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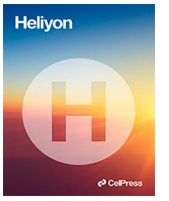

Research article

Exploring wine yeast natural biodiversity to select strains with enological traits adapted to climate change

Joana P. Guedes [a,1], Tiago Vidal Cardoso [a,1], Ticiana Fernandes [a], Filipa Mendes [a], M. Margarida Baleiras-Couto [b,c], Filomena L. Duarte [b,c], Maria João Sousa [a], Ricardo Franco-Duarte [a], Susana R. Chaves [a], Manuela Côrte-Real [a,*]

[a] CBMA - Centre of Molecular and Environmental Biology/ARNET-Aquatic Research Network, University of Minho, Campus of Gualtar, 4710-057, Braga, Portugal
[b] INIAV, IP - Instituto Nacional de Investigação Agrária e Veterinária, Pólo de Inovação de Dois Portos, Quinta da Almoinha, 2565-191, Dois Portos, Portugal
[c] BioISI – Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, 1749-016, Lisboa, Portugal

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ABSTRACT

Wine is widely consumed throughout the world and represents a significant financial market, but production faces increasing challenges. While consumers progressively value more complex flavor profiles, regional authenticity, and decreased use of additives, winemakers strive for consistency among climate change, characterized by rising environmental temperatures and sun burn events. This often leads to grapes reaching phenolic maturity with higher sugar levels, and increased microbial spoilage risk.

Herein, we addressed these dual concerns by investigating the use of autochthonous *Saccharomyces cerevisiae* strains for fermentations of grape musts resulting from these altered conditions. We characterized underexplored repositories of naturally-occurring strains isolated from different environments and geographical regions, regarding adequate enological properties (e.g., high cell growth, reduced production of H_2S, ethanol and acetic acid, increased SO_2 resistance, killer activity), and other less frequently investigated properties (resistance to osmotic stress, potassium and aluminium silicates and fungicides). The phenotypic data were organized in a biobank, and bioinformatic analysis grouped the strains according to their characteristics. Furthermore, we analyzed the potential of four Portuguese isolates to be used in fermentations of grape musts with high sugar levels, uncovering promising candidates. This research therefore contributes to ongoing efforts to increase sustainability and quality of wine production.

1. Introduction

Wine production is one of the most relevant industries in the Portuguese secondary sector. Besides international competition, the current climatic instability and pest pressure arise as significant threats to grapevine and wine, requiring strategies to adapt to the

* Corresponding author.
E-mail address: mcortereal@bio.uminho.pt (M. Côrte-Real).
[1] Equal contributors.

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stress conditions resulting from these changes. Indeed, climate change significantly affects grapevine growth and physiology, impacting not only yield but also grape berry chemistry [1]. As a result, alterations in grape must composition can lead to stuck fermentations or varied unwanted consequences in the properties of finished wines, which develop diversified flavors and mouthfeel [2,3]. One of the most pressing issues is increased grape sugar, which results in higher ethanol content and can also lead to stuck fermentations, decreased fixed acidity and increased levels of undesirable fermentation co-products, such as acetic acid. Yet another consequence of global warming is increased radiation, and thus the spraying of vineyards with sunscreen materials such as kaolin and potassium silicate has been increasing [4,5]. Wine regions also face an increased risk of pests and diseases, which will be aggravated by decreased pesticide use, necessary for sustainable agriculture and to meet consumer concerns. Indeed, decreased use of pesticides can lead to increased contamination from spoilage microorganisms, often resulting in spoiled wine. In addition, consumer health awareness and demand for organic products limit the use of additives such as sulphur dioxide (SO_2) directly to grape must or wine, aggravating this problem [6].

The number of studies analyzing yeast diversity in several locations worldwide has greatly increased, due to the recognition of the role of indigenous yeasts in wine differentiation and potential to solve emergent problems [7–9]. These studies show that many strains can be explored to impart positive wine organoleptic key characteristics through: *i)* increase of specific fermentation products such as glycerol and fixed acidity; *ii)* reduction of acetic acid content; *iii)* enhancement of the aromatic profile, namely of esters, higher alcohols and volatile thiols; *iv)* enrichment of wine aroma specifically by secreted esterases, *β*-glucosidases, lipases, proteases, and others; *v)* reduction of ethanol content; *vi)* control of the spoilage microflora; and *vii)* improvement to the overall wine quality and complexity.

In summary, increasing changes in grape berry composition, due to climate alterations, led to changing properties of grape musts. As such, it becomes imperative to control the fermentation step to tailor wines of increased quality and typicity, envisaging the maintenance of product characteristics in a multi-year scale. Yeast selection is therefore one of the most influential post-harvest decisions regarding the fine tuning of a particular wine, and producers would thus greatly benefit from expanded choices of unexplored autochthonous yeast strains. This highlights the need for yeast biobanks containing a large number of well-characterized isolates with adequate properties to cope with alterations in grape must composition. In the present work, we aimed to select Portuguese yeast strains that could provide an alternative to available commercial *Saccharomyces cerevisiae* strains, especially concerning grape musts with changed chemical compositions.

2. Materials and methods

2.1. Strain collection

A total of 223 *S. cerevisiae* strains were used in this work, available at the collections of the Department of Biology/Centro de Biologia Molecular e Ambiental (CDB), Estação Vitivinícola Nacional/Instituto Nacional de Investigação Agrária e Veterinária (INIAV), and Instituto dos Vinhos do Douro e Porto (IVDP), isolated throughout several years from different origins (vineyard, grape must, wine, arbutus berry fermentation, other fermentation, baker, laboratory, soil, clinical and unknown) and including commercial strains (Supplementary table S1).

