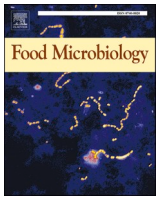
Food Microbiology 114 (2023) 104276

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm



Saccharomyces cerevisiae wine strains show a wide range of competitive abilities and differential nutrient uptake behavior in co-culture with *S. kudriavzevii*

Alba Contreras-Ruiz [a], Javier Alonso-del-Real [a], Eladio Barrio [a,b], Amparo Querol [a,*]

[a] *Departamento de Biotecnología de los Alimentos, Grupo de Biología de Sistemas en Levaduras de Interés Biotecnológico, Instituto de Agroquímica y Tecnología de Los Alimentos (IATA)-CSIC, Valencia, Spain*
[b] *Departament de Genètica, Universitat de València, València, Spain*

ARTICLE INFO

Keywords:
Saccharomyces species
Yeasts biodiversity
Competition
Wine fermentation
Nitrogen uptake
Fitness

ABSTRACT

The wine industry has implemented complex starters with multiple yeast species as an efficient method to improve certain wine properties. Strains' competitive fitness becomes essential for its use in such cases. In the present work, we studied this trait in 60 *S. cerevisiae* strains from different origins, co-inoculated with a *S. kudriavzevii* strain, and confirmed it to be associated with the strains' origin. To gather deeper knowledge about the characteristics of strains with highly competitive ability versus the rest, microfermentations using representative strains from each group were performed and the carbon and nitrogen sources uptake was analysed. Our results demonstrate that despite wine strains being the subclade with the highest competitive ability, they present a wide range of behaviors as well as nutrient uptake dynamics, which points to a heterogeneous nature of domestication processes. An interesting strategy was observed in the highly competitive strains (GRE and QA23), the nitrogen sources uptake in the competition was accelerated and the sugar fermentation was slowing despite the fermentation finish at the same time. Therefore, this competition study, using particular combinations of strains, expands the knowledge in the field of the usage of mixed starters in wine manufactured products.

1. Introduction

Saccharomyces cerevisiae is a widespread species found in highly diverse habitats such as soil, fruits, tree bark, or cacti (Wang et al., 2012), as well as in association with fermentative environments (Legras et al., 2018). The outstanding efficiency in fermenting sugars to ethanol in *S. cerevisiae* has made it the main species responsible for producing wine, bread, beer, and other fermented food products for thousands of years (Cavalieri et al., 2003; McGovern et al., 2004). Therefore, the variety of *S. cerevisiae* strains that can be found is extremely wide as a consequence of the different selective pressures they have faced during that time in each of the habitats they have colonized.

The adaptation to fermentative environments such as wineries or breweries is considered a domestication process associated with human activity and reveals the genome plasticity in *S. cerevisiae* (Gallone et al., 2016; Legras et al., 2018). These domesticated lineages have mosaic genomes due to crosses between strains from different origins, showing complex patterns of genetic differentiation within the species (Peter et al., 2018; Peter and Schacherer, 2016; Schacherer et al., 2009). In the particular case of the wine lineage, *S. cerevisiae* has adapted to the stresses associated with alcoholic fermentation, such as osmotic stress, high ethanol concentration, or nitrogen starvation, becoming strains of high biotechnological interest (Querol et al., 2003; Guillamón and Barrio, 2017), and contributing to make it the main microorganism that prevails during fermentation (Pretorius, 2000). Nevertheless, classically, this has been attributed to its well-known strategy based on the Crabtree effect (Crabtree, 1928; Hagman et al., 2014). As a result, *S. cerevisiae* has a competitive advantage, depleting sugars faster (Fleet, 2003) while producing high levels of ethanol. This can cause nutrient consumption restrictions as well as toxicity to the other microorganisms, displacing them from the fermentative environment at advanced stages of fermentation (Piškur et al., 2006).

Ecological studies regarding the domination phenomenon become of special interest due to the trends in the use of co-cultures in wineries,

* Corresponding author. Carrer del Catedràtic Agustín Escardino Benlloch, 7, 46980, Paterna, Valencia, Spain.
E-mail address: aquerol@iata.csic.es (A. Querol).

https://doi.org/10.1016/j.fm.2023.104276
Received 28 December 2022; Received in revised form 3 April 2023; Accepted 3 April 2023
Available online 6 April 2023

which has grown in recent times as a strategy to solve the current problems faced by the industry, specifically; the consumer demands more aromatic and low alcoholic wines; and problems associated with climate change that are causing alterations in the grapes ripening process, leading to an excessive ethanol content in wines (Jones et al., 2005; van Leeuwen and Darriet, 2016; Pérez-Torrado et al., 2017a,b; Querol et al., 2018).

Recently, the use of co-cultures at different temperatures (17, 24, and 31 °C) employing *S. cerevisiae* together with other species from the *Saccharomyces* genus, such as *S. kudriavzevii* and *S. uvarum*, has been shown to achieve wines with a lower alcoholic content (Alonso del Real et al., 2017a, 2017b). *S. kudriavzevii*, due to its cryophilic nature, competed better and was able to stay with *S. cerevisiae* in similar proportions during fermentation at 12 °C (Alonso del Real et al., 2017a,b). Strikingly, despite so, the presence of a wine *S. cerevisiae* strain provoked a negative effect on *S. kudriavzevii* growth at 12 °C when inoculated in co-cultures, but no defect in growth was observed in *S. cerevisiae*. Contrarily, a *S. cerevisiae* wild strain did suffer a negative impact under the same conditions. This difference in the competitive ability of the two *S. cerevisiae*strains was hypothesized to be due to wine strain gain of this trait by adaptation to the fermentative habitat (Alonso del Real et al., 2017a,b).

The mechanisms involved in this phenomenon remain yet poorly described. Recent advances in the study of yeast interactions evidence the existence of other kinds of indirect as well as direct interactions, apart from the accumulate-make-consume strategy ethanol. In direct interactions, cell-to-cell contact and the *FLO* gene family play an important role in influencing population dynamics (Rossouw et al., 2018; Petitgonneta et al., 2019). On the other hand, as examples of indirect interactions, a study carried out by Branco et al. (2014) showed how *S. cerevisiae* produced peptide fragments from the protein glyceraldehyde 3-phosphate dehydrogenase constitutively linked to the cell wall that exhibits antimicrobial activity. The endogenous production of sulphite and SO_2 resistance in highly competitive strains would be another indirect interaction (Pérez-Torrado et al., 2017a,b). Also, Bisson (1999) suggested that quorum sensing takes place in yeast interactions during winemaking. Later on, other studies found that particular molecules that may be acting as quorum sensing molecules, such as ammonium cations (Palkova et al., 1997) or tyrosol (Chen et al., 2004), and thus be involved in the complex communicative process that takes place between microorganisms in a fermentative environment (Winters et al., 2019). Furthermore, killer factors were described by Pérez et al. (2001), Roudriguez-Cousiño et al. (2011), in *S. cerevisiae* and other non-*Saccharomyces* strains, and how their production also influenced the growth of other species. Interestingly, several studies have focused on the production of vesicles as a method of communication between the different microorganisms presents in fermentation (Raposo and Stahl, 2019; Mencher et al., 2021). Finally, different authors have described a competitive response based on the competition for nutrients in mixed cultures with non-*Saccharomyces* yeasts (Curiel et al., 2017; Yan et al., 2020; Zilelidou and Nisiotou 2021), which agrees with the results reported by *S. cerevisiae - S. kudriavzevii* interaction (Alonso del Real et al., 2019).

Strikingly, as well as the above mentioned case of the better competitive fitness of a wine strain than a wild strain, in this work, only the wine strain showed this behaviour, and a wild strain did not. Particularly, carbon and nitrogen sources seemed to be the main metabolites at stake. However, one of the limitations common to all mentioned studies is the generalization of the conclusions obtained from one or a few *S. cerevisiae* strains to all of the species. In the present work, we aimed to elucidate how the competitive fitness against the yeast *S. kudriavzevii* is distributed among the *S. cerevisiae* species through the study of 60 strains from diverse origins, especially wine isolates. Furthermore, we analysed whether the mechanism based on faster nutrient uptake correlates with the competitive fitness of the selected strains.

2. Material and methods

2.1. Yeast strains and growth media

We used a total of 60 *S. cerevisiae* strains, which came from different origins and sources of isolation: 22 of them were wine strains; 3 wild strains, 4 flor yeast strains; 4 clinical strains; 23 strains isolated from fermented food or drinks such as cocoa, chicha or sake among others; and 4 bioethanol-producing strains (Fig. 1A). All the detailed information of each strain is listed in Table S1 of the Supporting Information.

Furthermore, we included the strain *S. kudriavzevii* CR85, a natural isolate from oak tree bark in Agudo, Ciudad Real province, Spain. This strain was selected because it showed efficient fermentation kinetics in wine according to a previous study (Peris et al., 2016), and it has already been used in other competitiveness studies against *S. cerevisiae* (Alonso-del-Real et al., 2017a; 2017b).

Cryogenically preserved (−80 °C) strains were cultured and maintained on GPY plates (2% glucose, 2% agar, 0,5% peptone, 0,5% yeast extract) and stored at 4 °C. GPY liquid medium (2% glucose, 0.5% peptone, 0.5% yeast extract) was used for overnight growth of precultures.

2.2. Synthetic must fermentation in multi-well plates

Synthetic must (Rossignol et al., 2003) was used in microvinification experiments, with 100 g/L glucose, 100 g/L fructose, and 300 mg/L of YAN (yeast assimilable nitrogen).

Micro-fermentations were performed in multi-well plates (Non-Tissue Culture Treated Plate, 48 Well, Flat Bottom with Low Evaporation Lid; FALCON) in 500 μL of SM. Each *S. cerevisiae* strain was inoculated in single cultures and co-cultures of equal proportions with *S. kudriavzevii* CR85. CR85 was also inoculated in monoculture. Five replicates of each fermentation were carried out, as shown in Fig. 1B.

Overnight pre-cultures were grown in falcon tubes with 20 mL of GPY medium at 25 °C, 100 rpm. After 15 h of culture, cells were washed with mQ water and quantified by cytometry (MUSE Cell Analyser). It was calculated the volume of synthetic must that needed to be added to the cell pellet to inoculate the initial concentration of 10^6 cells/mL for each strain in a fixed volume.

Plates were incubated for 96 h at 12 °C and agitated in a plate shaker (Mini-shaker, PSU-2T, Biosan) at 100 r.p.m. Yeast cells were collected, cell density measured by cytometry, and kept at −20 °C for the subsequent total DNA isolation.

2.3. DNA isolation and qPCR competition analysis

Following the protocol described by Querol et al. (1992), the total DNA from yeast samples after fermentation was extracted. The concentration of the DNA samples was measured in a Nanodrop spectrophotometer ND-1000 (Nanodrop Technologies TM, Wilmington, DE. USA) and adjusted to 17 ng/μL (Fig. 1C).

