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Growth response of *Saccharomyces cerevisiae* strains to stressors associated to the vine cycle

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ABSTRACT

Saccharomyces cerevisiae isolates from grapes, soil, vine bark and buds collected at seven phenological stages of an annual growth cycle, were molecular typed by Microsatellite Multiplex PCR. Subsequently 30 *S. cerevisiae* genotypes were selected and the effect of vineyard environmental stressors, in both sublethal upper and lower levels, on their growth parameters was evaluated. The effect of low and high temperature (7–40 °C), pH (2.5–8.0), glucose concentration (3.0–300.0 g/L), nitrogen concentration (0.008–8.0 g/L), and copper presence (24 mg/L) were modelled individually using the reparametrized Gompertz equation. Multivariate ANOVA and Generalized Procrustes Analysis were used to determine the environmental stressor's influence over the lag phase (λ) and the maximum specific growth rate (μ_{max}). Both parameters were significantly affected by the *S. cerevisiae* genotype, the treatments, and the interaction between them. Despite a generalized reduction in μ_{max} and a variable answer in λ, the 30 *S. cerevisiae* genotypes were able to overcome all the treatments. Extreme glucose limitation, copper presence and low temperature had the highest impact over the growth parameters. Interestingly, ten genotypes mostly distributed in the vineyard were the least affected, suggesting a greater acclimatization fitness and the possibility to persist in the changing conditions of the vine annual cycle.

1. Introduction

Saccharomyces cerevisiae is the most efficient yeast in completing wine fermentation. It is widely accepted that the vineyard (primarily the grapes) and spontaneous or natural fermentations environments, are the typical habitats of *Saccharomyces* species (Alsammar & Delneri, 2020; Fleet, 2008). Grape yeasts populations in many viticultural regions worldwide are isolated, characterized and preserved as reservoirs of genetic variability (Fleet, 2008; Sipiczki, 2016). Numerous biogeographical studies about the genetic diversity of *S. cerevisiae* strains in grapes have emphasized the dynamic and polyclonal nature of their populations (Knight & Goddard, 2015; Mercado et al., 2011; Viel et al., 2017). In general, the variability of grapes microbial populations is associated with the grapevine high potential for adaptation to different climate and soil conditions (Lavee, 2000). Moreover, *terroir* factors contributing to *S. cerevisiae* genetic diversity have been identified: grape variety, vineyard age, cultural practices and farming management, climatic and geographical conditions, among others (Cordero-Bueso et al., 2011a; Cordero-Bueso, Arroyo, Serrano, & Valero, 2011b, c; Ribéreau-Gayon, Dubordieu, Donèche, & Lombaud, 2006).

During the fruiting season, *S. cerevisiae* may disperse, either passively or through an insect. Numerous studies have probed the role of birds, fruit flies and bees as vectors for *S. cerevisiae* (Francesca, Canale, Settanni & Moschetti, 2012; Goddard, Anfang, Tang, Gardner, & Jun 2010; Stefanini et al., 2012). Moreover, the importance of water as a vehicle for yeast dissemination has been also described (Valero, Schuller,

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Cambon, Casal, & Dequin, 2005). It is still unclear which are the reservoirs where yeasts can overwinter and subsist when no grapes are available, and whence they can be taken by carrier vectors to spread in the vineyard (Ramírez, López-Piñeiro, Velázquez, Muñoz, & Regodón, 2019). The vineyard soil has also been suggested as a potential source of grape yeasts since berries fall on the ground during ripening and harvest. Nonetheless, *S. cerevisiae* has been only isolated from vineyard soil right after grape harvest, to thereafter become undetectable (Cordero-Bueso, Arroyo, Serrano, & Valero, 2011c; Ramírez et al., 2019). Knight and Goddard (2016) have proposed that *S. cerevisiae* could sporulate in vineyard soil as a strategy to survive. Alternatively, it has also been proposed that the mummified berries left on the vines behave as reservoir for yeasts overwintering (Sipiczki, 2016). As a deciduous species, the vine needs a specific sequence of environmental conditions to annually renew all its tissues through a growth cycle comprising two main stages: dormancy and active growth (Lorenz et al., 1995; Zapata, Salazar-Gutierrez, Chaves, Keller, & Hoogenboom, 2017). In a previous study, our group evaluated the vineyard ecological reservoirs and the relation between *S. cerevisiae* yeast populations with the vine phenological stages. Specifically, the study addressed *S. cerevisiae* abundance, biodiversity, and persistence on grapes, soil, buds, and vine bark of a Malbec vineyard through seven stages (maturity, post-harvest, winter pruning, sprouting, early and advanced veraison and maturity) of the 2010–2011 growth cycle (González et al., 2020). In the mentioned study, vineyard niches were defined by combining the type of sample and the phenological stage of isolation, thus 13 *S. cerevisiae* vineyard niche populations were studied. Overall, a dynamic change in *S. cerevisiae* populations was confirmed during the vine annual cycle. Analysis of populations genetic diversity revealed a high differentiation between the vineyard niches and most of the variation was found within these populations. A total of 94 molecular patterns were obtained from 1047 *S. cerevisiae* isolates. Forty-five patterns were recovered from ripen grapes, 26 from bark samples, 12 from vineyard soil, 6 from vine buds and 11 from irrigation water (González et al., 2020). Soil's low biodiversity suggested its scarce contribution as a *S. cerevisiae* reservoir. Moreover, the lowest biodiversity was registered during winter dormancy where *S. cerevisiae* was only isolated from vine buds. In contrast, the biodiversity of *S. cerevisiae* in bark samples remained stable from sprouting to harvest. The results suggested that vine bark could be the main reservoir of *S. cerevisiae* strains that may colonise grapes in the next harvest (González et al., 2020). These findings are in line with the preliminary observations of Cordero-Bueso et al., 2011c.