2.2. Culture media and treatments

Cells were pre-inoculated overnight aerobically in synthetic grape must (SM medium) pH 3.3–3.4 [10], containing 200 g/L of sugars (SM200, 100 g/L fructose+100 g/L glucose) or 300 g/L of sugars (SM300, 150 g/L fructose+150 g/L glucose) in 96-well plates and incubated at 30 °C, before each of the further tests. Cells were then diluted in the respective fresh medium with the desired treatments by using a replicator, in 96-well plates, and incubated at 26 °C. For each treatment, cell growth was evaluated after 24 h and 48 h by measuring the OD at 600 nm and compared with cell growth in medium without any treatment (control). Treatments were as follows: SM200 medium containing acetic acid (80 mM, 120 mM and 160 mM), ethanol (12 % and 14 %, v/v), the fungicides tebuconazole (62.5 μg/mL and 125 μg/mL) or cymoxanil (125 μg/mL and 250 μg/mL), sodium metabisulfite (3.5 mM and 4.5 mM), kaolin (5 %, w/v) or potassium silicate (6.5 mM), To test resistance to osmotic pressure, we assessed growth in SM medium with 200 g/L glucose and 200 g/L fructose (SM400 medium), as described by Stratford et al. [11]. To assess temperature growth dependency, yeast strains were grown in SM200 medium at different temperatures (15, 18, 22, 26 and 37 °C). To attribute scores for phenotypic tests, and to facilitate the mathematical/statistical tests as in Mendes et al. [12], the yeast cell growth under each treatment conditions was catalogued in four growth scores, namely: score 0 - $OD_{600nm} \leq 10$ % OD_{600nm} max; score 1–10 % OD_{600nm} max $< OD_{600nm} \leq 25$ % OD_{600nm} max; score 2–25 % OD_{600nm} max $< OD_{600nm} \leq 75$ % OD_{600nm} max; score 3 - $OD_{600nm} > 75$ % OD_{600nm} max. OD_{600nm} max, maximum OD_{600nm} of the control condition. All experiments were performed according to Mendes et al. [12], Franco-Duarte et al. [13], Barbosa et al. [14], and Ruiz et al. [15] and carried out between March 2022 and March 2023.

2.3. H_2S production assessment

We assessed reduction of sulphite to sulphide on BIGGY solid medium [16]. Strains were inoculated on BIGGY medium using a pin replicator, incubated at 30 °C and the color alteration was monitored over 48 h. The phenotypic scores attributed to the yeast strains in BIGGY solid medium were: 0, no color alteration; 1, cream color; 2, brown color; and 3, dark brown color. To control for consistency, results were compared with those obtained previously with 300 *S. cerevisiae* strains [17].

2.4. β-glucosidase activity assessment

We assessed β-glucosidase activity, which may increase the aromatic wine profile, using Esculin Bile Agar. Strains were inoculated on Esculin Bile Agar medium (Merck®, Kenilworth, NJ, USA) using a pin replicator, incubated at 30 °C and the halo formation was monitored over 48 h, according to Silva-Sousa et al. [18]. The phenotypic scores attributed to the yeast strains on Esculin Bile Agar were: 0 – does not show a halo; 1 – halo size ≤0.4 cm (negative control); 2–0.4 < halo size >0.6 cm; 3 – halo size >0.6 cm.

2.5. Killer activity assessment

Cells were pre-inoculated overnight aerobically in YPD medium (2 % glucose, 1 % peptone and 0.5 % yeast extract) and incubated at 30 °C, 200 rpm. Plates were seeded with the killer *S. cerevisiae* Z157 strain or sensitive *S. cerevisiae* Z10 strain at $OD_{600\ nm} = 1$. After, yeast strains were inoculated (1 mL) at $OD_{600\ nm} = 0.1$ in the killer and sensitive plates. The plates were then incubated at 30 °C and the visual alterations were monitored over 48 h, following the protocol of Silva-Sousa et al. [18]. The absence/presence of a halo indicates a non-killer/killer strain (score 0/3) whereas the absence/appearance of blue color indicates a non-sensitive/sensitive strain (score 0/3).

2.6. Growth curves

For growth curves, cells were grown overnight aerobically in SM200 or SM300, pH 3.3–3.4 in 96-well microplates, and incubated at 30 °C. Cells were then diluted in SM200 or SM300 medium at an approximate OD_{600nm} of 0.05, in 96-well microplates. Cells were incubated at 22 °C in a microplate reader (Victor Nivo TM, PerkinElmer) and their growth was evaluated hourly during 72 h by measuring optical density at 600 nm (OD_{600nm}). The data were plotted in semilogaritmic graphs of the OD_{600nm} versus time.

2.7. Fermentations

Pre-cultures were prepared in synthetic grape must (SM200 medium) and incubated overnight aerobically at 30 °C. Cells were then diluted in fresh SM200 medium or in SM300 medium at an $OD_{600} = 0.05$ and incubated at 22 °C, 50 rpm. Fermentations were carried out in Erlenmeyer flasks (50 mL), in triplicate, using a ratio of 1:2.5. Cell growth was evaluated after 3, 6 and 9 days by measuring OD_{600nm}. The fermentative profile of each strain was evaluated by HPLC, according to the method described in [19]. Briefly, to deproteinize samples, 2 % (v/v) perchloric acid was added, samples were kept on ice for 30 min and then centrifuged at 12,000×*g* for 10 min. Prior to analysis, the supernatants were filtered through a 0.22 μm pore filter and then subjected to analysis using a Carbohydrate H+ 9 μm HyperRez XP column to quantify glucose, fructose, glycerol, acetic acid, succinic acid and ethanol. The analysis was carried out at a constant flow rate of 0.5 mL/min over 30 min at 40 °C. The concentrations of the detected compounds were determined using an internal standard method with arabinose (10 g/L) as the internal standard.

2.8. Data analysis

The results from three independent experiments performed for each test were compiled in a matrix. Scores between 0 and 3 were used to represent all phenotypic results, as explained in the corresponding sections and according to Mendes et al. [12], in order to facilitate and validate the multivariate analysis. Phenotypic inter-strain variability was evaluated by principal component analysis (PCA), and FreeViz [20], available in the Orange data mining suite software v. 3.25.0 [21]. Statistical analysis was performed using Student's *t*-test (GraphPad Prism, USA, Boston).