To study the competitive fitness of the included strains, we analysed the proportion of each species after co-culture fermentation by a relative quantification assay based on quantitative PCR (qPCR). To do this, as in previous work (Alonso del Real et al., 2017a,b), the DNA samples from the co-culture fermentations were amplified in triplicate using specific primers for each species independently.

PCR amplification was performed in a 10 μL final volume that contained 2.5 μL of the DNA template, 1.5 μL MilliQ water, 0.2 μM of each primer, and 5 μL of LightCycler© 480 SYBR Green I Master (Roche). Reactions were performed in 96-well plates in a LightCycler© 480 (II) PCR amplification and detection instrument with an initial denaturalization step at 95 °C for 5min, followed by 45 cycles of 95 °C for 10 s, 54 °C for 10 s, and 72 °C for 14 s. The C_T values were calculated automatically by this instrument.

The samples from pure cultures fermentation were used as a positive

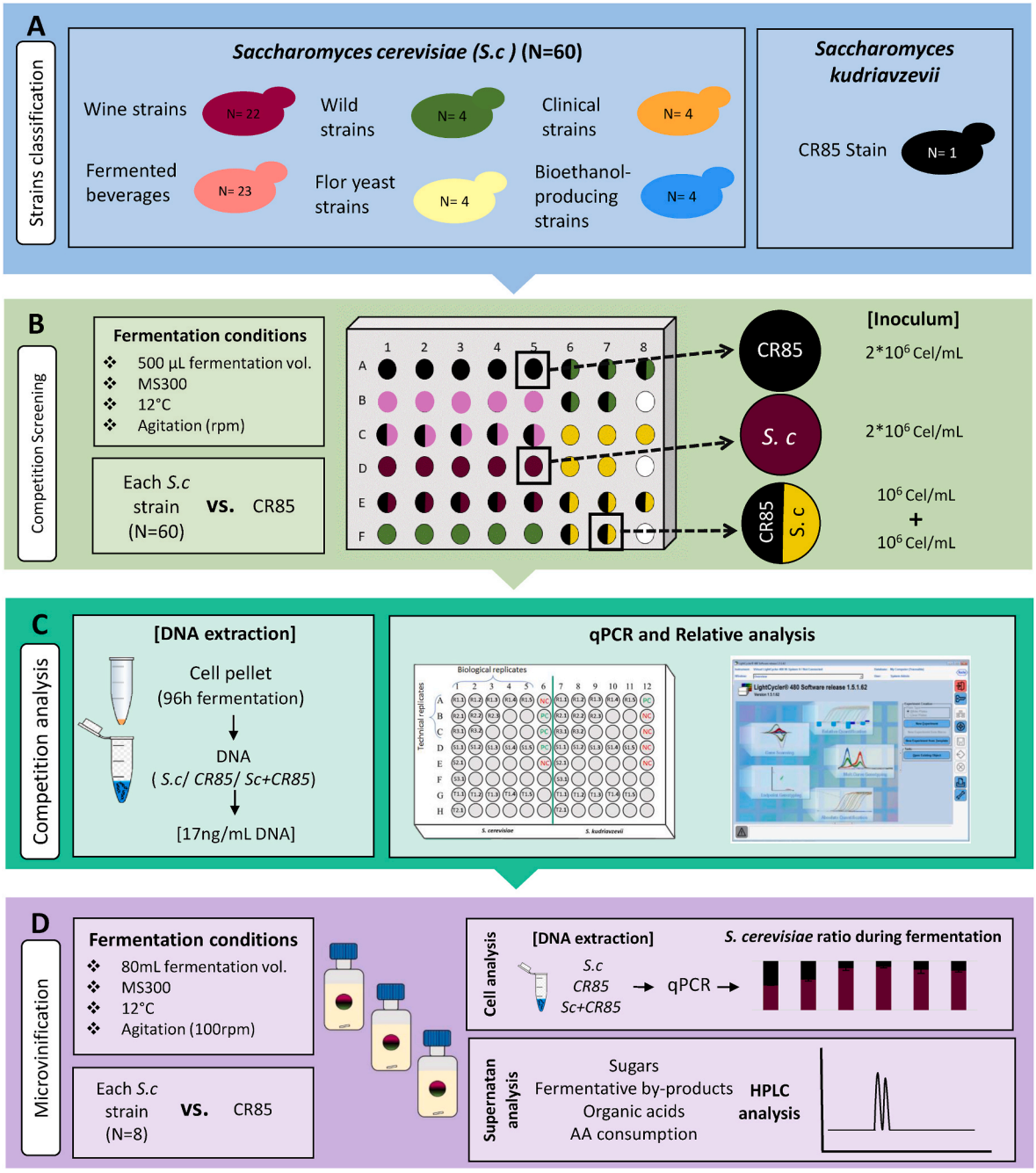


Fig. 1. Outline of the experimental design and methodology used. **A)** Yeast groups according to their environmental origins and number of studied strains included in each group: Wine (n = 22), fermented beverages (n = 23), wild (n = 3), flor yeast (n = 4), clinical (n = 4) and bioethanol-producing (n = 4). **B)** Multi-well plates fermentation for competition screeing. **C)** Competition analysis, including DNA extraction, qPCR and qPCR data analysis. **D)** Microvinification stage, including cellular and supernatant analyses.

or negative control for cross amplification. Also, mQH$_2$O was used as a control for reactive contamination.

To calculate the amplification efficiency of primers, the programs LC480Conversion and LinRegPCR were used. After that, LightCycler 480 instrument software 1.5 (RocheDiagnosis, Darmstadt, Germany) was used to calculate the relative strain proportion in each sample (Fig. 1 C).

2.4. Microvinifications in bottles

Micro-fermentations in 100 mL bottles containing 80 mL MS300 were carried out at 12 °C with 100 rpm agitation (Fig. 1D). Each *S. cerevisiae* strain was set up in monoculture and co-culture with CR85 (*S. kudriavzevii* strain) in three replicates. Also, three replicates of CR85 were included in monoculture (Fig. 1D). Müller valves were used to control the fermentation phase by weight loss until a constant weight was reached, at which point it was considered to be finished.

Samples were taken at 22 h, 72 h, 96 h, 168 h, 216 h, 260 h, 336 h, 360 h, 384 h, 432 h, and 500 h covering the growth phases: lag phase, exponential growth, and stationary growth and also the fermentation kinetics.

Yeast cells and supernatant were collected and separated for deep-freezing and kept at −80 °C until being processed and analysed.

The fermentation curves were adjusted by non-linear regression to Gompertz equation, for bacterial growth curves, with the following

expression, as proposed by Pérez et al. (2021):

$$y = A * \exp\{ -\exp[((\mu \max e / A) * (\lambda - t)) + 1]\}$$

Where λ is the lag phase (the time in hours when fermentation starts vigorously), μmax, the maximum specific growth rate (h−1), and A, is the maximal asymptotic y-value, the maximal consumption of sugar (200 g/L).

In this study, biological parameters were reassigned to kinetic parameters of fermentation curves being λ, the time when fermentation starts vigorously; *Vmax*, the maximum specific fermentation rate, and *A*; the maximal sugar consumption.

2.5. HPLC analysis

Sugars (glucose, fructose), fermentative by-products (glycerol, ethanol, 2,3 butanediol, and erythritol), and organic acids (acetate, succinate, tartrate, citrate, and malate) were determined by HPLC (Thermo Fisher Scientific, Waltham, MA) using a refraction index detector and UV/VIS (210 nm) detector equipped with a HyperREZTM XP Carbohydrate H+ 8 mm column (Thermo Fisher Scientific, Waltham, MA) and HyperREZTM XP Carbohydrate Guard (Thermo Fisher Scientific, Waltham, MA). Samples were filtered through a 0.22-μm nylon filter (Symta, Madrid, Spain) and the analysis conditions were: eluent, 1.5 mM of H2SO4; 0.6 ml. min-1 flux, and a 50 °C oven temperature.

Determination of amino-acids and ammonia (yeast assimilable nitrogen) was carried out by derivatization of a volume of supernatant followed by UPLC (Dionex Ultimate 3000, Thermo Fisher Scientific, Waltham, MA) equipped with an Accucore C18 10 × 4.6 mm 2.6 μm Defender guards (Thermo Fisher Scientific, Waltham, MA). Derivatization process consisted of mixing 400 μl of the sample with 430 μl borate buffer (1 M, pH 10.2), 300 μl absolute methanol, and 12 μl of diethyl ethoxymethylenemalonate (DEEMM), and ultra-sonicating for 30 min at 20 °C. After ultra-sonicating, samples were warmed up at 80 °C for 2 h to allow the complete degradation of excess DEEMM. After the derivatization process, samples were filtered with 0.22 μm filters. The analysis conditions used in the UPLC are available in Table S2, Support Information.

2.6. Killer factor analysis

The eight selected representative *S. cerevisiae* strains were assessed for killer factor toxin-producing capacity, as well as for their resistance or sensitivity to these factors by the seeded-agar-plate technique, following the protocol described in Lopes and Sangorrín (2010). For more detailed information, see supplementary material.

2.7. Statistical analysis

Differences in the proportions reached by *S. cerevisiae* strains in co-cultured fermentations, both in the plate experiments during screening and in the bottle fermentation, were statistically analysed by ANOVA, Tukey's significant difference test ($\alpha = 0.05$). Sugars and amino acids consumption differences were also analysed using this statistical test.

Moreover, principal component analysis (PCA), as a multivariate methodology, was applied to determine which variable contributes the most to the differences observed. All statistical analyses and plots were obtained by the use of Infostat software, version 2011 (Grupo Infostat, Cordoba, ′ Argentina), and GraphPad Prism version 8.0 (Graph-Pad Software, Inc., La Jolla, CA).

3. Results

3.1. Screening for the competitive ability of S. cerevisiae strains

60 *S. cerevisiae* strains isolated from different origins and described in Table S1 were used in a competitive fitness study. They were grouped according to their origin into six groups: (I) wine strains, (II) wild (natural) strains, (III) clinical strains, (IV) strains isolated from traditional beverages or foods (traditional ferments), (V) flor-yeast strains and, (VI) bioethanol producing strains. We used the strain CR85 as a representative of *S. kudriavzevii* because is one of the best strains fermenting musts according to previous data (Peris et al., 2016). It should be noted that the difference in the size of the study groups has been taken into account when analysing the data. The wine (I) and traditional ferments (IV) groups have a larger number of species as they were considered the most interesting to analyse, always taking into account a possible industrial application point of view.

