

Isolation, identification and characterization of natural yeasts for brewing

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Introduction

In recent years, there has been a worldwide increasing interest in craft beers and in microbreweries. Producers and consumers continuously require new beers with new aroma, flavours, alcohol or sugar content, etc. that can be in part conferred by using other yeast instead of the ones supplied by the yeast companies [1].

The objective of this study is to analyse the *Saccharomyces cerevisiae* biodiversity in Andalusia, studying them at the genetic as well as at the phenotypic level. Thus yeasts with different properties to those currently used in the brewing industry could be found. With this, we would be able to create a catalogue of autochthonous yeasts with different features for microbreweries, so they could chose the one that suits their procedures or their beer best, taking into account the fermentation conditions and the characteristics that they can contribute to the final product [2].

Yeast isolation

- Samples of wine must from Andalusian wineries were centrifuged and incubated in different conditions: grape must or malt extract, with and without hop and ethanol, at 16 and 22 °C in falcon tubes with airlocks (Figure 1A).
- The samples were periodically weighed to develop the growth curve based on the CO₂ released (Figure 1B).
- Samples were streaked and *S. cerevisiae* were identified by checking their inability to grow on lysine media (Figure 1C)

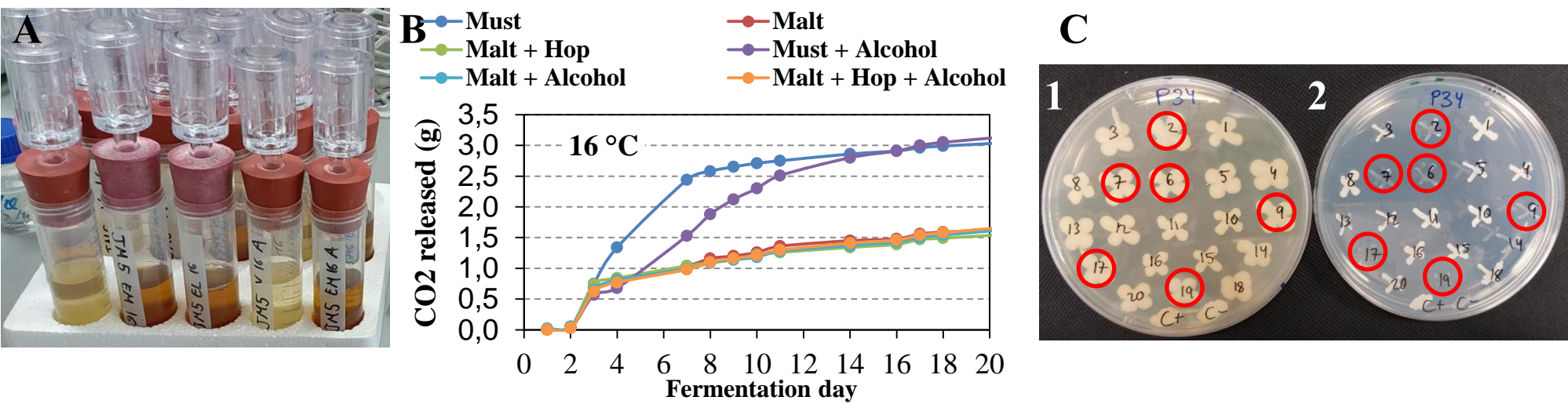


Figure 1. A. Samples during the fermentation process in falcon tubes with airlocks. B. Grow curve of one of the samples at 16°C. C. YPD plate (1) and YNB-Lysine plate (2). *S. cerevisiae* strains (lysine deficient) are marked in red.

Genetic identification: SSR-PCR

171 isolated *S. cerevisiae* (Table 2) were identified by Simple Sequence Repeats (SSR) analysis (Figure 2).