3. Results and discussion

A total of 223 *S. cerevisiae* strains available at the collections referred in Material and Methods were characterized in detail. Supplementary Data S1 details the environments and technological applications from where the strains derived: wine environments, commercial wine strains, arbutus berry, clinical, laboratory, other fermentative beverages, other natural environments (fruits, soil woodland and plants), baker, and two isolates from an unknown origin. A phenotypic screen was devised to evaluate intra-strain specific patterns among the 223 strains for a set of 23 physiological tests that are important for winemaking strain selection. While some are standard, others were included to mimic the conditions of altered grape musts, brought about by alterations in grape berry composition caused by climate change. All tests were selected from relevant literature that unravels phenotypic diversity of natural yeast populations [12,13,22–25]. To the extent of our knowledge, this constitutes one of the largest groups of *S. cerevisiae* strains that were tested for such an extensive set of phenotypic and fermentative tests.

The global profile of intra-strain phenotypic variation was evaluated using principal component analysis (PCA) of the data generated in the screening approach, as shown in Fig. 1. PCA results show the segregation of the 223 isolates (Fig. 1A – scores) and the 23 phenotypic variables (Fig. 1B – loadings), in the first two PCA components.

Strain variability is explained by the first two principal components, up to a total of 30.4 % (PC1 - 20.4 % and PC2 - 10.0 %). Each strain was categorized according to its origin (specific environment or technological application) and using different colors, in order to evaluate a potential relation between the strain origin and phenotypic behavior. While all the variables contribute to explain the diversity of the strains, the phenotypes responsible for the highest diversity observed appear to be related with resistance to 14 % (v/v)

ethanol, performance at different temperatures (especially 22 and 18 °C), and resistance to acetic acid, concerning the first component. Additionally, resistance to cymoxanil and sodium metabisulphite is associated with the second component (Fig. 1B). Of note, three strains showed a very heterogenous profile, depicted by their position in the top right of the PCA, even though they belong to three different categories, namely wine, arbutus berry and other fermentations. These three strains showed a very low tolerance to the conditions identified before as influential to the first component, especially growth at 22 °C and in 14 % (v/v) ethanol. Of particular importance is the discriminatory position of the temperatures test to separate these strains, in contrast to the neutral contribution of temperatures to discriminate other yeast isolates in our previous study [12]. The main difference is the wider range of temperatures introduced, allowing to unravel great strain heterogeneity at 22 °C, a value not tested in the previous study.

Wine strains mainly accumulate in the bottom part of the PCA (Fig. 1A), under the influence of the second component. They display considerable heterogeneity, but a distinct separation from the strains obtained from arbutus berry fermentation, which predominantly accumulate in the upper part of the PCA. Given its relatively unexplored nature, arbutus berry yeasts could be of great interest to produce differentiated products, mainly because of an apparent higher resistance to sugars and to low temperature (15 °C), as shown by the PCA of Fig. 1. While beyond the main scope of this work, these strains warrant further assessment of their fermentation

