Isolation, identification and characterization of natural yeasts for brewing

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Introduction

The beer market has experienced significant growth in recent years, impulsed by the consumption of a greater variety of beers with different characteristics. The role of yeasts in the aromatic and flavor profile of beer is essential, since they are responsible for most of the aromatic compounds present in the final fermented product (White et. al, 2010). Naturally, a multitude of yeast species are present in the environment that surrounds us. Many of them have the physiological properties needed to carry out the fermentation process for brewing. Through different isolation, identification and characterization techniques, it is possible to determine which of these natural yeasts are suitable to be used in the fermented beverage industry. For this reason, in this work we have focused on the search for yeasts, mainly *Saccharomyces cerevisiae*, present in different grape worts and other natural media, in order to create a collection of yeasts with a particular fermentative potential for the brewing beer.

Spontaneous fermentation of grape musts

As the main source of natural yeasts, we got five grape musts (Image 1) from different companies, collection times and locations, which contain a different autochthonous microbiota. These worts were subjected to spontaneous fermentation, at different temperatures (16, 18, 20 and 24ºC) in flasks with a volume of 50 ml. We check the evolution of the optical density (OD) and the CO2 released, Figure 2 and 3 show the evolution of one of those worst. Likewise, samples were taken throughout the process that we use for the subsequent isolation of the predominant yeasts at each point of the fermentation.

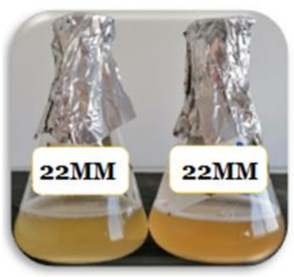

Figure 1. Flasks with 50 ml of 22MM worts at 24 ºC on day 3 and 5, respectively of fermentation.

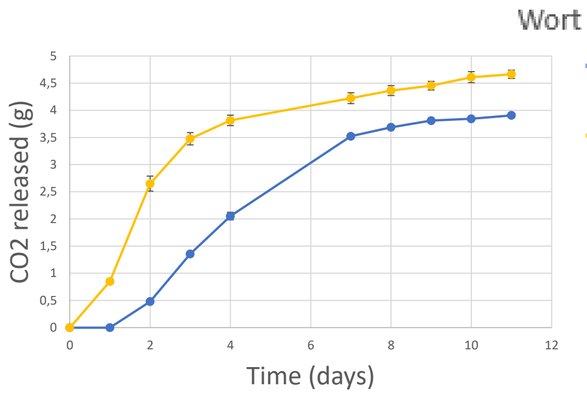

Figure 2. Evolution of CO2 during wort fermentation 22MM at 16 and 24ºC.

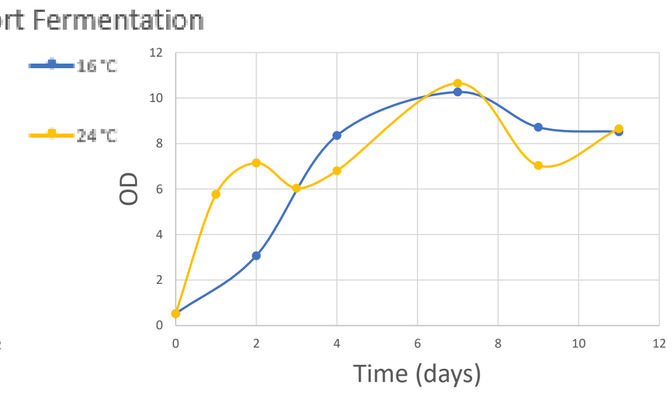

Figure 3. Evolution of OD during wort fermentation 22MM at 16 and 24 ºC.

Figure 4. YPD plate with isolated colonies from specific sample

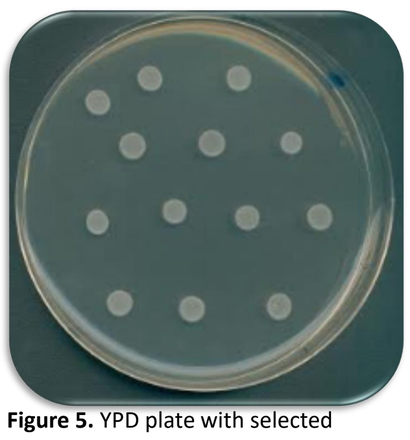

Figure 5. YPD plate with selected colonies from specific sample

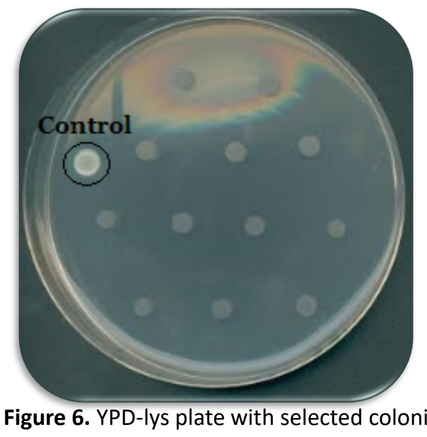

Figure 6. YPD-lys plate with selected colonies. Non Saccharomyces colonie was spotted as control.

Isolation and identification of yeasts

The samples taken during the fermentation were inoculated in rich medium plates (YPD) in order to isolate individual colonies present in the musts (Figure 4). The selected colonies were transferred onto new YPD plates (Figure 5) and YPD-lys plates (Figure 6). The absence of growth in YPD-lys (Walters and Thiselton, 1953) allowed us to determine those species belonging to Saccharomyces spp. since these do not grow in media with lys as the sole nitrogen source. Additionally, some colonies were plated in YPD medium + malt extract + 5% alcohol, to select those species capable of fermenting maltose and support high concentrations of alcohol in the medium.

