

Novel isolation campaign to explore microbial biodiversity associated to a *Pied de Cuve* for Amarone wine

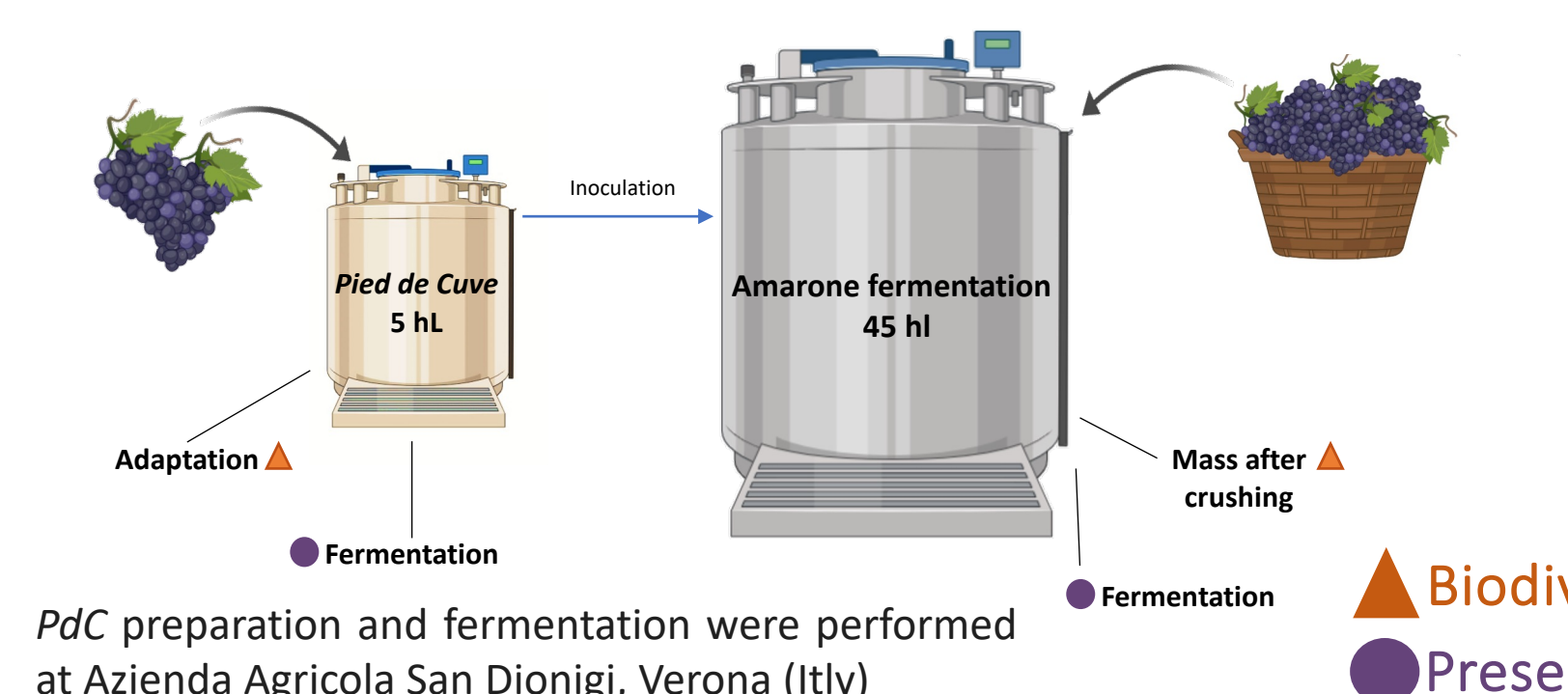
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Introduction

- Amarone della Valpolicella is a premium wine produced in the Verona province (Italy), characterized by a post-harvest grape withering process and the use of a unique blend of grape varieties (Corvina, Corvinone and Rondinella) [1].
- Alcoholic Fermentation can be controlled, with commercial active dry strains of *Saccharomyces cerevisiae* or spontaneous [2].
- Pied de Cuve* (PdC) is a winemaking technique where a limited amount of fermenting must be used to start the main fermentation. Spontaneous PdC fermentation, driven by resident microbiota, is considered to positively express the “*terroir*” concept [3].
- This study explores the microbial biodiversity of a PdC and “Amarone della Valpolicella” wine fermentation through culture-dependent methods, to determine presence, dominance and persistence of winemaking-related microbiota.