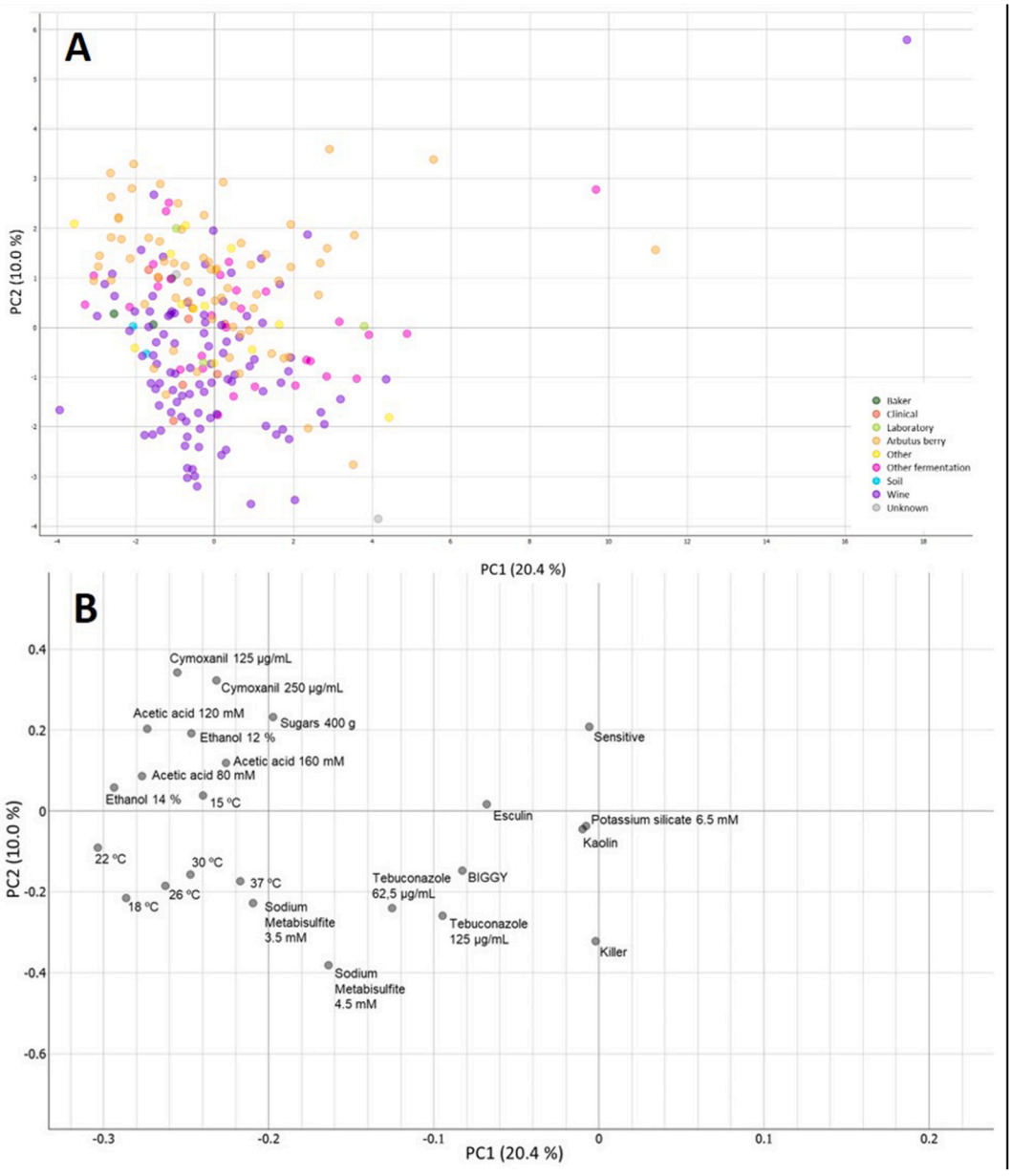

Fig. 1. Principal component analysis (PCA) of phenotypic data of 223 *S. cerevisiae* strains. (A) Scores – strains distribution. Colors represent the technological application or origin of the strains. (B) Loadings – 23 phenotypic tests.

performance.

The 96 winemaking strains analyzed in this study seem to be able to grow at different temperatures and are resistant to sodium metabisulphite and 14 % (v/v) ethanol (Fig. 1), which are relevant characteristics in the context of climate change. Though these characteristics were not found in a previous screening with *S. cerevisiae* strains [12], another study showed that some winemaking strains also exhibit resistance to potassium metabisulphite [19], but a good performance at high temperatures and to high percentages of ethanol was not as evident as herein. In fact, sulphur dioxide, caused by the decomposition of sodium or potassium metabisulphite, is widely used as a preservative and antimicrobial agent in various industries, causing a general inhibition of bacteria and yeast growth [26,27]. The cellular and molecular mechanisms responsible for the sulphur dioxide resistance of some yeast strains are fairly complex and under investigation, but it was shown to have a direct correlation with the pH, ethanol and sugar concentration in the media [27]. Interestingly, the strain collection studied herein rather showed an inverse correlation with the sugar and ethanol concentration,

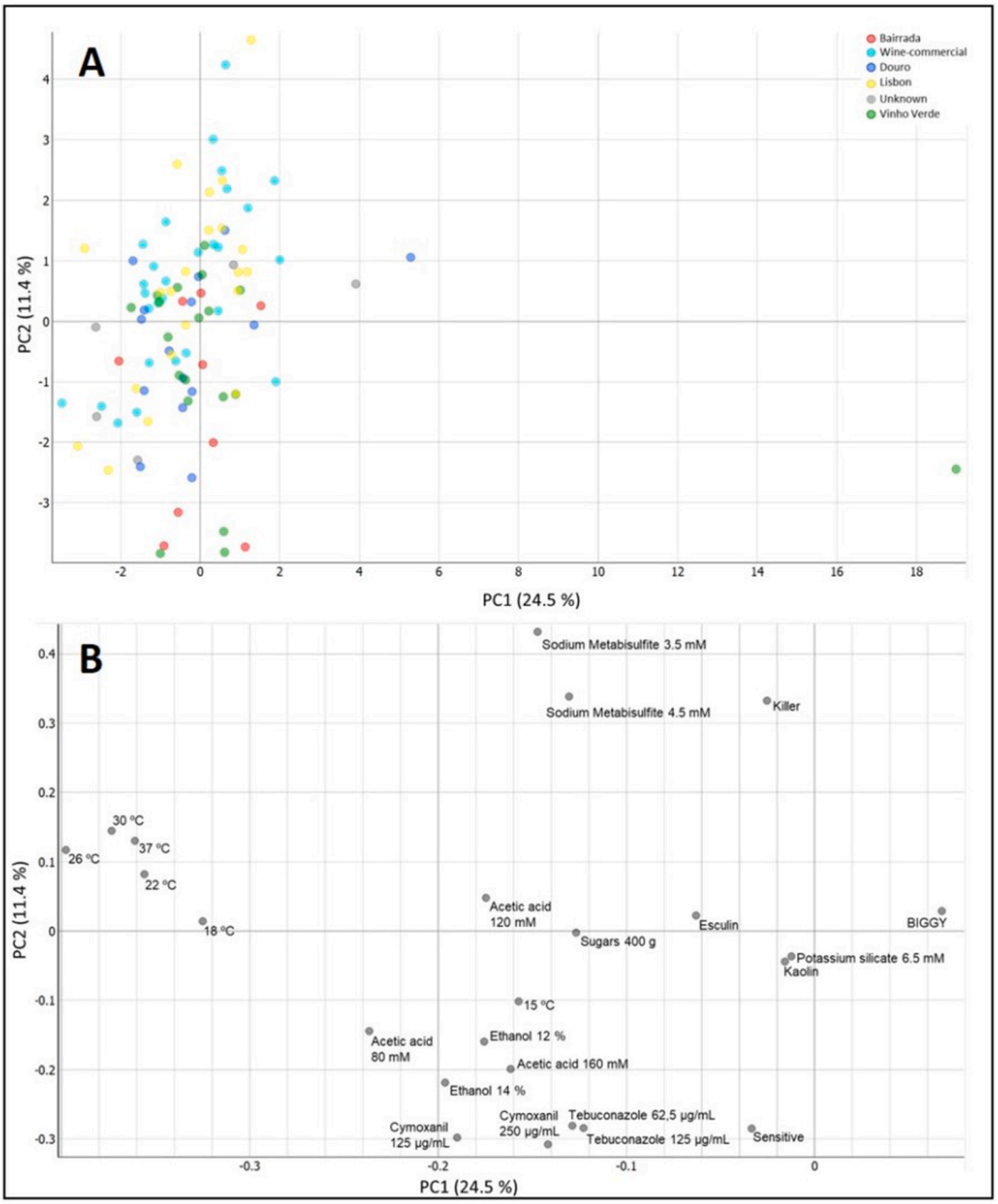

Fig. 2. PCA of phenotypic data of 96 *S. cerevisiae* wine strains (Portuguese wine isolates), in comparison with commercial *S. cerevisiae* strains. (A) Scores – strains distribution. Colors represent the geographical origin of the strains or their identification as commercial strains. (B) Loadings – 23 phenotypic tests.

though showing a direct correlation with the capacity to resist to higher temperatures (in particular to 37 °C).

