Flow Cytometry for Yeast Bioprospection

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Background

- *Metschnikowia pulcherrima* (*Mp*) is a non-*Saccharomyces* yeast used both to protect musts from microbial spoilage and to modulate the aromatic profile of wines.
- Using flow cytometry (FC), we characterized an autochthonous strain of *Mp* for use in the vinification of Chasselas must for the 2022 vintage.

Results

- FC with CFDA and Syto-41 dyes allowed to distinguish *Mp* from *Sc* in bench scale fermentations (Fig 1A-B).
- *Mp* cells showed high metabolic activity but did not consume sugars (Fig 1A-2A).
- *Mp* viability decreased upon inoculation of *Saccharomyces cerevisiae* (*Sc*) and initiation of alcoholic fermentation (AF; Fig 2B).

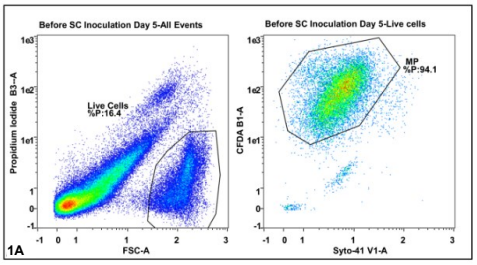

Fig.1A FC analysis of bench-scale fermentation. *Mp* cells are viable and active, as shown by CFDA staining.

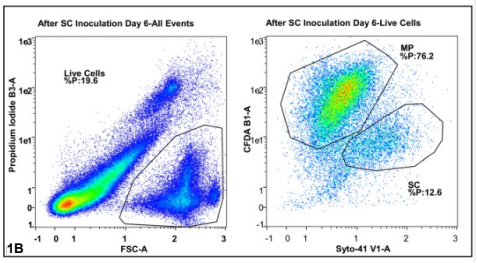

Fig.1B FC analysis of bench-scale fermentation. *Mp* and *Sc* cells (after inoculation) are identified by CFDA/Syto41 staining.

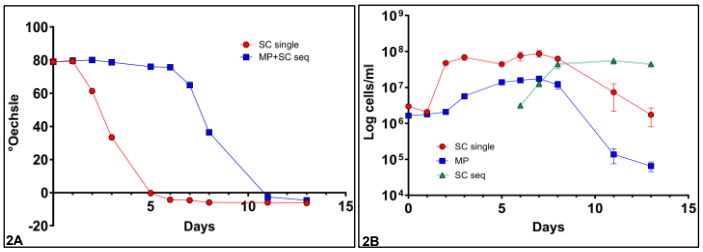

Fig.2A Sugar consumption as measured by densitometry. Fig.2B. FC follow-up of live cells (two fermentation conditions were prepared: SC and MP+SC after 5 days).

- In the 2022 harvest, 3 vinifications were prepared on a pilot scale (100L) : Classic cuve (Cc w/*Sc*), Pied-de-Cuve (PdC, spontaneous fermentation), Sequential Fermentations (*Mp*+*Sc* at day +5).
- *Mp* viability decreased after *Sc* inoculation (Fig 3A). Interestingly, yeasts in PdC did not show high metabolic activity as compared to the other conditions (Fig. 3B).

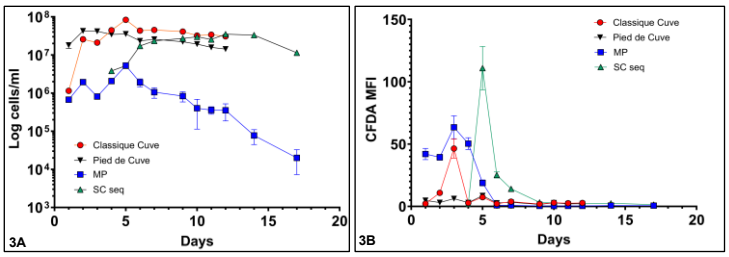

Fig.3A FC follow-up of live cells at pilot scale. 3B. Metabolic activity (as defined by CFDA fluorescence).

- DNA analysis revealed the complexity of PdC fermentation in terms of yeast populations and a possible bioprotective effect of *Mp* (Fig. 4).

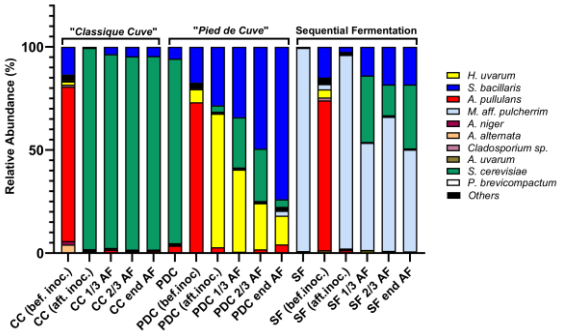

Fig.4 Relative abundance of yeasts in pilot vinifications at different time points (bef/aft. inoculum, 1/3, 2/3, end of AF).

- Sensory analysis showed that wine produced with *Mp* had more lactic flavor and was preferred over PdC (Fig. 5).

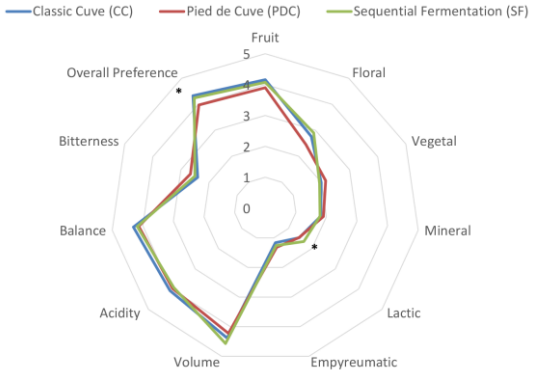

Fig.5 Results of sensory analysis. Overall preference: *p<.05 CC vs. PDC; p=.09 SF vs. PDC; Lactic: *p<.005 CC vs. SF; p<.005 PDC vs. SF.

- FC showed that *Mp* was viable and did not prevent *Sc* proliferation in bench-scale fermentations. This observation led us to perform pilot-scale assays in the 2022 vintage.
- *Mp* may have a bioprotective role, since no other yeast species (except the inoculated Sc) was detected in this condition compared to PdC.
- The wine produced with *Mp* had new characteristics compared to Cc and PdC, without drastically changing the character of the Chasselas.
- FC may guide the selection of microorganisms that can be effectively used in the winemaking process at different scales.

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