We analysed the presence of each *S. cerevisiae* strain in mixed cultures at 96 h of fermentation by qPCR. The results are shown in Fig. 2A, which includes the proportion of each strain in decreasing order. Table S3 shows the average proportion of 5 biological replicates of each strain and the standard deviation. Of the 60 strains included in the study, the highest implantation values were achieved by strains from the wine group. By contrast, the strains with lower proportions belonged to the wild group or traditional beverage and foods group (<10% proportion). Of the 15 strains with the lowest proportions, only one was a wine strain, the rest of them belonged to the wild and traditional beverage group (Fig. 2A, Table S3).

We studied differences in the competitive ability among groups, analyzing their proportions, and the results are illustrated in Fig. 2B. Wine group showed significantly better competition performance compared to wild, traditional beverages, and bioethanol groups. On the other hand, clinical and flor yeast groups do not differ significantly from the rest of the groups.

Strikingly, as shown in Fig. 2A, the strains belonging to the wine group have a wide range of proportions from 6% to 99%, which is indicative of high variability of the competitive fitness trait, not just at the species level but at the isolation source or phylogenomic subclade level.

Determination of *S. cerevisiae* representative strains proportion at five points along the co-inoculated microvinifications.

After the screening according to the strain's competitive ability, eight strains were selected as representative of the different behaviours: 6 wine strains; Lalvin ICV GRE and QA23 were selected as representatives of the group with a proportion higher than 70%, Lalvin EC1118, and T73 for the proportion between 50% and 30%, and Lalvin Clos and Lalvin ICV D254 for the proportions lower than 20%; and 2 wild strains, Chr96.2 and NCAIM Y00925 (Fig. 3A). The natural strains were selected because of their poor competitive ability and to compare their behaviour with wine strains that are also poor competitors but that we assumed they have undergone a domestication process. These strains were co-cultured with *S. kudriavzevii* CR85 and their prevalence was determined along micro-vinifications in 80 mL SM. The classification carried out in this study according to their competitive ability is always taking into account the study condition based on competition against a specific strain of *S. kudriavzevii* (CR85) and using a synthetic wine must.

To compare the selected strains in a similar metabolic and physiological state, and considering the percentage of sugar consumed as a more determinant variable than the fermentation time, the sampling points were selected according to the amount of sugars remaining in the medium, including the following: 20%, 30%, 50%, 95%, and, 100% of sugars consumed.

As depicted in Fig. 3B, the ability of *S. cerevisiae* to dominate over CR85 reaching a proportion of more than 60% during fermentation was dependent on the type of strain. The extensive analysis of the proportion of wine strains shows that they presented a variety of profiles in terms of their competitive ability, as suggested by the above presented screening outcome. In the case of ICV-GRE strain, it took hold in and was maintained along with the fermentation in proportions higher than 70%, as predicted by the screening. Concerning QA23, it had a growth peak in the first 24 h, then its proportion decreased and was maintained during

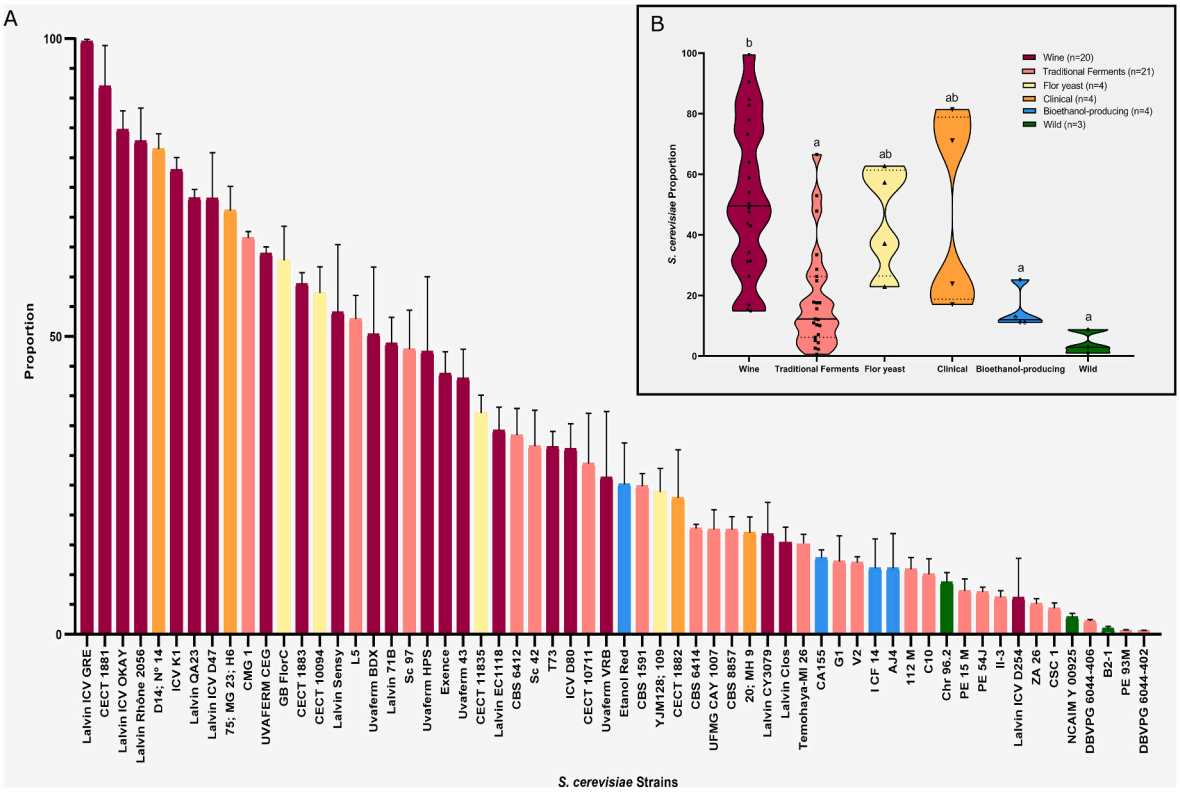


Fig. 2. A) Proportion of each *S. cerevisiae* **strain** at 96 h of fermentation in competition. The six groups related with the strain environmental origin are represented by different colors: wine (maroon), clinical (orange), fermented beverages (pink), flor yeast (yellow), bioethanol-producing (blue) and wild (green). **B)** Violin plot including *S. cerevisiae* strains proportion values grouped according to their environmental origins. Groups marked with different letters are significantly different ($p < 0.05$) according to the Tukey HSD test (ANOVA test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

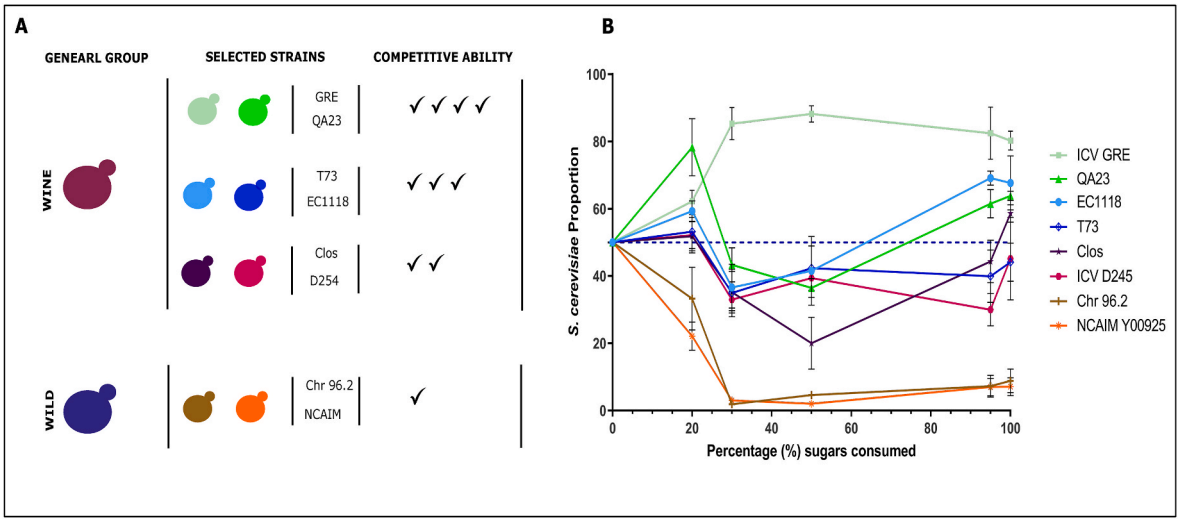


Fig. 3. A) Strain selection scheme including environmental origin group, strain name, and level of competitive ability. **B)** Temporal diagram showing *S. cerevisiae* strains proportions during competitive fermentations against CR85. Points represent mean values and error bars represent the standard deviation of the three replicates *S. cerevisiae* proportions in mixed cultures estimated by qPCR.

fermentation at around 40% and finally, it increased again at the end of fermentation, up to around 60%. About T73 and EC1118, classified as medium competitors, their proportion fluctuated around 40–60%. Clos and ICV D254 kept around 20–30% during fermentation but reached more than 50% at the end point.

On the other hand, Chr96.2 and NCAIM wild strains showed little competitive capability, being displaced by CR85 during the first hours of fermentation, and never exceeding 9% from 72 h (30% sugar consumed) onwards.

3.2. Characterization of nutrient uptake profiles: monoculture vs. co-culture

3.2.1. Sugar consumption profiles of S. cerevisiae strains in monoculture and co-culture with CR85

Sugar consumption was measured by HPLC along the fermentations to study how sugar consumption changes in a fermentation carried out by two co-cultured species compared to monoculture fermentations.

Total sugar consumption kinetics is depicted in Fig. 4 and a set of different patterns are found among the studied strains. Those classified as good competitors in the screening, GRE and QA23, showed a very similar sugar consumption rate as *S. kudriavzevii* strain. However, interestingly, the co-cultures dominated by *S. cerevisiae* up to a certain extent did not show a similar sugar consumption profile to its respective single *S. cerevisiae* strain, but slower kinetics (Fig. 4A and B). These differences in glucose and fructose consumption between monoculture and co-culture become significant after 96 h of fermentation (Tables S4 and S5). This sluggish fermentation effect was also observed in the strain EC1118 (Fig. 4D), one of the medium skilled competitor representatives, but this was not the case for T73, which showed a lower ability competitor in this group (see Fig. 4C). In this case, as well as in the bad competitors', the sugar consumption profile is very similar to that of *S. kudriavzevii* in single culture (Fig. 4C, E, 4F, 4G, and 4H).

The time to consume 95% of sugars (t_{95}) was calculated to highlight the observed phenomenon that for strains with higher competitive capacity, slower fermentations were seen in the mixed culture with respect to their monoculture (Fig. 5). The same happened for EC1118, but not for T73, which showed significantly accelerated consumption of sugars. However, wine strains with low competitive ability showed no significant differences between fermentations in single-culture and in co-culture with CR85, as well as wild strains in co-culture, whose t_{95} is quite similar to CR85 monoculture.