These results highlight the potential of the current yeast bio-databank, which gathers relevant phenotypic characterization of strains from different environmental and technological origins, including wine isolates, to be used successful in fermentations of grape musts with altered chemical composition.

To better evaluate diversity within the group of the 96 winemaking strains, a new PCA visualization was built, coloring strains according to their geographical origin (Fig. 2). This new PCA shows the segregation of strains in the two first principal components (PC1 - 24.5 % and PC2 - 11.4 %), representing a total of 35.9 % of variability explained.

PCA visualization shows no clear separation between the different origins (Douro, Bairrada, Lisboa and Vinho Verde) and the commercial wine strains (Fig. 2A). However, the distribution of Lisboa strains overlaps more with commercial strains in the upper part of the PCA, whereas the bottom mainly comprises strains from Vinho Verde and Bairrada. Douro strains are predominantly located in the central part of the PCA, with no strains in extreme positions. Other *S. cerevisiae* strains from Douro were previously shown to be more diverse, but regarding reduction of grape musts volatile acidity [28,29], a feature not assessed in this phenotypic screening. The natural isolates from Bairrada and Vinho Verde are more similarly distributed, namely regarding resistance to fungicides and sodium metabisulfite (Fig. 2B). This high resistance is in accordance with previous results regarding other strains from Bairrada [30] and Vinho Verde [12] regions. This may be related with similarities in climate conditions such as humidity, which influence viticulture and

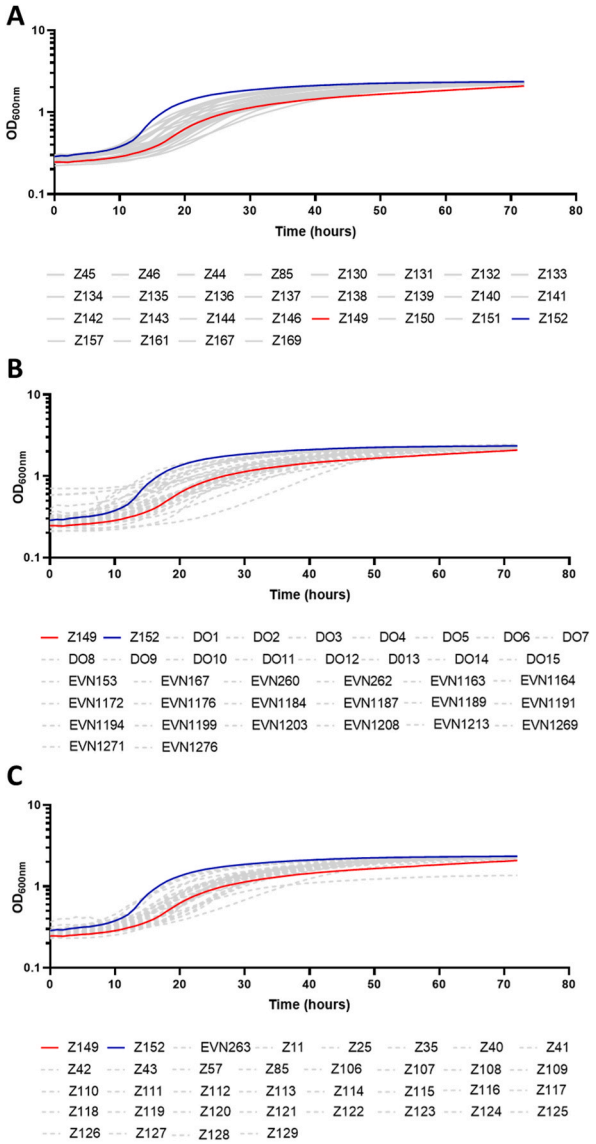

Fig. 3. Growth curves of the 96 *S. cerevisiae* winemaking strains in SM300 media, during 72 h of growth. A) Commercial strains are represented by full lines, where the best and worst strain in terms of faster and slowest growth, after 72h, are coloured in blue (Z152) and red (Z149) respectively; B) and C) natural isolates are represented by dotted lines, together with Z152 and Z149.

oenological practices. In contrast, the hotter and drier climate, along with associated practices, may drive the differentiation of Lisbon strains, and potentially also of some commercial winemaking strains.

Since our results indicate that natural winemaking isolates from all the regions may present similar features to the commercial strains, especially under normal concentration of sugars (with 200 g/L in synthetic must, SM200), we next evaluated how the 96 *S. cerevisiae* winemaking strains in the collection (both natural isolates and commercial strains) would perform in the presence of a higher concentration of sugar (300 g/L in synthetic must, SM300). For this purpose, growth curves were built with the 68 isolates and 28 commercial winemaking strains using SM300 media in 96 microplate wells, during 72 h of growth at 22 °C (Fig. 3). Though the specific growth rates determined under these conditions are lower than those obtained using flasks with agitation, they allowed the comparison between the growth behavior of the natural isolates and the commercial strains. Growth of the commercial strains (full lines) was less heterogeneous than that of the natural isolates (broken lines) in SM media containing 300 g/L of sugars. Interestingly, the growth behavior of some of the natural isolates was better than some of the commercial strains. Indeed, most natural isolates displayed shorter lag phases and/or higher specific growth rates than the commercial strains (Supplementary table S2).

