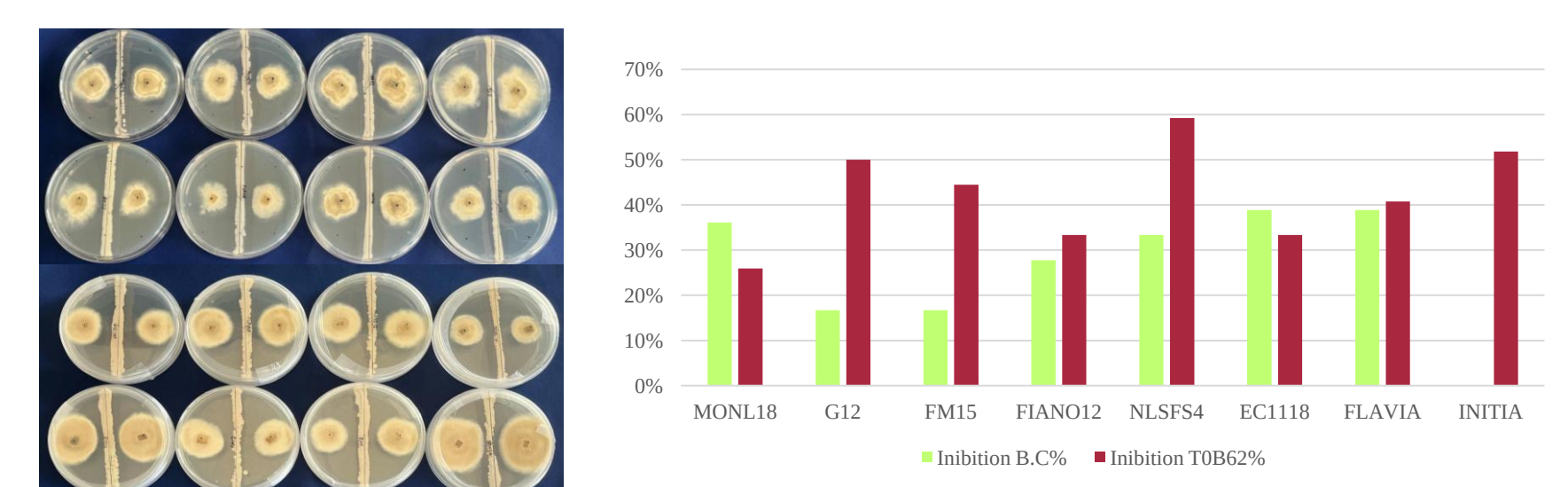
Most common profiles: IL, HI (each 6.6%).

Strains Selection

Strain code	Isolation			β -Glucosidase	β -Lyase	H ₂ S	Pulcherrimin
	Geographic area	Year	Source				
FIANO 12	Puglia, IT	2015	Fresh grape must	0	100	3.00	4
NLSFS4	Veneto, IT	2020	Withered grape must	100	100	2.5	4
G12	Veneto, IT	2020	Dried fruits;	100	100	2.5	4
FM15	Veneto, IT	2020	Withered grape must	100	100	2.00	3
MONL18	Veneto, IT	2019	Withered grape must	100	100	1.00	4