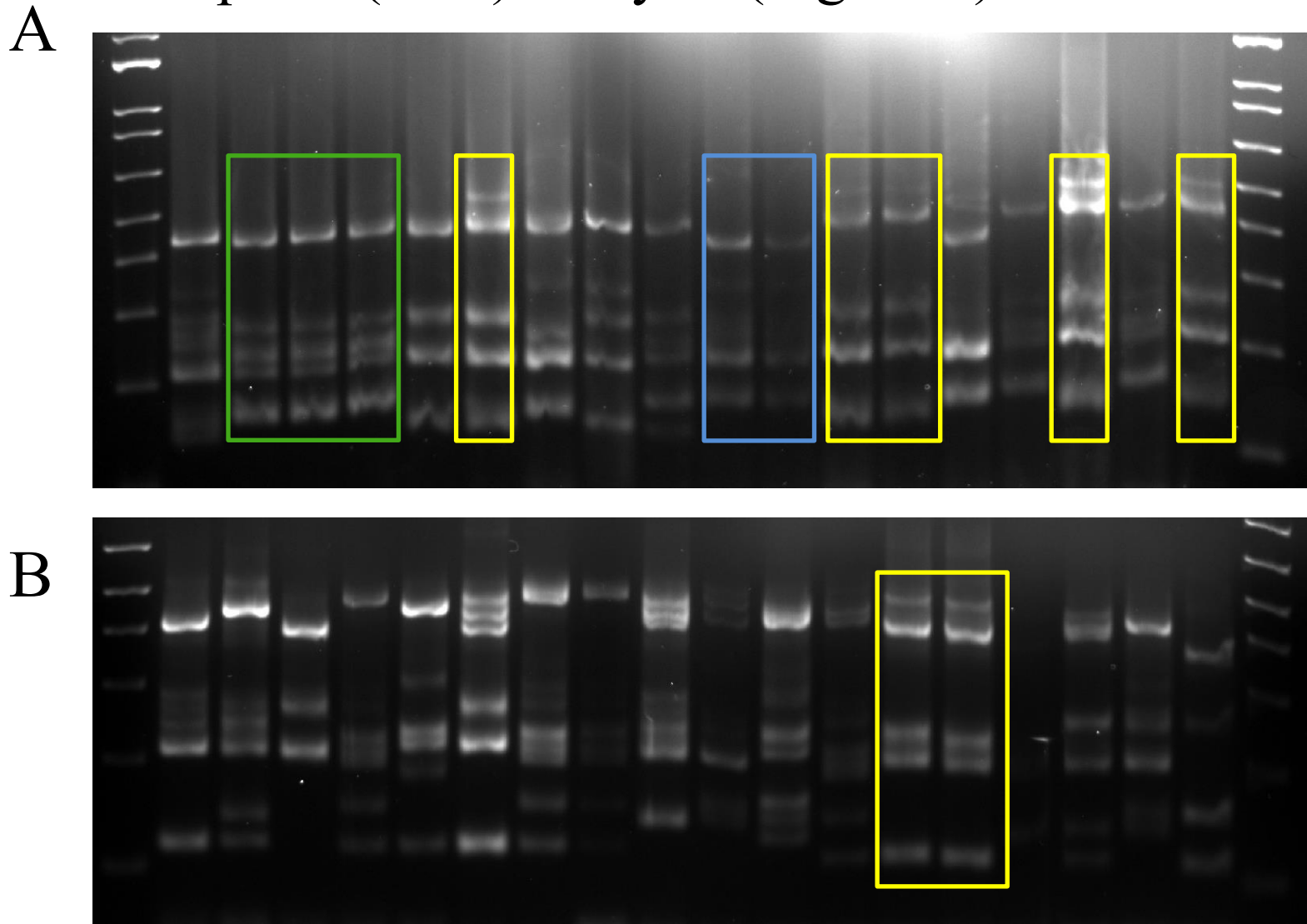


Figure 2. SSR patterns from 36 *S. cerevisiae* isolated strains. Samples with low (A) or high variability of patterns (B) are shown. Identical patterns are marked in colored boxes.

Results

Until now, 288 yeasts have been isolated, 171 of them were *S. cerevisiae*. (Table 2). Out of them 90 different putative strains have been identified by SSR-PCR (Figure 3).