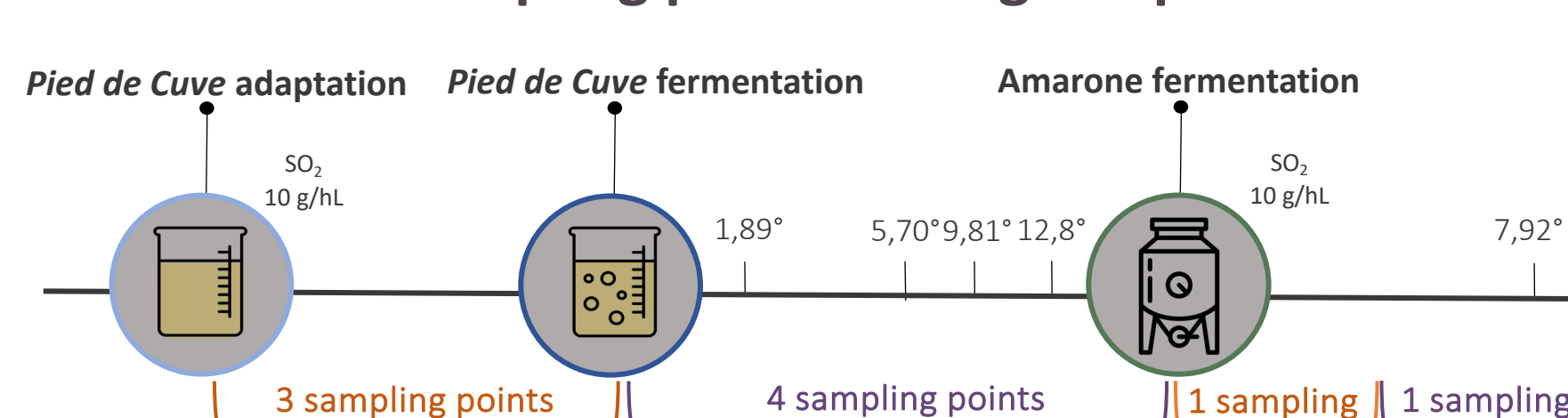
Materials and methods

Pied de Cuve inoculation for Amarone fermentation

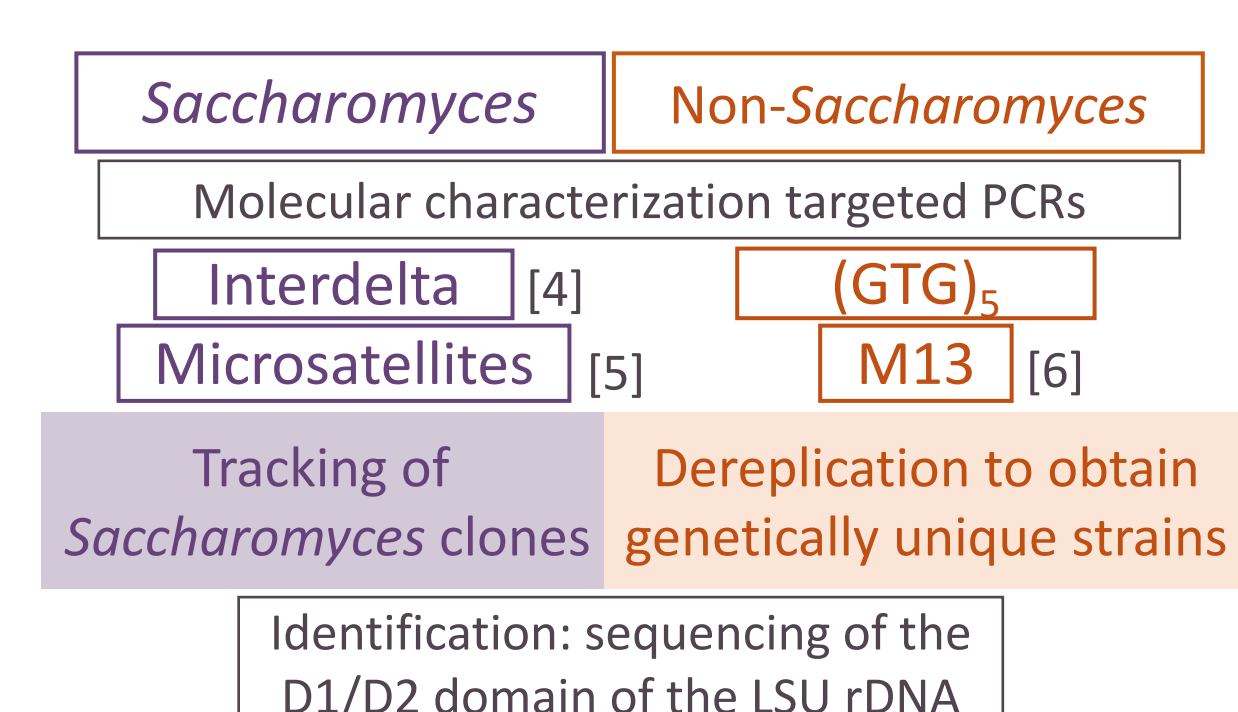


PdC preparation and fermentation were performed at Azienda Agricola San Dionigi, Verona (Italy)