The sugar consumption kinetics being similar to *S. kudriavzevii* has been observed in mixed cultures with bad *S. cerevisiae* competitors as can be concluded according to the proportion results of the two species (Fig. 4). In these cases, *S. kudriavzevii* would be outcompeting *S. cerevisiae* and thus, sugar consumption profile would be due to the former activity. Intriguingly, medium and good competitors presented the opposite behaviour, despite dominating the fermentation culture, they slowed the sugar uptake from the medium (Fig. 5).

In addition, at the end of the fermentation process, HPLC analysis was carried out to confirm that fermentation was completed and that the sugars in the must have been completely consumed. In addition, fermentation products such as glycerol, ethanol, and acetic acid were determined (Table S6). Glycerol production was higher in competitive fermentations than in monoculture fermentations due to the presence of CR85, which is a high glycerol-producing strain. Ethanol production was around 12% for all fermentations, including competitive and monoculture fermentations. Finally, acetic acid was produced in lower amounts in all competing fermentations compared to the fermentation of each of the strains in monoculture, except for QA23.

3.2.2. Amino acid consumption profiles

Amino acids consumed by the different strains in monoculture as well as in co-culture were determined after 24 and 72 h of fermentation for the above reported fermentations. At 72 h, practically all amino acids were consumed in all the fermentations. As has been described in the "Screening for the competitive ability of *S. cerevisiae* strains" section, all the strains present similar cell values (see Table S3). So, the amino acids consumption was not obtained related to the cell/mL in each point, the data show consumption in terms of percentage abundance patterns and overall nutrient concentrations.

Concerning the 24 h, first, it has to be noticed that for all of the studied mixed cultures the consumption patterns were more similar to its respective *S. cerevisiae* monoculture than to *S. kudriavzevii* (Support Information, Fig. S1). Despite no significant differences between *S. cerevisiae* and *S. cerevisiae* co-cultured with CR85 in the consumption of any single amino acid existed, there was an observable trend of higher consumption in mixed fermentations as we can see in Fig. 6. Furthermore, it should also be noted that the only amino acid with a higher consumption by the co-cultures in all the strains analysed is glutamine, which is one of the main amino acids in the must. However, for ammonium and arginine, which are the other two main sources of nitrogen, we do not observe a clear pattern as some strains consume more in monoculture and others in co-culture.

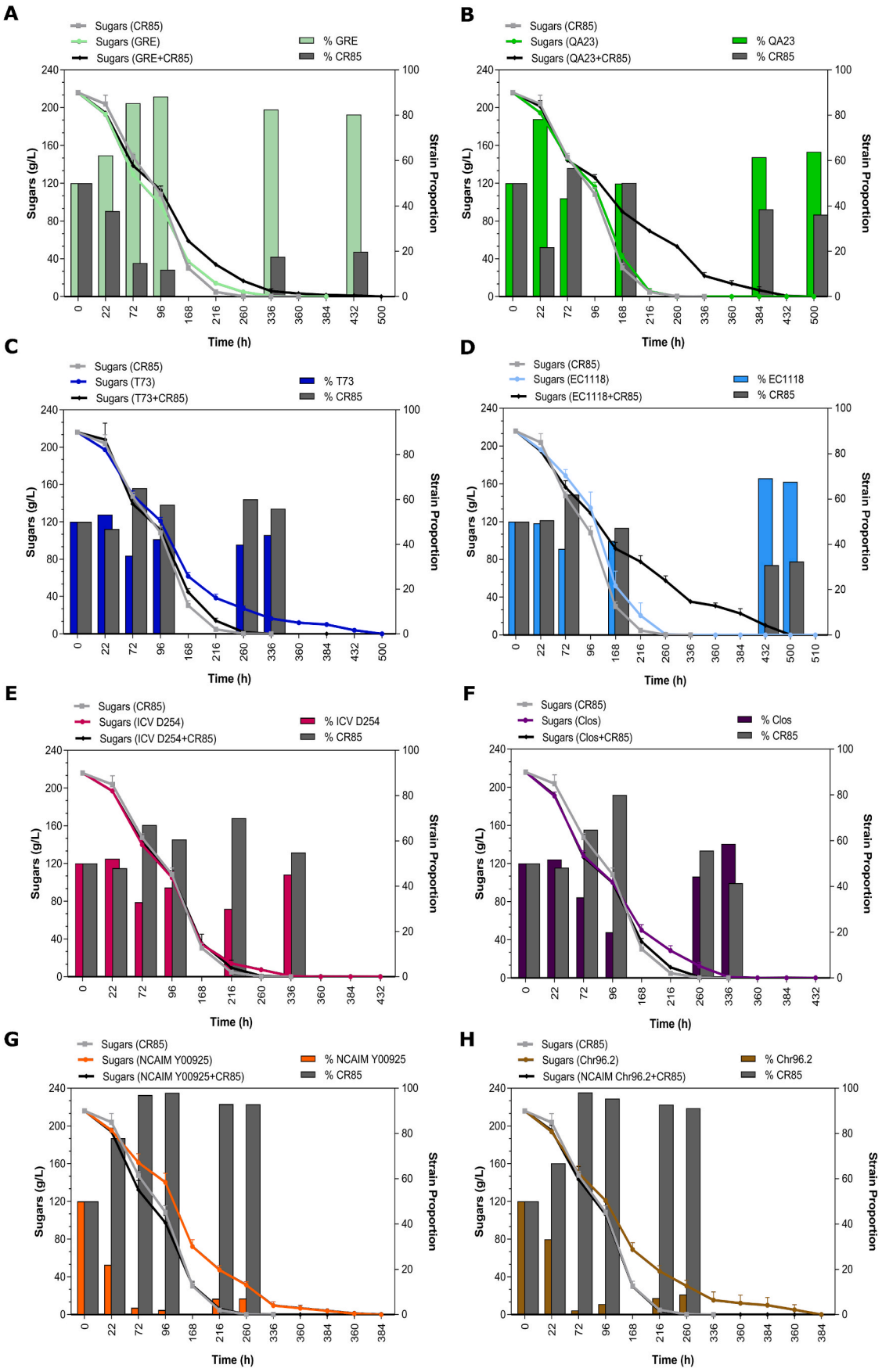
Since only one point of amino acid consumption had been truly informative, a new set of fermentations was carried out replicating the same conditions, and samples were analysed at short times: 10 h, 24 h, and 48 h (Fig. 7, Table S7, Table S8). In this experimental round, we observed that in *S. cerevisiae* strains the consumption of most of the amino acids was between 20 and 70% so a large part of the amino acid consumption must take place between 48 h and 72 h (when consumption was total according to our first experimental round). From the results obtained in this, it is interesting to note that the highest glutamine consumption by co-cultures compared to the monocultures is observed after 48 h instead of 24 h. That may indicate an important role of glutamine uptake during competitive growth.

Focusing on *S. cerevisiae* monoculture fermentations, we observed different amino acid consumption patterns with different preferences for each strain, regardless of their competing abilities. Also, co-inoculated fermentations showed their own consumption patterns different from their respective monocultures (Fig. 7). These results indicate that amino acid consumption depends on the strain and growing conditions. Despite this pattern for monocultures, for some co-cultures the consumption patterns show a higher similarity towards one of the strains present in the co-culture, probably matching the strain found in the highest proportion.

In the cases of GRE strain (Fig. 7A), at 10 h we observed a generally higher consumption of the amino acids by the co-inoculated fermentation, most clearly observed comparing the total YAN consumption: by GRE was around 9%, by the co-culture, 14%, and by CR85, 1%, probably because showed better competitions fitness to have the Killer factor. Also at 24 h, GRE + CR85 co-culture presented a consumption profile more similar to GRE monoculture. For example, analysing glycine, if we compare the consumption between GRE, CR85, and co-culture, we observe that CR85 had the lowest consumption, which could indicate that the consumption observed in the co-culture is due to GRE activity. In the case of histidine, again the profile followed by the co-culture is more similar to the profile shown by GRE since both show lower consumption than the CR85 strain in monoculture.

The EC1118 strain, which is the second strain with the best fermentative fitness (Fig. 3), which did not present the killer factor, showed an interesting nitrogen uptake pattern. At 10 and 24 h in co-cultures, the amino acid consumption profile is more similar to EC1118 monoculture than CR85. It is interesting to note that it consumes arginine, one of the main amino acids in must, more quickly in co-culture at 10 h than in monocultures, and at 24 h is more effective uptaking arginine, aspartate, isoleucine, leucine, serine, and threonine than monocultures. Concerning the T73 strain (Fig. 7C), at 10 h, we observed a unique pattern in the co-culture with respect to both single strains, consisting of a slower consumption. However, at 24 and 48 h, the profile is more similar to T73 than to CR85.

Finally, for the low competitive fitness strain NCAIM (Fig. 7G), we observe that, in monoculture fermentation, the consumption of amino acids is relatively low. Also, the consumption profile observed in the mixed culture is much more similar to CR85 consumption profile, and, what is more, the competition promotes a higher amino acid consumption than its respective monocultures, similar to the case of Clos (Fig. 7F). This acceleration of amino acid consumption is observed from 24 h in Clos and NCAIM, wine and wild strains, respectively, with almost 100% consumption of YAN in the co-cultures, which is a consumption even higher than in CR85 monoculture. That behaviour could be related



(caption on next page)

Fig. 4. Sugars consumption profiles during fermentation with single cultures of *S. cerevisiae*, *S. kudriavzevii* (CR85), and competition co-cultures (represented by points joined by lines). Values are the mean HPLC values for the three replicates, and error bars represent standard deviation. Grey lines represent CR85 in monoculture, black lines represent competition co-culture, and the other colors (green, blue, purple and brown) lines represent *S. cerevisiae* strains. **A** (GRE) and **B** (QA23) are wine strains with high competition capacity. **C** (T73) and **D** (EC1118) are wine strains with medium competition capacity. **E** (D254) and **F** (Clos) are wine strains with low competition capacity. **G** (NCAIM) and **H** (Chr96.2) are wild strains with low competition capacity. The bars represent the mean of the proportion achieved by each strain in the three replicates in 6 points of the competitive fermentation carried out by CR85 + *S. cerevisiae* co-cultures. The ratio at time 0 is not measured by qPCR. It is assumed to be 50%–50% because the same amount of cells from each species is inoculated. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

