Detection of Lodderomyces elongisporus yeast in Rioja DOCa wine

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Introduction

Yeast identification in wineries is becoming an increasingly important task, since the species present in the initial inoculum used will determine the quality of the fermentation and, consequently, the organoleptic properties of the final product. In order to identify these microorganisms in wine, the RFLP technique (Restriction Fragment Length Polymorphism) was implemented as a routine analysis by the Oenological Station of Haro, La Rioja. This molecular method analyzes the sequences of the gene coding for the 5.8s ribosomal RNA, flanked by two ITS regions (*Internal Transcribed Spacers*). The fragment of gene amplified present a great genetic variability between strains of different species and is widely used by several authors in order to the identify a different yeast species [1].

Material and Methods

1. **Yeast isolation.**
- YEPD agar (Method OIV-MA-AS4-01).
2. **DNA Purificación and PCR.**
- DNA Purificación (GEN-IAL® Simplex® Easy Wine Kit, Ref: Q-300, R-Biopharm).
- PCR Method **(Figure 1)** [2].
  - Equipment: Heal force K640.
3. **Restriction Analysis.**
- Enzymes: *Cfo I* (ER1851), *Hae III* (ER0151) and *Hinf I* (ER0801), Thermo Sc.
- DNA Leadder 50pb (PanReac Ref: A8368) and Gene Ruler 50pb (Thermo Sc.™ Ref:10314340).
- E-Gel™ with SYBR™ Safe 4% (Thermo Sc. Ref: G401004)
  - Equipment: E-Gel™ Power Snap Electrophoresis. (Thermo Sc. Ref: G8300).

Figure 1. PCR-amplified rRNA gene region using ITS1 and ITS4 primers.

Results

Torulaspora delbrueckii, *Candida parapsilosis* and *Candida glaebosa*, among other species, were isolated during routine analysis in different wine samples. An important finding was the identification of *Lodderomyces elongisporus* in one of the wines analyzed because this species is usually masked as *Candida parapsilosis* **(Figure 2)**. The result was confirmed by sequencing of the D1/D2 fragment from the 26S rRNA gene.

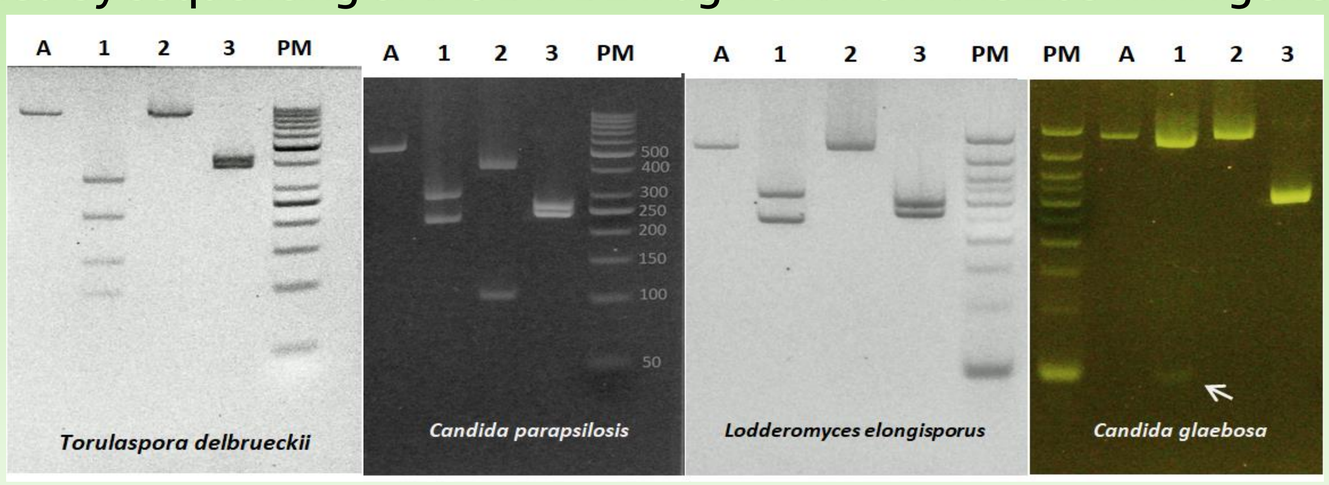

Figure 2.- Restriction patterns of the different yeast species:
(A) Amplicon
(1) Cfo I
(2) Hae III
(3) Hinf I
(PM) Gene Ruler 50pb and DNA Ladder 50pb

Conclusions

This finding suggests further studies with the aim to explore the establishment of this unconventional fermentative yeast species in the DOCa Rioja.
Lodderomyces elongisporus was reported in apple must in 2018 [3]. In wine, it was reported for the first time in 2019 as part of the fermentative microbiota, especially in the initial stage of wine fermentation, and may be involved in wine quality [4].

References

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