Conditions	Temperature	Total yeasts	<i>Saccharomyces</i>
Must	16°C	30	25
Malt	16°C	20	6
	22°C	30	24
Malt + Hop	16°C	30	5
	22°C	30	14
Must + Alcohol	16°C	30	28
Malt + Alcohol	16°C	30	7
	22°C	30	26
Malt + Hop + Alcohol	16°C	28	10
	22°C	30	26
Total		288	171

Table 2. Summary of different conditions and yeasts isolated

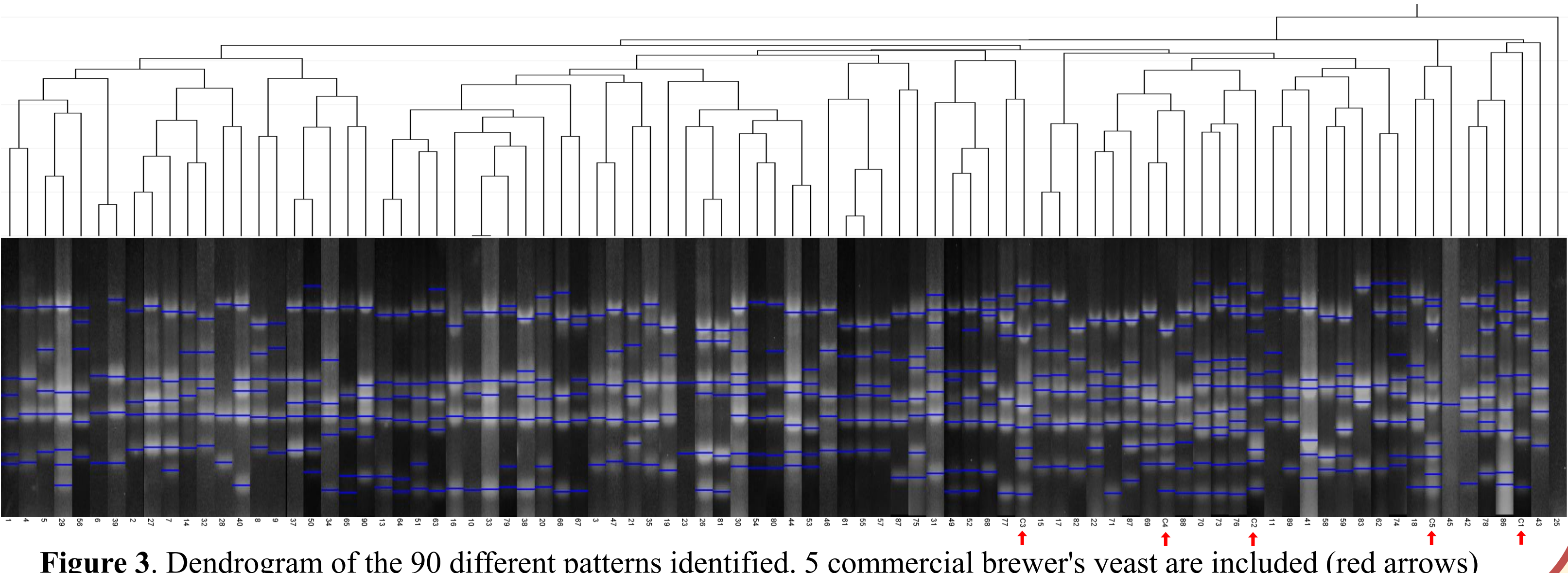


Figure 3. Dendrogram of the 90 different patterns identified. 5 commercial brewer's yeast are included (red arrows)

Conclusions and Ongoing

At this point of the project, we can conclude that there are numerous *Saccharomyces* strains present in nature in Andalusia that we can characterize to make them available to microbreweries.

Now we will study phenotypically the strains identified in order to analyze their growth capacity in different conditions such as temperature, alcohol or sugar sources. After this, beers will be produced with selected strains to evaluate their effect on the organoleptic characteristics.

References

[1] Cubillos, F. A., Gibson, B., Grijalva-Vallejos, N., Krogerus, K., & Nikulin, J. (2019). Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. In *Yeast* (Vol. 36, Issue 6, pp. 383–398). John Wiley and Sons Ltd. <https://doi.org/10.1002/yea.3380>

[2] Meier-Dörnberg, T., Finlays, M. M., & Hutzler, M. (2017). Genetic and Phenotypic Characterization of Different Top-fermenting *Saccharomyces cerevisiae* Ale Yeast Isolates.. *BrewingScience* 70: 9-25