Yeasts biodiversity is considered a "microbiological footprint" of wine *terroir* (Belda, Zarraonaindia, Perisin, Palacios, & Acedo, 2017; Bokulich, Thorngated, Richardsone, & Mills, 2014). Furthermore, as vineyard is not a closed ecosystem and it is influenced by other *S. cerevisiae* vector-borne strains (Buser, Newcomb, Gaskett, & Goddard, 2014; Goddard et al., 2010), it is important to study the physiological traits that allow vineyard strains to persist and eventually dominate the grape berry microbiota.

Numerous reports have assessed the effect of stress factors on the growth and survival of *S. cerevisiae* strains associated to winemaking, many of them using predictive modelling techniques (Arroyo-López, Orlić, Querol, & Barrio, 2009; Grangeteau, David, Hervé, Guilloux-Benatier, & Rousseaux, 2017; Lemos Junior et al., 2017; Salvadó et al., 2011; Vendramini et al., 2017). Since grapevines evolve through an annual cycle guided by environmental conditions, the resistance of *S. cerevisiae* vineyard populations to environmental stressors has both genetic and ecological components (García-Ríos, López-Malo, & Guillamón, 2014; Legras et al., 2018). Indeed, to gain insight and better understand the mechanisms involved in the abundance, persistence, and plausible acclimatization of *S. cerevisiae* to the vineyard ecological niches, it is necessary to study the effect of vineyard environmental stressors on the growth of *S. cerevisiae* strains isolated from various phenological stages.

Therefore, the aim of the present study was to evaluate the growth of 30 selected *S. cerevisiae* genotypes, previously isolated from different vineyard ecological niches, under different stressing factors associated with meteorological conditions (temperature), substrate composition (glucose, nitrogen, and pH) and vineyard management (copper fungicides), occurring during the vine growth cycle.

2. Materials and methods

2.1. S. cerevisiae isolates

This study was applied on a representative set of 120 *S. cerevisiae* isolates obtained from a cv. Malbec vineyard from INTA-EEA Mendoza (33°00′17.1"S 68°51′12.8"W, Luján de Cuyo, Mendoza, Argentina) during seven stages of a complete grapevine growth cycle. The group included representative isolates from 13 vineyard niches composed by sample type - Soil (S), vine bark (BK), vine bud (BD) and grape berry (G) – and sampling stages -Harvest (H10 and H11); Senescence or Post-Harvest (PH), Dormancy or Winter pruning (PR), Sprouting (SPR), Early Veraison (EV) and Advanced Veraison (AV)-. Further details on the sampling design, yeast isolation, and preliminary molecular characterization, are specified in González et al. (2020).

2.2. Molecular genotyping

To confirm the previous strain differentiation obtained by Interdelta-PCR (González et al., 2020), *S. cerevisiae* isolates from all the vineyard niches and 4 commercial wine yeast (EC1118, D-254, TANGO and QA23) were genotyped using 3 highly polymorphic microsatellite loci (SC8132X, YOR267C and SCPTSY7) (Vaudano & Garcia-Moruno, 2008). DNA extraction was performed as described by Ruiz-Barba, Maldonado-Barragán, and Jiménez Díaz (2005), amplifications were performed adapting the protocol of Vaudano et al. (2019) for MyTaq™ DNA Polymerase (Bioline). PCR products were separated at 100 V for 180 min on agarose gels (2% w/v in 1X TAE buffer) stained with ethidium bromide (5 μg/mL) and using 1 Kb Plus DNA ladder (Invitrogen, Thermo Fisher Scientific). MM-PCR data were processed with Bionumerics software (Applied Maths, Kortrijk, Belgium), clustering was performed by UPGMA, based on Dice coefficient, with cut-off of 80% similarity for strains identity and 40% similarity to select the final set of *S. cerevisiae* genotypes with the greatest genetic differences.