The natural isolate 469 and the commercial Z152 strains and the natural isolate EVN260 and Z130 strains exhibited the highest and lower OD_{600nm} 72h after growth in SM300 medium, respectively (Supplementary Data S2), but nearly all strains grew well (Fig. 3). Among the 20 strains exhibiting a decrease in the specific growth rate <30 % in relation to the fastest strain, there were 13 natural

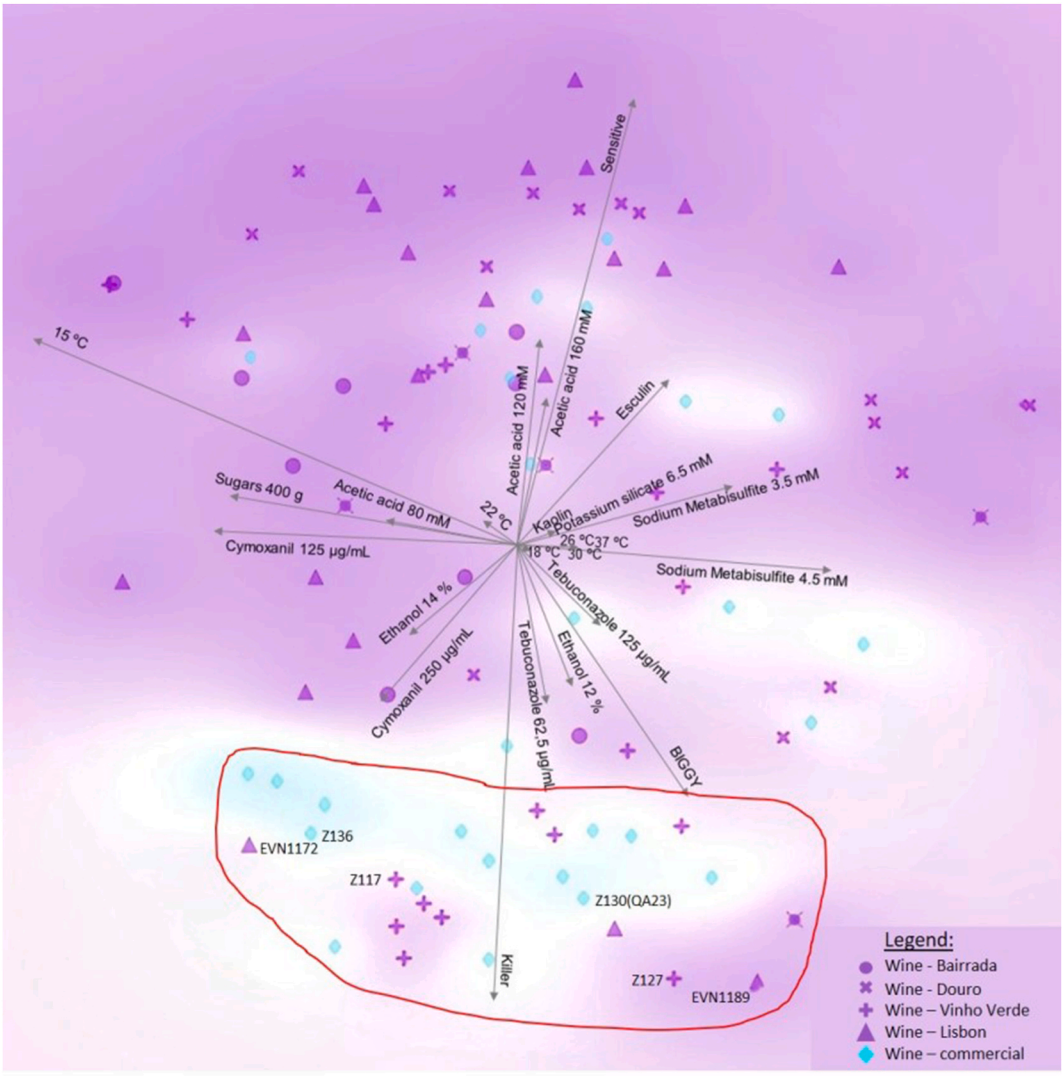

Fig. 4. FreeViz plots of multivariate projections, using phenotypic data (23 tests) of the 96 *S. cerevisiae* isolates from wine environments (natural isolates and commercial strains). Symbol shapes represent the strains' geographical origin, and colors identify natural or commercial winemaking strains. The direction and size of each vector indicates the relative prevalence of that feature to explain group stratification. Background color is an intensity gradient related to cluster positioning and size, and indicates the relative prevalence of that feature to explain group stratification. The six identified strains within the figure were chosen for further analysis.

isolates and 7 commercial strains (Supplementary Data S2). On the other hand, if the comparison is made in relation to the strain with lowest lag phase duration, the top 20 strains include 17 natural isolates and 3 commercial strains (Supplementary Data S2). As culture growth rate is determined by both the specific growth rate and the lag phase duration, taking both these parameters into account, the list of 20 strains with faster growth includes 14 natural isolates and 6 commercial strains (Supplementary Data S2). These data suggest that some of the natural isolates tested are similar or even better suited to ferment grape musts with higher sugar levels than most commercial strains.

To further verify the contribution of each phenotypic test to the differences observed between winemaking strains, and especially comparing wild and commercial strains, a multivariate projection using the FreeViz algorithm was used (Fig. 4), considering all the phenotypic tests and the 96 winemaking strains.

In FreeViz, each strain is plotted as a single point on a two-dimensional surface, with the distance between two points determined by their overall similarity. The background colour is displayed as an intensity gradient related to cluster positioning and the size of the axis. This multivariate visualization allowed us to evidence further properties of strains, not revealed in the previous analysis performed, with high impact in separation, such as the killer phenotype that grouped the majority of the wine commercial strains together in the bottom area. This analysis was already used in our work with similar purposes [31]. Killer activity seemed to be the most segregating phenotype, separating them from the wine environments strains. However, strains from this latter group located near the commercial strains (Fig. 4, cluster delineated in the red), indicating their killer activity and thus increased suitability for commercial exploitation. These strains were obtained mainly in the Vinho Verde region, with the exception of three strains from Lisbon, one from Bairrada and one from Douro.