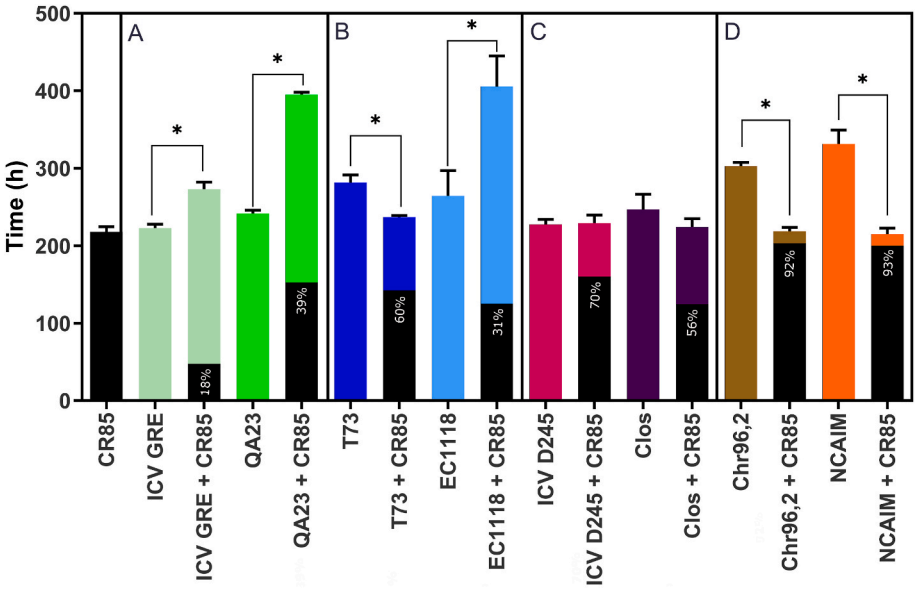


Fig. 5. Mean of consumption time of 95% sugar for each strain in monoculture and in co-culture with CR85. This parameter was calculated using the kinetic parameters calculated by the reparametrised Gompertz equation. For each co-cultured fermentation, the CR85 proportion is plotted in black. **A:** Wine strains with highly competitive ability. **B:** Wine strains with medium competitive ability. **C:** Wine strains with low competitive ability. **D**: Wild strains with low competitive ability. The error bar corresponds to the deviation between the replicates and * represents significant differences between mono- and co-culture ($P < 0.05$) derived from Tukey's analysis of variance test.

to a competitive response by CR85 in which amino acid consumption could be involved.

In summary, it is interesting to remark that wine *S. cerevisiae* strains have different amino acid consumption profiles, specific to each strain, as well as having different competition abilities. In co-cultures, strains with high competition ability have a consumption profile more similar to *S. cerevisiae* monoculture and are more efficient in the uptake of some amino acids than strains with low competition ability that showed a profile more similar to CR85.

4. Discussion

The use of mixed cultures with both *S. cerevisiae* and *non- S. cerevisiae* strains is one of the strategies used in industrial fermentations (Pérez-Torrado et al., 2017a,b). Therefore, the study of the interaction of different yeast species, as well as their competition mechanisms, becomes crucial. In this context, cold resistant *Saccharomyces* species have been tested in competition against *S. cerevisiae* strains from natural and wine origins. Results pointed to a better competitive fitness of *S. cerevisiae* industrial strains in high-sugar media, even at low temperatures (Williams et al., 2015; Alonso del Real et al., 2017a,b). However, the low amount of strains included in these studies did not allow for to draw of statistically supported conclusions. To answer this issue and expand knowledge on how different strains respond to a competitor, we performed a competitive fitness screening on *S. cerevisiae* strains *(n = 60)* from a variety of origins at low temperature winemaking conditions, typical of white wines fermentation processes, against *S. kudriavzevii* CR85, a cryophilic species. This way, we set up an industrial-relative system, but keeping such harsh conditions for *S. cerevisiae* as the survival in the presence of a cryotolerant yeast fully able to conduct fermentation at low temperatures.

Analysing the groups of strains studied, we observed that wild strains are not able to cope with *S. kudriavzevii* during fermentation, reaching a low prevalence level. More interestingly, the strains isolated from traditional fermented beverages generally showed a very modest competitive ability, being displaced by *S. kudriavzevii* in the first hours of fermentation (96 h). Wine strains constitute a group presenting a wide range of prevalence ratios with respect to its competitor, but also confirmed the greatest competitive ability against *S. kudriavzevii* when compared with the rest of the *S. cerevisiae* groups. These results are not surprising, since previous competition studies carried out by Alonso del Real et al., (2017a, 2017b) showed that the implantation ability of *S. cerevisiae* in a co-cultured fermentation with *S. kudriavzevii* or *S. uvarum* at 12 °C was higher for strains whose origin was associated with a fermentative environment. One plausible explanation for these results is that wine yeast has been selected and adapted for centuries to a fermentative environment (Querol et al., 2003), being more resistant to different stresses, such as osmotic pressure, high alcohol concentrations (Arroyo-López. et al., 2010; Navarro-Tapia et al., 2016) or nitrogen limiting conditions (Crépin et al., 2014). Therefore, the domestication process of wine strains could involve also a better competition ability. This has been described numerous times in the literature involving non-*Saccharomyces* species that are naturally present in grapes and compete at the beginning of fermentation (Nissen and Arneborg, 2003; Medina et al., 2012; Lleixa et al., 2016). However, wine strains do not conform to a homogeneous group. As we demonstrated, not all of them competed effectively, despite being a defined phylogenetic subgroup (Liti et al., 2009; Legras et al., 2018). This highlights the importance of co-culture studies devoted to each strain of interest to elucidate what is driving a yeast to be highly competitive, in a particular, industrially relevant environment.

Several mechanisms are involved in the competitive ability of each strain, (Kemsawasd et al., 2015; Branco et al., 2017b, 2018; Luyt et al., 2021; Alonso del Real et al., 2019). Our results suggest, by analysing the *S. cerevisiae* GRE strain, that the ability to produce killer factors is a great advantage for implantation in a co-cultured fermentation with a

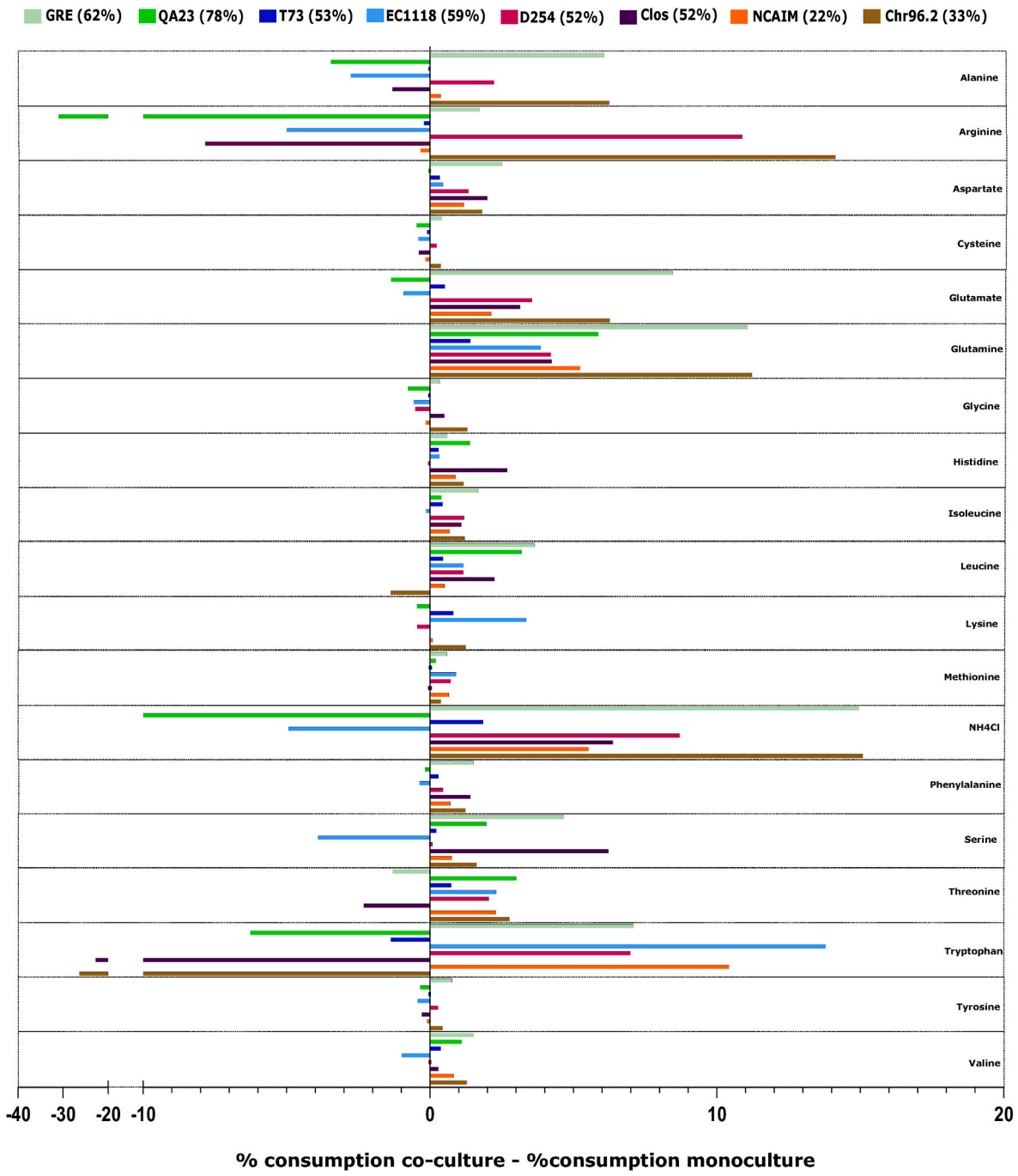


Fig. 6. Bar graph showing the difference between the percentage of consumption of the co-culture (different *S. cerevisiae* strains against *S. kudriavzevii*) and the percentage of consumption of the monoculture for each *S. cerevisiae* strain and for each amino acid. Positive numbers mean a higher consumption by the coculture. Each strain is represented in a colour, and the 8 study strains are grouped by amino acid analysed. The colour legend for each *S. cerevisiae* strain includes the proportion achieved by that *S. cerevisiae* in the co-culture in the time represented. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

sensitive-killer factor strain, as is the case of strain CR85. Also, our results indicate that nutrient uptake can be another mechanism associated with good competitive fitness as we observed in *S. cerevisiae* strains that did not produce killer factor. In brief, we observed that each *S. cerevisiae* consumes a large part of the amino acid between 48 and 72 h (the data show consumption in terms of percentage abundance patterns and overall nutrient concentrations), and more sluggishly than CR85. *S. cerevisiae* strain has its own amino acid consumption pattern and different patterns are emerging in co-cultured fermentations. However, our results suggest that those were more similar to *S. cerevisiae* strains when it comes to good competitor strains, with greater similarities being seen after 24 h of fermentation. And also that the amino acid consumption is higher in co-culture fermentation with the exception of T73.