Sampling points during the process



Molecular characterization of isolates



Results

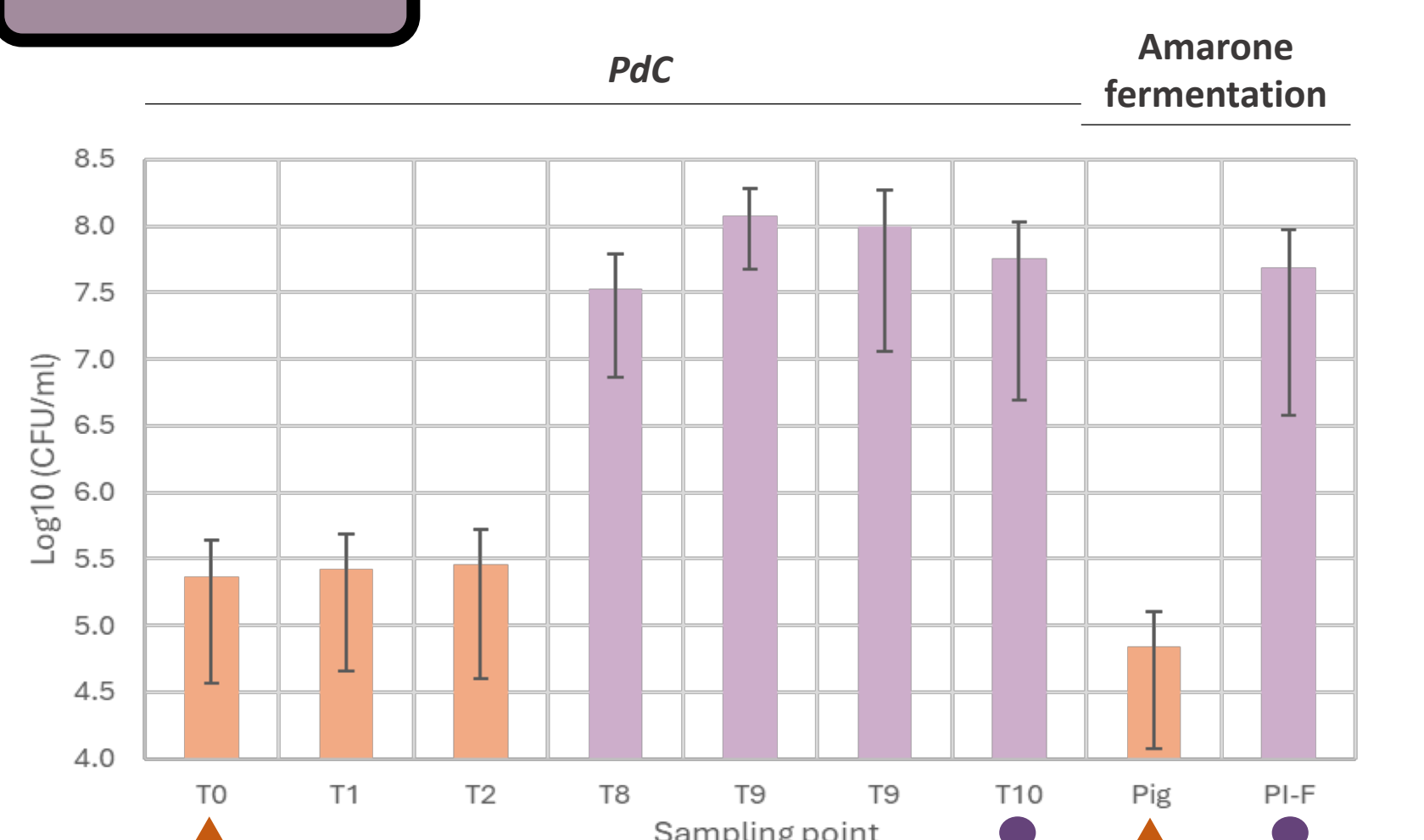


Figure 1. Yeast populations (CFU/mL) for *Saccharomyces* spp. (violet) and non-*Saccharomyces* spp. (orange) at the different sampling points.

Dereplication of non-*Saccharomyces* isolates (Figure 2) revealed 12 different genotypes, identified as *Starmerella bacillaris*, *Starmerella apicola*, *Hanseniaspora uvarum*, and *Hanseniaspora vineae*.