To characterize the analytical profiles of the fermentations performed by the Portuguese natural isolates, especially those showing promising profiles in comparison with commercial isolates, individual higher-volume fermentations were performed using SM200 and SM300 media. For this experiment, eight *S. cerevisiae* wine isolates were chosen: four natural isolates – two from Lisbon region – EVN1172 and EVN1189 –, and two from Vinho Verde region – Z117 and Z127; four commercial strains – Z130 (which corresponds to the commercial Portuguese QA23 strain from Vinho Verde region), Z136, Z152 and Z169. These isolates were chosen from the cluster bounded by the red line in Fig. 4, since this highlights the natural isolates with a similar phenotypic profile to commercial strains. Though the four natural isolates 469, 503, Z11 and Z43 exhibited the best growth performance (Supplementary table S2), they were not selected for these assays as all lack killer activity, which is a relevant feature for their potential application as starter cultures.

The sugar consumption profile of the strains is presented in Fig. 5. All strains, with the exception of Z127, displayed a similar sugar consumption profile, with both sugars exhausted after 6 and 9 days in SM200 and SM300, respectively. When comparing SM200 and SM300 media, the main differences were observed in fructose consumption (Fig. 5B and D). Indeed, while glucose consumption occurred after 6 days in both SM200 and SM300 for all the strains except Z127, fructose consumption was delayed 3 days in MS 300 in comparison with MS200. To further characterize the inter-strain fermentative variability, the concentration of succinic acid, glycerol, acetic acid and ethanol was then assessed after 6 and 9 days in SM200 and SM300, respectively.

The four commercial strains have similar behaviour in SM200 regarding the production of succinic acid, glycerol, acetic acid and ethanol. Nonetheless, among these, Z136 produced the highest amount of succinic acid and Z130 higher levels of acetic acid. In SM

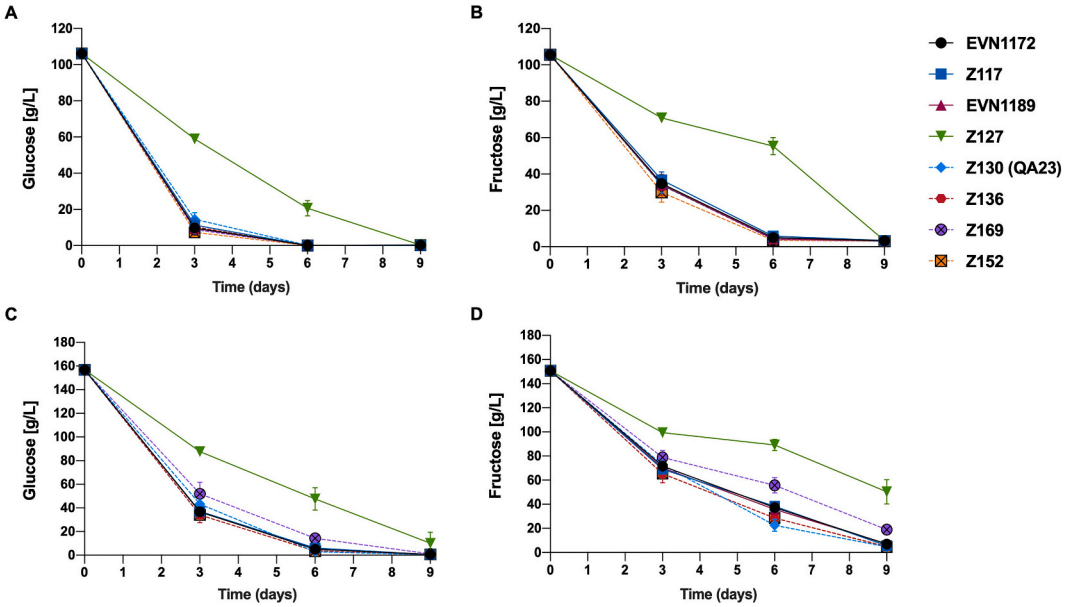

Fig. 5. Glucose (A and C) and fructose (B and D) consumption (g/L) profiles in SM200 (A and B), and SM 300 (C and D) media by four natural *S. cerevisiae* wine strains (EVN1172, Z117, EVN1189 and Z127) and four commercial ones (Z130, Z136, Z169 and Z152). Values represent the average glucose and fructose consumption from the three replicates for each strain.

300, the exceptions are strain Z136 which produced more succinic acid and less acetic acid, and strain Z152, which produced less succinic acid. Taking this into account the behavior of the four natural isolates was statistically compared with Z136, the commercial strain displaying the best performance and with Z130 (QA23) since this is the only commercial strain of Portuguese origin. Fig. 6 shows that the natural isolate Z127 produces more succinic acid than the commercial strain Z130, but not than strain Z136, in both SM200 and SM300, despite neither having completed glucose nor fructose consumption, while strains Z117 and Z136 exhibit this same phenotype only in SM200. In contrast, strain EVN1189 produces less succinic acid than Z130 and Z136, but only in SM200. Moreover, the Z117 strain produced higher concentrations of glycerol than Z130 and Z136 in SM200, but not in SM300, and EVN1189 produced lower concentrations of glycerol than Z130.

As already pointed, strain Z127 showed a distinct behaviour from the other strains, in particular regarding a slower glucose and fructose consumption (Fig. 5) and a significantly lower production of acetic acid concentrations, both in SM200 and SM300 in comparison to Z130 and Z136 (Fig. 6). To understand this different behavior we carried out a genomic DNA sequencing of this isolate and found that it contained DNA from *Pichia kudriavzevii*. This finding raises the hypothesis that it may be a hybrid between this species and *S. cerevisiae*.