In the case of wild strains such as NCAIM, their low capacity to uptake nitrogen sources was shown in monoculture, resulting in very low consumption. This could be directly related to the poor competitive ability of the strain due to the impossibility to outgrow a faster competitor such as CR85 in this case. The low presence of *S. cerevisiae* in the co-inoculated fermentation was reflected in the amino acid consumption, since the observed pattern was similar to CR85, indicating

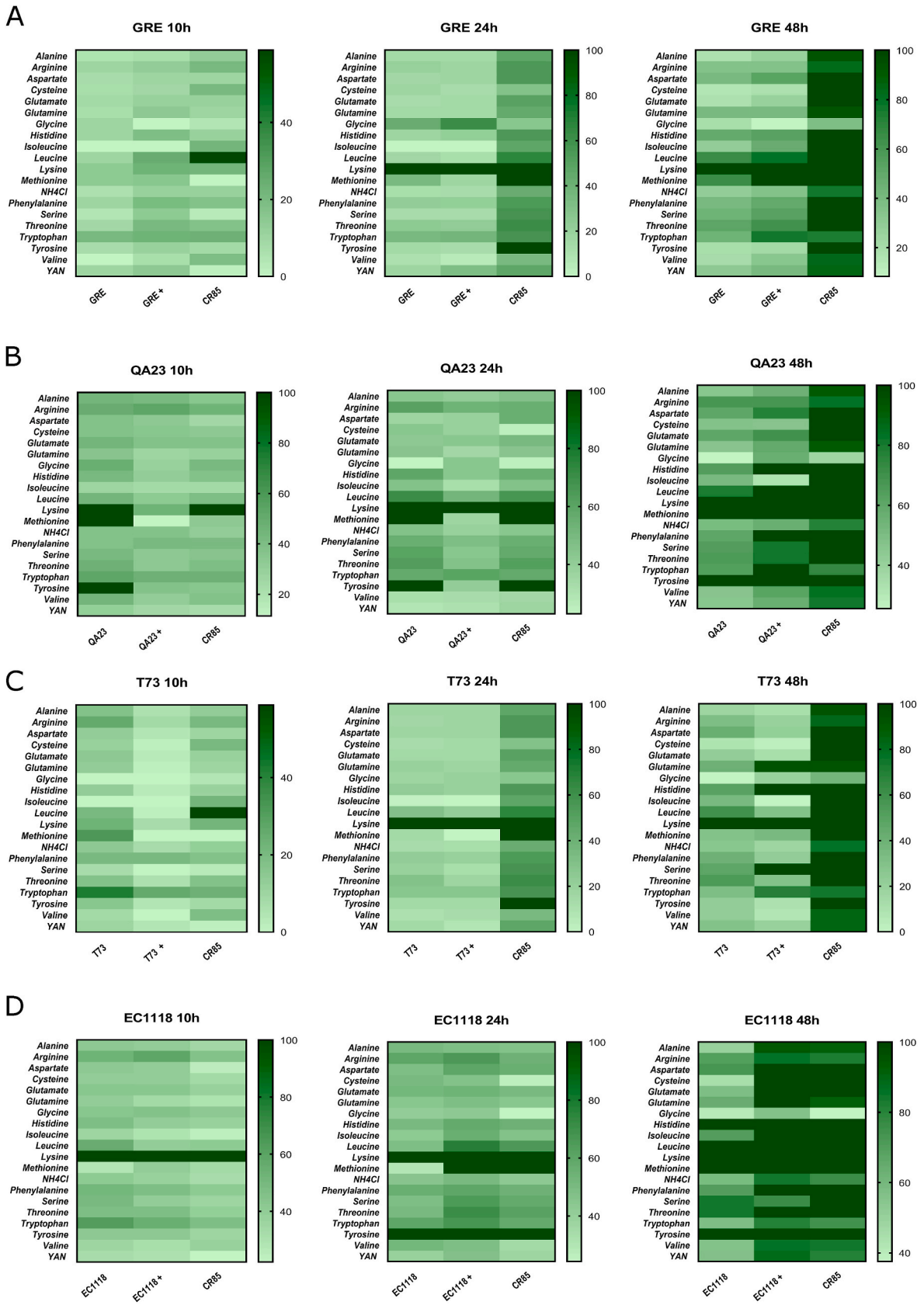


Fig. 7. The heatmap of the order of consumption rate of the different nitrogen sources including the total YAN consumption. The three points analysed, 10 h, 24 h, and 48 h for each study strain are represented. The colour range represents in light green a minimum consumption and gradually increases the colour shade to dark green as the consumption increases, being the darkest tone the total consumption. Each heatmap includes the amino acid consumption for the *S. cerevisiae* strains in monoculture, the *S. cerevisiae* - *S. kudriavzevii* co-culture, represented with the name of the *S. cerevisiae* strain next to a + sign and CR85 in monoculture. Analysed at the 3 sampling points 10 h, 24 h and 48 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

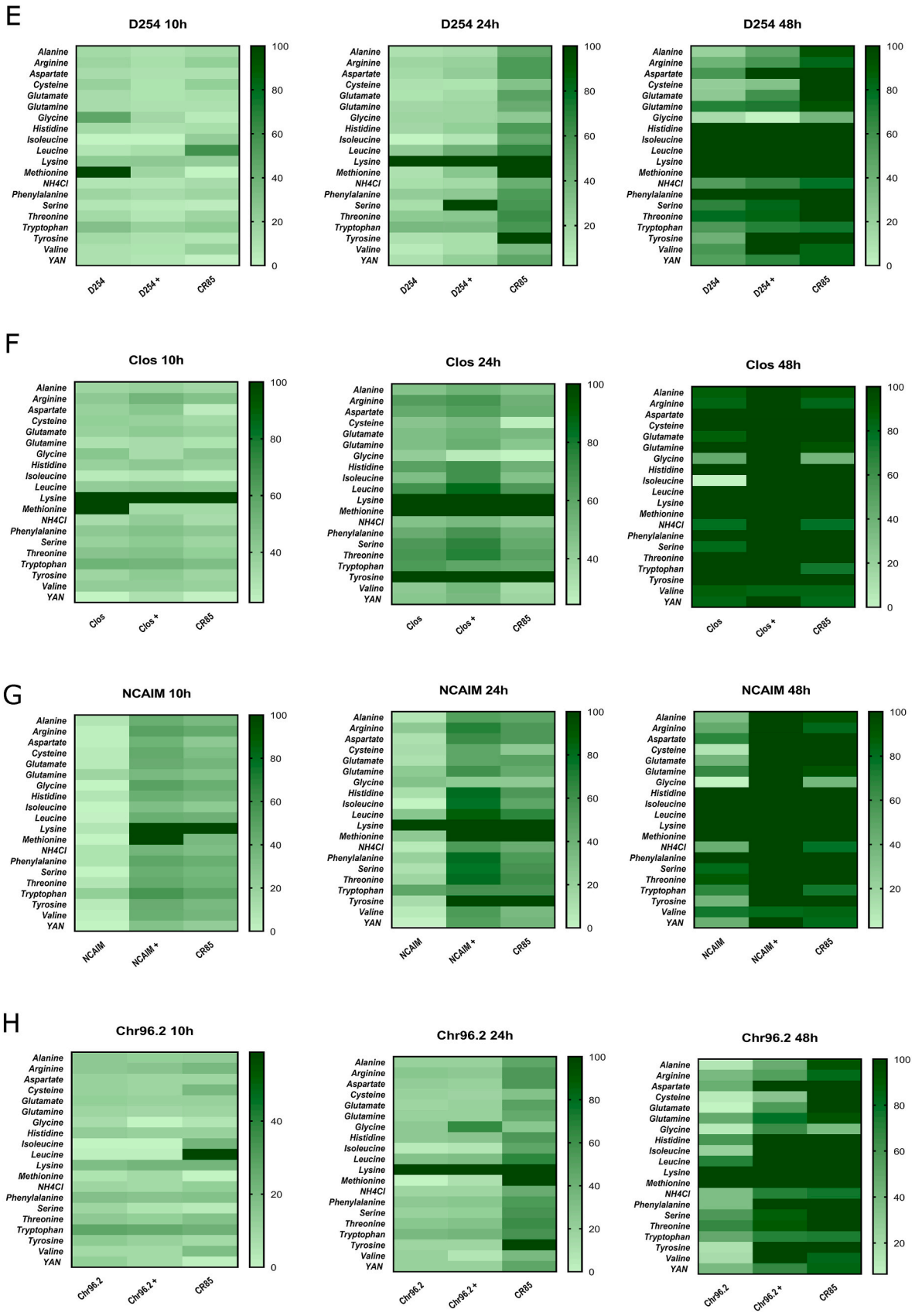


Fig. 7. (*continued*).

that the co-inoculated fermentation was being carried out mostly by CR85. Focusing the study not only on the consumption of amino acids but also on the consumption of sugars, Balsa-Canto et al. (2020) suggested that the cells would have a predetermined pattern of nutrient consumption, as well as Minebois et al. (2021) showed a different metabolic uptake between domesticated and wild wine strains. In our research, we show how the nutrient uptake pattern is differential even within strains of the same subclade, as is the case in our tested wine strains. These consumption differences could be related to the competitive fitness of the strain, however, there is not a clear pattern to be identified from our results in specific amino acids uptake, suggesting a very complex behaviour, except for the slow down in sugar consumption of some of the wine strains.

Here, T73′s sugar consumption behaviour, accelerated in co-culture, is worth to be mentioned. In our study, where we consider T73 as a medium competitive ability strain, it consumes and uses glucose faster than other strains, leading to an acceleration of the fermentation process, as we can see in the time it takes to finish fermentation. These results are consistent with those obtained by Alonso del Real (2017a, 2017b, 2019), who observed an acceleration in the consumption of sugars in conjunction with the reprogramming of gene expression during the early stages of fermentation when it competed with another strain. This reprogramming resulted in more efficient sugar uptake and was associated with a modification of the plasma membrane composition. However, we did not observe an acceleration of amino acid consumption. Other studies support the hypothesis of accelerated nutrient uptake when mixed fermentations are carried out. For instance, Shekhawat et al. (2019) revealed, with transcriptomics data, that the presence of other yeast in the medium affected genes that primarily belonged to two groups: genes that can be linked to the competition for certain trace elements such as copper and iron, as well as genes required for cell wall structure and integrity. Moreover, Tronchoni et al. (2017) performed mixed fermentations between *S. cerevisiae* and *non-saccharomyces* species such as *T. delbrueckii*, *C. sake,* or *H. uvarum*. Besides the overexpression of genes related to the sugar pathway, as seen by Alonso del real et al., (2019), they also found overexpression of genes related to the stress response and cell replication. This reprogramming was also observed by Conacher et al. (2022) who found that strains grown in mixed fermentations overexpressed genes related to the stress response, but that this response varied depending on which strains competed, and that the overexpression was even more notable when the co-culture was between three rather than two specie.