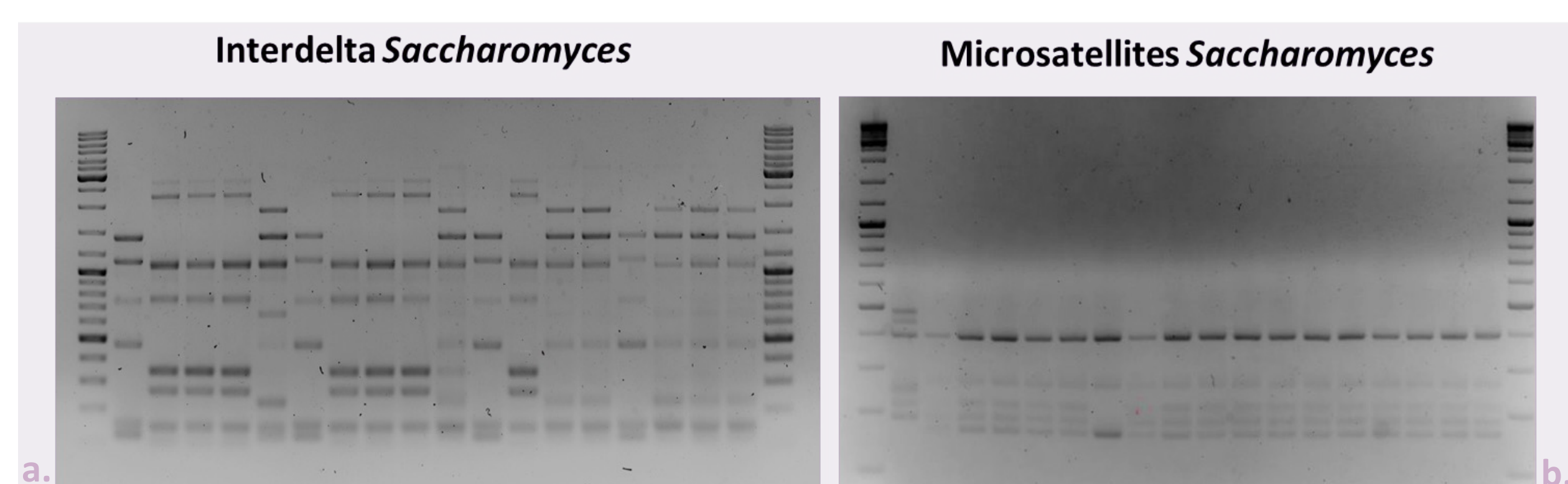


Figure 3. Comparison of *Saccharomyces* isolates fingerprinting using a. primers delta12-delta21 and b. microsatellites

The total population belonging from the first three samplings made during the process remained near the 5,5 log (CFU/mL) until the starting of the fermentation of PdC. In sampling point T8, at 1,89° alcohol (v/v), the total viable population reached up to 8 log (CFU/mL).