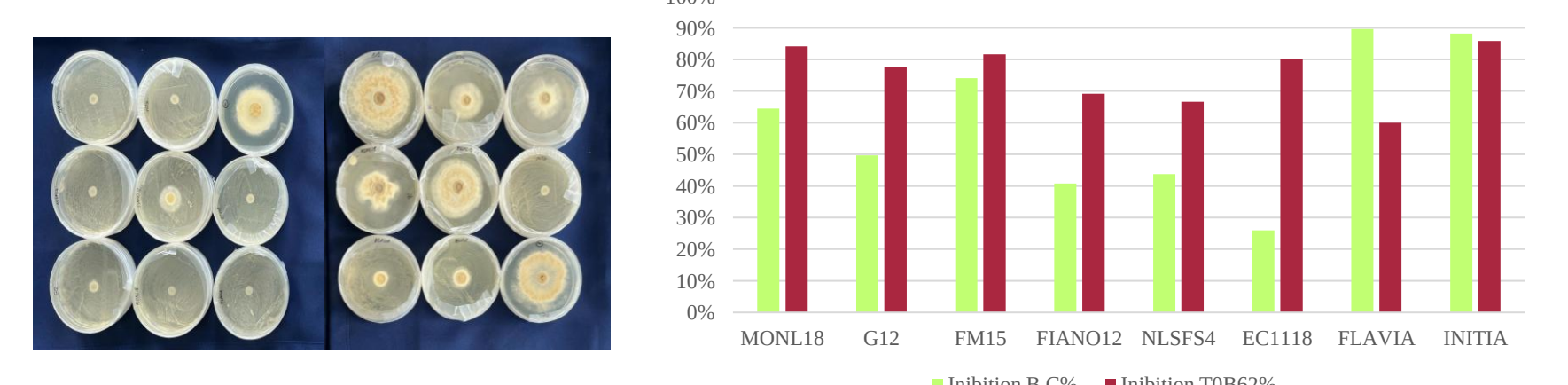
Biocontrol Assays:

- In vitro* dual culture:



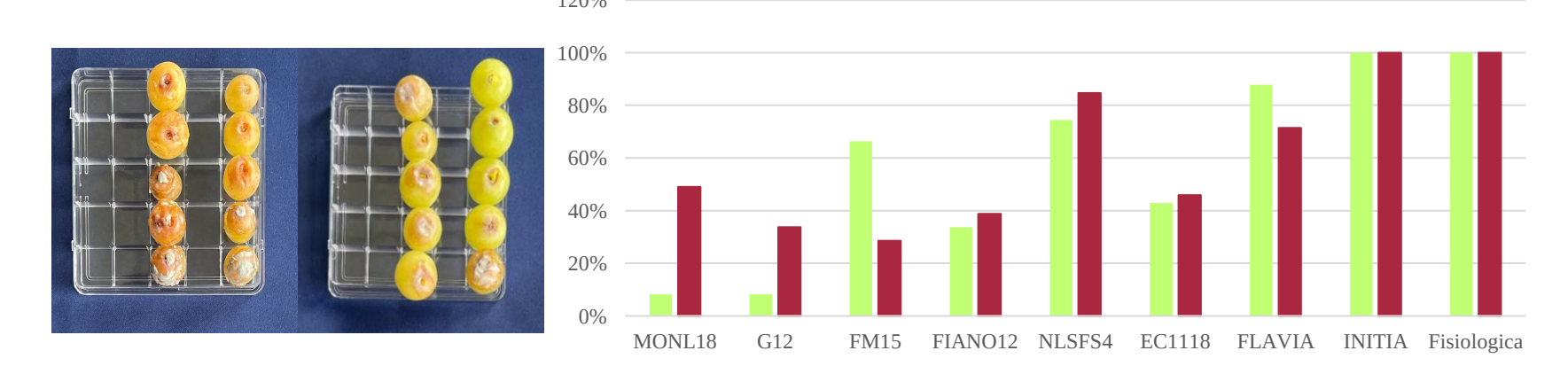
NLSFS4 and Level² Flavia showed the highest inhibition (up to 40%), confirming the more substantial biocontrol potential of certain strains, followed by G12(up to 30%).

- VOC-mediated inhibition:



Level² Flavia and Initia exhibited the highest inhibition rates against *B. cinerea* (up to 75%), confirming their strong biocontrol potential. Among the VUCC-DBT isolates, MONL18 and FM15 showed the best performance, reaching inhibition levels up to 70%.

- In vivo* test:



Level² Initia was the most effective strain, fully inhibiting both *B. cinerea* isolates (100%). Level² Flavia and NLSFS4 also showed strong activity (88–75% on B.C., 71–85% on TOB26). FM15 performed well on B.C. (66%), but poorly on TOB26 (29%).

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References

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