In our study we also observed these differences in behaviour, and even though these results are consistent with the studied strain T73, the strategy followed by GRE and QA23 (highly competitive strains), and EC1118 (medium competitive strains), show the opposite behaviour. The analysis of monoculture fermentation shows how the nutrient consumption of these three strains is very similar to the consumption of CR85. Nevertheless, when analysing the co-culture, we observed a slowdown in sugar consumption but a higher consumption of amino acids at 48 h with respect to the pure cultures. A possible explanation could be that these strains follow a different strategy and focus their energy resources on capturing more amino acids, the most limiting nutrient, to favour its growth and multiplication, leaving sugars aside. That is striking since it would disagree with the accepted paradigm that a strong crabtree effect is the main tool of *S. cerevisiae* in competition.

Nevertheless, the mechanism related to nutrient uptake must not be the only one involved. Another important part is the medium used to carry out the fermentation. The medium used in this work is a synthetic must with 300 mg of assimilable nitrogen which simulates a nitrogen concentration present in the natural must, but different outputs in the competition cannot be ruled out under different conditions, as suggested previously (Vendramini et al., 2017; Su et al., 2019).

In conclusion, wine strains are the *S. cerevisiae* subclade with the highest competitive ability against the strain of *S. kudriavzevii* studied, probably due to the domestication in the fermentative environment. However, we have demonstrated the high variability in that trait as well as in nutrient uptake strategies that exists within this group despite the homogeneity at the genomic level. The use of CR85 (a *S. kudriavzevii* strain; with interesting characteristics when fermenting wine musts at low temperatures) together with *S. cerevisiae* in mixed cultures has proved to be a simple model of study that has allowed us to broaden our knowledge in the use of consortium cultures. In a field of increasing interest in diversifying its products by the use of new strains or microbial consortia, such as winemaking, where systematic approaches to predict wine composition are needed, the broadening of the knowledge of the behaviour of particular combinations of strains constitutes a basic step to the application of mixed cultures as starters industrial level.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships to influence the work reported in this paper.

Acknowledgments

AC was supported by a FPU contract from Ministerio de Ciencia, Innovación y Universidades (ref. FPU18/02933). This project has received funding from the Spanish government MCIN/AEI and EU ERDF-FEDER projects RTI2018-093744-B-C31 and Grant PID2021-126380OB-C31 funded by MCIN/AEI/10.13039/501100011033 and EU ERDF-FEDER to AQ and RTI 2018-093744-B-C32 and PID 2021-126380OB-C33 to EB. Thanks to the Spanish government MCIN/AEI to the Center of Excellence Accreditation Severo Ochoa (CEX 2021-001189-S/funded by MCIN/AEI710.13039/501100011033).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2023.104276.

References

Alonso-del-Real, J., Lairón-Peris, M., Barrio, E., Querol, A., 2017a. Effect of temperature on the prevalence of *Saccharomyces* non *cerevisiae* species against a *S. cerevisiae* wine strain in wine fermentation: competition, physiological fitness, and influence in final wine composition. Front. Microbiol. 8, 150. https://doi.org/10.3389/fmicb.2017.00150.

Alonso-del-Real, J., Contreras-Ruiz, A., Castiglioni, G.L., Barrio, E., Querol, A., 2017b. The use of mixed populations of *Saccharomyces cerevisiae* and *S. kudriavzevii* to reduce ethanol content in wine: limited aeration, inoculum proportions, and sequential inoculation. Front. Microbiol. 8, 2087.

Alonso-del-Real, J., Pérez-Torrado, R., Querol, A., Barrio, E., 2019. Dominance of wine *Saccharomyces cerevisiae* strains over *S. kudriavzevii* in industrial fermentation competitions is related to an acceleration of nutrient uptake and utilization. Environ. Microbiol. 21 (5), 1627–1644. https://doi.org/10.1111/1462-2920.14536.

Arroyo-López, FN, Salvadó, Z., Tronchoni, J., Guillamón, JM., Barrio, E., Querol, A., 2010. Susceptibility and resistance to ethanol in Saccharomyces strains isolated from wild and fermentative environments. Yeast 27 (12), 1005–1015. https://doi.org/10.1002/yea.1809.

Balsa-Canto, E., Alonso-Del-Real, J., Querol, A., 2020. Temperature Shapes Ecological Dynamics in Mixed Culture Fermentations Driven by Two Species of the *Saccharomyces* Genus. Front Bioeng Biotechnol. 21 (8), 915. https://doi.org/10.3389/fbioe.2020.00915.

Bisson, L.F., 1999. Stuck and sluggish fermentations. Am. J. Enol. Vitic. 50, 107–119.

Branco, P., Francisco, D., Chambon, C., Hebraud, M., Arneborg, N., Almeida, M.G., et al., 2014. Identification of novel GAPDH-derived antimicrobial peptides secreted by *Saccharomyces cerevisiae* and involved in wine microbial interactions. Appl. Microbiol. Biotechnol. 98, 843–853.

Branco, P., Kemsawasd, V., Santos, L., Diniz, M., Caldeira, J., Almeida, M.G., et al., 2017. *Saccharomyces cerevisiae* wine strains show a wide range of competitive abilities and differential nutrient uptake behavior in co-culture with *S. kudriavzevii* accumulates GAPDH-derived peptides on its cell surface that induce death of non-*Saccharomyces* yeasts by cell-to-cell contact. FEMS Microbiol. Ecol. 93, fix055. https://doi.org/10.1093/femsec/fix055.

Branco, P., Albergaria, H., Arneborg, N., Prista, C., 2018. Effect of GAPDH-derived antimicrobial peptides on sensitive yeasts cells: membrane permeability, intracellular pH and H+−influx/−efflux rates. FEMS Yeast Res. 18, foy030.