- ▲ 26 non-*Saccharomyces* isolates
- 88 *Saccharomyces* isolates (44 at T10 and 44 at PI-F)

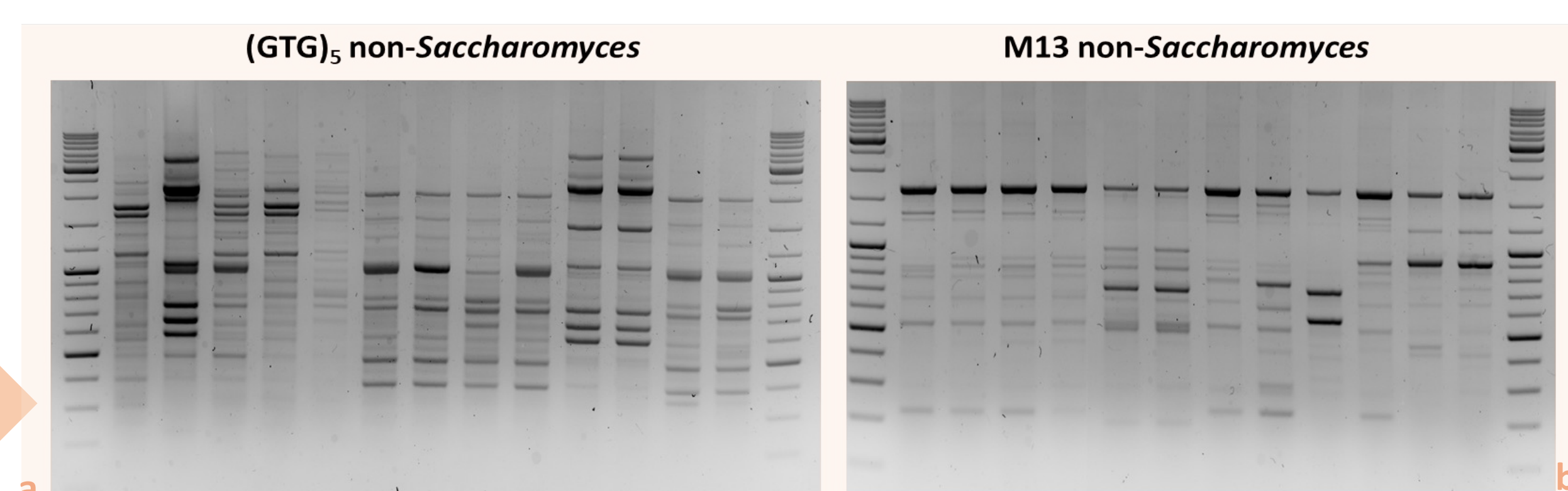


Figure 2. Comparison of non-*Saccharomyces* isolates fingerprinting using a. Rep-PCR (GTG)₅ and b. RAPD-PCR M13

Typing of *Saccharomyces* spp. isolates (Figure 3) resulted in 14 distinct profiles, and one profile (F) was dominant and persistent from PdC to must fermentation. It was confirmed to belong to *S. cerevisiae*.

Profile F accounted 32/88 isolates:

- 16 out of 44 isolates during the fermentation of PdC
- 16 out of 44 isolates during Amarone wine fermentation.

Discussion and conclusions

- Non-*Saccharomyces* yeasts isolated belong to the *Starmerella* and *Hanseniaspora* genera.
- Fermentation of Amarone wine inoculated with a spontaneous PdC contained different genotypes of *Saccharomyces* spp.
- One genotype of *Saccharomyces cerevisiae*, profile “F”, drove the fermentation process and persisted from PdC to the Amarone fermentation. The *Saccharomyces* spp. strains will be evaluated for enological properties.
- The molecular characterization of *Saccharomyces* spp., through analysis of both electrophoretic patterns, suggests that analysis of microsatellites could be used first for the dereplication, while interdelta for genotyping.

References

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