Molecular identification

Colonies selected and identified as Saccharomyces spp. were subjected to molecular analysis. For this, DNA extraction was carried out initially by means of an adapted protocol with Llalzyme (Ibeas J.I. personal communication). Subsequently, we performed the genotypic analysis of microsatellites at loci 13, amplifying regions by PCR by using the oligos: SCYOR267C, SCPTSY7, ScAAT3, SC8132X and C5 (Lautebarch et al., 2019). Finally, we performed an electrophoresis of the amplified fragments that shows a specific pattern of bands (Figure 7) for each of the *Saccharomyces cerevisiae* strains, allowing us to select organisms with a different genotype and potential phenotype. Electrophoretic lanes with a unique band pattern (Figure 7) were selected for their physiological analysis and possible interest for brewing (Figure 8).

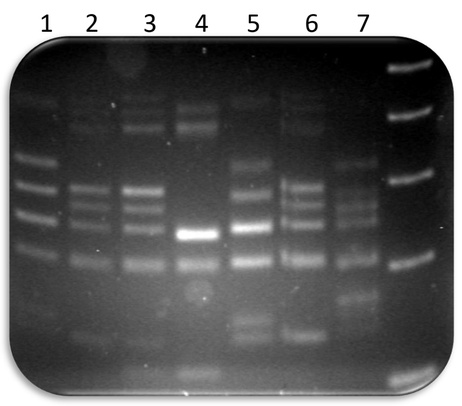

Figure 7. Agarose gel electrophoresis (2.5%) of the PCR product of 7 selected colonies of 22MM must at 24 ºC on day 9 of fermentation.

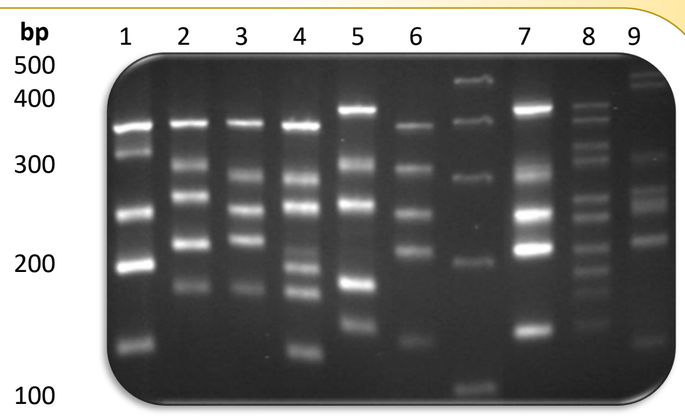

Figure 8. Agarose gel electrophoresis (2.5%) of the PCR product for nine selected strains.

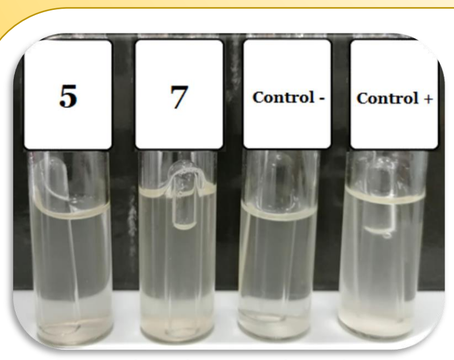

Figure 9. Vials with YNB-malt medium inoculated with selected strains 5 and 7 (maltose - and + fermenter, respectively) and control strains after 48 hours of incubation at 28 ºC w/o shaking.

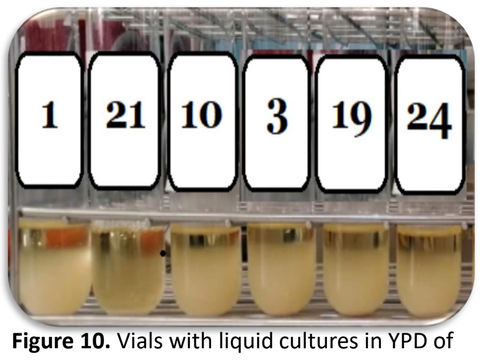

Figure 10. Vials with liquid cultures in YPD of different strains selected after 48 hours of incubation at 28 ºC in shaking. Flocculation state after 2 hours in static.

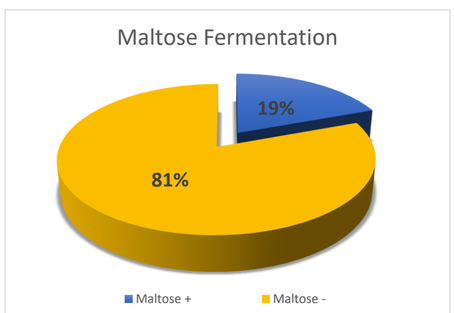

Figure 11. Percentage of maltose fermenting (81%) and non-fermenting (19%) strains.

Physiological characterization

For the physiological characterization, the strains identified with a determined band pattern were analyzed for their ability to ferment maltose (Figure 11) and their level of flocculation. To do this, the strains were inoculated into sterile tubes containing YNB-maltose liquid medium and a Durham tube upside down (Figure 9). Those cultures that formed gas bubbles inside the Durham tubes will be candidates for future brewing due to their ability to ferment maltose. For the flocculation test, 48 hours cultures in YPD media (Figure 10) were quantified for precipitation time.

Future tests

- Growth curves for the selected strains in different media including brewing conditions
- Time / temperature correlation between samples taken from fermenting grape musts.
- 2 liters brewing and testing with selected strains.

Conclusions

- There is a high diversity of natural yeasts in the environment.
- Most of the strains isolated from grape musts belong to the *Saccharomyces cerevisiae* species.
- Most isolated strains ferment maltose and thus they are suitable for brewing.

References

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