4. Conclusions

In conclusion, we characterized a collection of *S. cerevisiae* natural isolates from different environmental and technological origins,

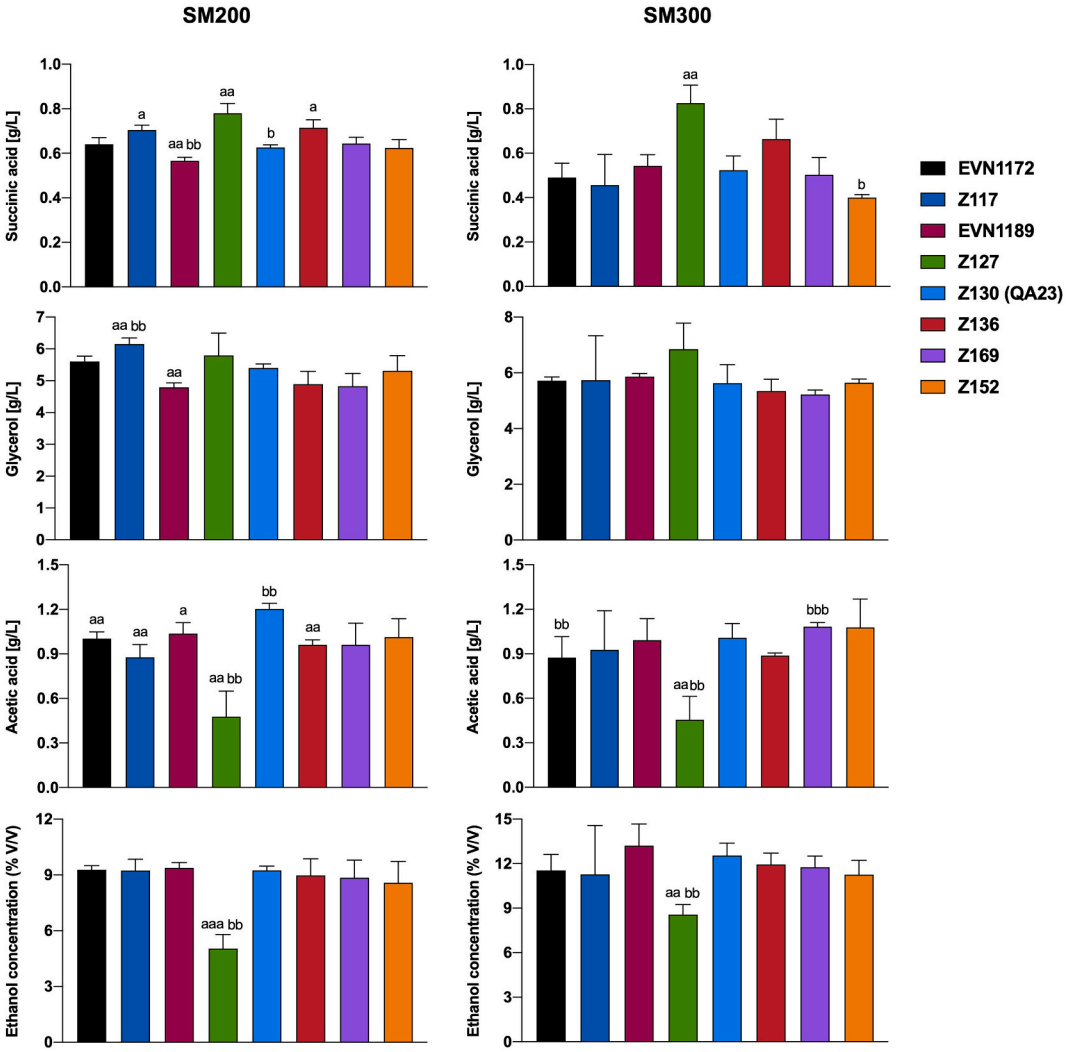

Fig. 6. Production of succinic acid, glycerol, acetic acid and ethanol by four natural (EVN1172, Z117, EVN1189 and Z127) and four wine and commercial *S. cerevisiae* strains (Z130, Z136, Z169 and Z152) 6 and 9 days after individual fermentation in SM200 (panels A, C, E and G) and SM300 (panels B, D, F and H) media. Results represent the mean values of three replicates./#, **/##, ***/###: $P < 0.05$, 0.01, 0.001, respectively, in comparison with Z130 (*) or Z136 (#).

and further analyzed the potential application of wine yeasts from major winemaking Portuguese regions to ferment grape musts with high sugar concentrations in comparison with commercial strains. The initial phenotypic characterization showed that the natural isolates may present similar desirable technological features as the commercial strains and presented even wider property variations, proving to be an important repository of biodiversity. We further found that growth performance of a number of commercial strains is negatively affected when sugar concentrations were elevated, while many autochthonous yeasts performed similarly or even better regarding increased fixed acidity and glycerol levels, and decreased volatile acidity and ethanol levels. These enological traits of some of the natural isolates herein characterized support their potential to be selected for commercialization as Portuguese starter cultures, particularly for the Portuguese winemaking market. Small scale fermentations with natural grape must comparing the promising natural isolates with different commercial strains may indicate that some can produce wines with better sensory profiles, which warrants further research.

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Availability of data and material

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

CRediT authorship contribution statement

Joana P. Guedes: Writing – review & editing, Methodology. **Tiago Vidal Cardoso:** Writing – review & editing, Software, Methodology. **Ticiana Fernandes:** Writing – review & editing, Software, Methodology. **Filipa Mendes:** Writing – review & editing, Software, Methodology. **M. Margarida Baleiras-Couto:** Writing – review & editing. **Filomena L. Duarte:** Writing – review & editing, Conceptualization. **Maria João Sousa:** Writing – review & editing, Writing – original draft, Conceptualization. **Ricardo Franco-Duarte:** Writing – review & editing, Writing – original draft, Software, Conceptualization. **Susana R. Chaves:** Writing – review & editing, Writing – original draft, Software, Methodology. **Manuela Côrte-Real:** Writing – review & editing, Writing – original draft, Software, Conceptualization.

Declaration of competing interest

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

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