Cavalieri, D., McGovern, P.E., Hartl, D.L., Mortimer, R., Polsinelli, M., 2003. Evidence for *S. cerevisiae* fermentation in ancient wine. J. Mol. Evol. 57.
Chen, H., Fujita, M., Feng, Q., Clardy, J., Fink, G.R., 2004. Tyrosol is a quorum-sensing molecule in *Candida albicans*. Proc. Natl. Acad. Sci. USA 101, 5048–5052.
Conacher, C.G., Naidoo-Blassople, R.K., Rossouw, D., Bauer, F.F., 2022. A transcriptomic analysis of higher-order ecological interactions in a eukaryotic model microbial ecosystem. mSphere 7. https://doi.org/10.1128/msphere.00436-22.
Crabtree, H.G., 1928. The carbohydrate metabolism of certain pathological overgrowths. Biochem. J. 22, 1289–1298.
Curiel, J.A., Morales, P., González, R., Tronchoni, J., 2017. Different *non-Saccharomyces* yeast species stimulate nutrient consumption in *S. cerevisiae*Mixed. Front. Microbiol. 8, 2121.
Fleet, G.H., 2003. Yeast interactions and wine flavour. Int. J. Food Microbiol. 86, 11–22.
Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B., et al., 2016. Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. Cell 166, 1397–1410.e16.
Guillamón, J.M., Barrio, E., 2017. Genetic polymorphism in wine yeasts: mechanisms and methods for its detection. Front. Microbiol. 4 (8), 806. https://doi.org/10.3389/fmicb.2017.00806.
Hagman, A., Torbjörn, S., Piškur, P., 2014. Analysis of the yeast short-term Crabtree effect and its origin. FEBS J. 281, 4805–4814. https://doi.org/10.1111/febs.13019.
Jones, G.V., White, M.A., Cooper, O.R., Storchmann, K., 2005. Climate change and global wine quality. Clim. Change 73, 319–343.
Kemsawasd, V., Branco, P., Almeida, M.G., Caldeira, J., Albergaria, H., Arneborg, N., 2015. Cell-to-cell contact and antimicrobial peptides play a combined role in the death of *Lachanchea thermotolerans* during mixed-culture alcoholic fermentation with *Saccharomyces cerevisiae*. FEMS Microbiol. Lett. 362 (14), fnv103. https://doi.org/10.1093/femsle/fnv103.
Legras, J.L., Galeote, V., Bigey, F., Camarasa, C., Marsit, S., Nidelet, T., et al., 2018. Adaptation of *S. cerevisiae* to fermented food environments reveals remarkable genome plasticity and the footprints of domestication. Mol. Biol. Evol. 35, 1712–1727.
Liti, G., Carter, D.M., Moses, A.M., Warringer, J., Parts, L., James, S.A., et al., 2009. Population genomics of domestic and wild yeasts. Nature 458 (7236), 337–341.
Lleixa, J., Manzano, M., Mas, A., Portillo, M.C., 2016. *Saccharomyces* and non-*Saccharomyces*Competition during microvinification under different sugar and nitrogen conditions. Front. Microbiol. 5, 1959. https://doi.org/10.3389/fmicb.2016.01959.
Lopes, C.A., Sangorrín, M.P., 2010. Optimization of killer assays for yeast selection protocols. Revista Argentina de Microbiologia.
Luyt, N.A., Beaufort, S., Divol, B., Setati, M.E., Taillandier, P., Bauer, F., 2021. Phenotypic characterization of cell-to-cell interactions between two yeast species during alcoholic fermentation. World J. Microbiol. Biotechnol. 28 (11), 186. https://doi.org/10.1007/s11274-021-03154-8.
McGovern, P.E., Zhang, J., Tang, J., Zhang, Z., Hall, G.R., Moreau, R.A., et al., 2004. Fermented beverages of pre- and proto-historic China. Proc. Natl. Acad. Sci. U. S. A. 101, 17593–17598.
Medina, K., Boido, E., Dellacassa, E., Carrau, F., 2012. Growth of non-*Saccharomyces* yeast affects nutrient availability for *Saccharomyces cerevisiae* during wine fermentation. Int. J. Food Microbiol. 157, 245–250.
Mencher, A., Morales, P., Tronchoni, J., Gonzalez, R., 2021. Mechanisms Involved in Interspecific Communication between Wine Yeasts. Foods 10 (8), 1734. https://doi.org/10.3390/foods10081734.
Minebois, R., Lairón, M., Barrio, E., Pérez-Torrado, R., Querol, A., 2021. Metabolic differences between wild and domesticated wine *Saccharomyces cerevisiae* strains during fermentation unveiled by multi-omic analysis. Environ. Microbiol. 23 (6), 3059–3076.
Navarro-Tapia, E., Nana, R.K., Querol, A., Pérez-Torrado, R., 2016. Ethanol cellular defense induce unfolded protein response in yeast. Front. Microbiol. 7, 189.
Nissen, P., Arneborg, N., 2003. Characterization of early deaths of non-*saccharomyces* yeasts in mixed cultures with *Saccharomyces cerevisiae*. Arch. Microbiol. 180, 257–263. https://doi.org/10.1007/s00203-003-0585-9.
Palkova, Z., Janderova, B., Gabriel, J., Zikanova, B., Pospisek, M., Forstova, J., 1997. Ammonia mediates communication between yeast colonies. Nature 390, 532–536.
Pérez, F., Ramírez, M., Regodón, J.A., 2001. Influence of killer strains of *Saccharomyces cerevisiae* on wine fermentation. Antonie van Leeuwenhoek. Int J Gen Mol Microbiol 79, 393–399.
Pérez, D., Jaehde, I., Guillamón, J.M., Heras, J.M., Querol, A., 2021. Screening of *Saccharomyces* strains for the capacity to produce desirable fermentative compounds under the influence of different nitrogen sources in synthetic wine fermentations. Food Microbiol. 97.
Pérez-Torrado, R., Rantsiou, K., Perrone, B., Navarro- Tapia, E., Querol, A., Cocolin, L., 2017a. Ecological interactions among *Saccharomyces cerevisiae* strains: insight into the dominance phenomenon. Sci. Rep. 7, 43603.
Pérez-Torrado, R., Barrio, E., Querol, A., 2017b. Alternative yeasts for winemaking: *Saccharomyces non-cerevisiae* and its hybrids. Crit. Rev. Food Sci. Nutr. https://doi.org/10.1080/10408398.2017.1285751.
Peris, D., Pérez-Través, L., Belloch, C., Querol, A., 2016. Enological characterization of Spanish *Saccharomyces kudriavzevii* strains, one of the closest relatives to parental strains of winemaking and brewing *Saccharomyces cerevisiae* x *S. kudriavzevii* hybrids. Food Microbiol. 53, 31–40. https://doi.org/10.1016/j.fm.2015.07.010.
Peter, J., Schacherer, J., 2016. Population genomics of yeasts: towards a comprehensive view across a broad evolutionary scale. Yeast 33, 73–81.
Peter, J., De Chiara, M., Friedrich, A., Yue, J.-X., Pflieger, D., Bergström, A., et al., 2018. Genome evolution across 1,011 Saccharomyces cerevisiae isolates. Nature 556 (7701), 339–344. https://doi.org/10.1038/s41586-018-0030-5.
Petitgonneta, D., Kleina, G.L., Roullier-Galla, C., Schmitt-Kopplinb, P., Quintanilla-Casasd, B., Vichid, S., et al., 2019. Influence of cell-cell contact between *L. thermotolerans* and *S. cerevisiae* on yeast interactions and the exo-metabolome. Food Microbiol. 83, 122–133.
Piškur, J., Rozpedowska, E., Polakova, S., Merico, A., Compagno, C., 2006. How did *Saccharomyces* evolve to become a good brewer? Trends Genet. 22, 183–186. https://doi.org/10.1016/j.tig.2006.02.002.
Pretorius, I.S., 2000. Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. Yeast 16, 675–729.
Querol, A., Barrio, E., Huerta, T., Ramón, D., 1992. Molecular monitoring of wine fermentations conducted by active dry yeast strains. Appl. Environ. Microbiol. 58, 2948–2953.
Querol, A., Fernández-Espinar, M.T., del Olmo, M., Barrio, E., 2003. Adaptive evolution of wine yeast. Int J Foof Microbiol S 86 (1–2), 3–10. https://doi.org/10.1016/s0168-1605(03)00244-7.
Querol, A., Pérez-Torrado, R., Alonso-del Real, J., Minebois, R., Stribny, J., Oliveira, B., Barrio, E., 2018. New trends in the uses of yeasts in oenology (Academic Press). Adv. Food Nutr. Res. 85, 210.
Raposo, G., Stahl, P.D., 2019. Extracellular vesicles: a new communication paradigm? Nat. Rev. Mol. Cell Biol. 20, 509–510.
Rodriguez-Cousiño, N., Maqueda, M., Ambrona, J., Zamora, E., Esteban, R., Ramírez, M., 2011. A new wine *Saccharomyces cerevisiae* killer toxin (Klus), encoded by a double-stranded RNA virus, with broad antifungal activity is evolutionarily related to a chromosomal host gene. Appl. Environ. Microbiol. 77, 1822–1832.
Rossignol, T., Dulau, L., Julien, A., Blondin, B., 2003. Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. Yeast 20, 1369–1385.
Rossouw, D., Meiring, S.P., Bauer, F.F., 2018. Modifying *Saccharomyces cerevisiae* adhesion properties regulates yeast ecosystem dynamics. mSphere 3.
Shekhawat, K., Patterton, H., Bauer, F.F., Setati, M.E., 2019. RNA-seq based transcriptional analysis of *Saccharomyces cerevisiae* and *Lachancea thermotolerans* in mixed-culture fermentations under anaerobic conditions. BMC Genom. 20, 145.
Su, Y., Origone, A., Rodríguez, M.A., Querol, A., Guillamón, J.M., Lopes, C.A., 2019. Fermentative behaviour and competition capacity of cryotolerant *Saccharomyces* species in different nitrogen conditions. Int. J. Food Microbiol. 291, 111–120.
Tronchoni, J., Curiel, J.A., Morales, P., Torres-Pérez, R., González, R., 2017. Early transcriptional response to biotic stress in mixed starter fermentations involving *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*. Int. J. Food Microbiol. 241, 60–68.
van Leeuwen, C., Darriet, P., 2016. The impact of climate change on viticulture and wine quality. J. Wine Econ 11 (1), 150–167.
Vendramini, C., Beltran, G., Nadai, C., Giacomini, A., Mas, A., Corich, V., 2017. The role of nitrogen uptake on the competition ability of three vineyard *Saccharomyces cerevisiae* strains. Int. J. Food Microbiol. 258, 1–11, 2017.
Wang, Q.-M., Liu, W.-Q., Liti, G., Wang, S.-A., Bai, F.-Y., 2012. Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity. Mol. Ecol. 21, 5404–5417.
Williams, K.M., Liu, P., Fay, J.C., 2015. Evolution of ecological dominance of yeast species in high-sugar environments. Evolution 69, 2079–2093. https://doi.org/10.1111/evo.12707.
Winters, M., Arneborg, N., Appels, R., Howel, K., 2019. Can community-based signalling behaviour in *Saccharomyces cerevisiae* be called quorum sensing? A critical review of the literatura. FEMS Yeast Res. 19, foz046.
Yan, G., Zhang, B., Joseph, L., Waterhouse, A.L., 2020. Effects of initial oxygenation on chemical and aromatic composition of wine in mixed starters of Hanseniaspora vineae and *Saccharomyces cerevisiae*. Food Microbiol. 90, 103460 https://doi.org/10.1016/j.fm.2020.103460.
Zilelidou, E.A., Nisiotou, A., 2021. Understanding wine through yeast interactions. Microorganisms 9 (8), 1620.

Further reading

Albergaria, H., Arneborg, N., 2016. Dominance of *Saccharomyces cerevisiae* in alcoholic fermentation processes: role of physiological fitness and microbial interactions. Appl. Microbiol. Biotechnol. 100, 2035–2046. https://doi.org/10.1007/s00253-015-7255-0.
Beltran, G., Rozès, N., Mas, A., Guillamón, J.M., 2006. Effect of low-temperature fermentation on yeast nitrogen metabolism. World J. Microbiol. Biotechnol. 23, 809–815.
Chen, H., Fink, G.R., 2006. Feedback control of morphogenesis in fungi by aromatic alc hols. Genes Dev. 20, 1150–1161.
Gómez-Alonso, S., Hermosín-Gutiérrez, I., García-Romero, E., 2007. Simultaneous HPLC analysis of biogenic amines, amino acids, and ammonium ion as aminoenone derivatives in wine and beer samples. J. Agric. Food Chem. 55, 608–613.
Goold, H., Kroukamp, H., Williams, T., Paulsen, I., Varela, C., Pretorius, I., 2017. Yeast's balancing act between ethanol and glycerol production in low-alcohol wines. Microb. Biotechnol. 10, 264–278.
Henriques, D., Alonso-del Real, J., Querol, A., Balsa-Canto, E., 2018. *Saccharomyces cerevisiae* and *S. kudriavzevii* synthetic wine fermentation performance dissected by predictive modeling. Front. Microbiol. 9, 88.
Oliveira, B.M., Barrio, E., Querol, A., Pérez-Torrado, R., 2014. Enhanced enzymatic activity of glycerol-3-phosphate dehydrogenase from the cryophilic. *Saccharomyces kudriavzevii*. PLoS ONE. 30;9(1):e87290.
Pérez-Nevado, F., Albergaria, H., Hogg, T., Girio, F., 2006. Cellular death of two non-*Saccharomyces* wine-related yeasts during mixed fermentations with *Saccharomyces*

cerevisiae. Int. J. Food Microbiol. 108, 336–345. https://doi.org/10.1016/j.ijfoodmicro.2005.12.012.

Pérez-Torrado, R., Oliveira, B.M., Zemancikova, J., Sychrová, H., Querol, A., 2016. Alternative glycerol balance strategies among *Saccharomyces* species in response to winemaking stress. Front. Microbiol. 7, 435. https://doi.org/10.3389/fmicb.2016.00435.

Pérez-Través, L., Lopes, C.A., Barrio, E., Querol, A., 2014. Stabilization process in *Saccharomyces* intra and interspecific hybrids in fermentative conditions. Int. Microbiol. 17, 213–224. https://doi.org/10.2436/20.1501.01.224.

Salvado, Z., Arroyo-Lopez, F.N., Barrio, E., Querol, A., Guillamón, J.M., 2011. Quantifying the individual effects of ethanol and temperature on the fitness advantage of *Saccharomyces cerevisiae*. Food Microbiol. 28, 1155–1161. https://doi.org/10.1016/j.fm.2011.03.008.

Thomson, J.M., Gaucher, E.A., Burgan, M.F., De Kee, D.W., Li, T., et al., 2005. Resurrecting ancestral alcohol dehydrogenases from yeast. Nat. Genet. 37, 630–635. https://doi.org/10.1038/ng1553.

Varela, C., Sengler, F., Solomon, M., Curtin, C., 2016. Volatile flavour profile of reduced alcohol wines fermented with the non-conventional yeast species *Metschnikowia pulcherrima* and *Saccharomyces uvarum*. Food Chem. 209, 57–64. https://doi.org/10.1016/j.foodchem.2016.04.024.

Wang, C., Mas, A., Esteve-Zarzoso, B., 2015. Interaction between Hanseniaspora uvarum and *Saccharomyces cerevisiae* during alcoholic fermentation. Int. J. Food Microbiol. 206, 67–74.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K., 1990. Modeling of the bacterial growth curve. Appl. Environ. Microbiol. 56, 1875–1881.