2.3. Experimental design and growth conditions

The global approach included the evaluation of 5 variables representative of growth stressors found in the vineyard ecosystem during the vine annual cycle: changes in the plant substrate (glucose, nitrogen, pH), seasonal environmental fluctuations (temperature) and traditional vineyard practices (copper fungicides). Two sublethal levels were assayed for each variable to induce stress and still allow yeast growth (Table 1). YNB (Yeast Nitrogen Base, DifcoTM) and YCB (Yeast Carbon Base: 20 g/L glucose, 1 g/L potassium phosphate, 0.5 g/L of magnesium sulfate heptahydrate) were used as basal culture media and the standard growth condition for *S. cerevisiae* were applied.

Yeasts growth was registered by measuring the optical density (OD) of liquid basal media contained in microtiter plates using an automatic spectrophotometer model Bioscreen C (Labsystem, Finland). The 30 *S. cerevisiae* genotypes selected (Table 2) were grown overnight on 5 mL YPD broth (20 g/L glucose, 10 g/L bacteriological peptone and 10 g/L yeast extract) at 25 °C and 150 rpm. Triplicate wells were filled with 290 μL of each treatment media (Table 1) and 10 μL of each yeast culture to reach an initial OD_{600} of 0.1 (approximately 10^6 CFU/mL). The assays were run at 25 °C, except for high and low temperature conditions. OD measurements were performed after a pre-shaking of 8 s every 2 h for 7 days, with wideband filter (520–600 nm). Growth curves (10 treatments x 30 genotypes x 3 replicates) were obtained and analysed.

Table 1
Stressor variables, levels and growth media used for yeast acclimatization evaluation.

Variable	Level	Category	Code	Growth conditions
Control	–	–	C	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
Temperature	40 °C	High	TA	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at **40 °C**.
	7 °C	Low	TB	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at **7 °C**.
Glucose concentration	300 g/L	High	GA	6.7 g/L of **YNB** added with **300 g/L glucose**, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
	3 g/L	Low	GB	6.7 g/L of **YNB** added with **3 g/L glucose**, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
Nitrogen concentration	8 g/L	High	NA	**YCB** added with **8 g/L ammonium sulfate**, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
	0.008 g/L	Low	NB	**YCB** added with **8 mg/L ammonium sulfate**, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
pH	2.5	Low	PHA	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, **adjusted to** pH **2.5** by chloride acid and incubated at 25 °C.
	8.0	High	PHB	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, **adjusted to** pH **8** by sodium hydroxide and incubated at 25 °C..
Cupper (organic fungicide)	0 mg/L	Absence	C	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate and 18 mg/L inositol, adjusted to pH 3.5 by chloride acid and incubated at 25 °C.
	24 mg/L	Presence	CU24	6.7 g/L of **YNB** added with 20 g/L glucose, 5 g/L ammonium sulfate, 18 mg/L inositol, **24 mg/L copper sulfate pentahydrate**, pH 4.44 and incubated at 25 °C.

2.4. Modelling and statistical analyses

Yeast growth parameters were calculated from each growth curve directly by fitting OD measurements *vs* time to the re-parameterized Gompertz equation (Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990):

$$y = A \times \exp\left\{ -\exp\left[\left(\frac{(\mu_{max} \times e)}{A(\lambda - t)}\right) + 1\right]\right\} \quad (1)$$

In equation (1), $y = \ln(OD_t/OD_0)$, where OD_0 is the initial OD and OD_t is the OD at time t, A is the maximum asymptotic, equivalent to $\ln(OD_{max}/OD_0)$, μ_{max} is the maximum specific growth rate (h^{-1}), and λ is the lag phase period (h). These parameters were obtained by a nonlinear regression procedure, minimizing the sum of squares of the difference between the experimental data and the fitted model, i.e., loss function (observed–predicted), with nonlinear module of the Statistica 7.1 software package (StatSoft Inc, Tulsa, OK, USA) and its Quasi-Newton option. Fit adequacy was checked by the proportion of total variance explained by the model (R^2) with respect to experimental data.

Statistical differences among the biological growth parameters obtained from the different treatments tested for each *S. cerevisiae* genotype were assessed by variance analysis and Fisher LSD *Post-Hoc* test using the multivariate ANOVA (MANOVA) module of Statistica 7.1 software. The study of the influence of genotype and growing conditions was also achieved by the Generalized Procrustes Analysis (GPA, Gower, 1975). This technique provides optimal comparability by matching the individual matrices (point configurations) with appropriate transformations (translation, rotation, reflection, and isotropic readjustment) and Procrustes is a measure of their similarities. The consensus structure was obtained as the average of all transformed individual matrices. Then, a 500 permutations test was performed to verify that the GPA configuration is a true consensus (Rc value). Next, individual matrices and the GPA consensus were subjected to a Principal Component Analysis (PCA) where they were visualized and compared. In this representation, the vectors distances from the origin illustrate the responses of the different *S. cerevisiae* genotypes. Finally, variance analysis (PANOVA) was carried out to identify the relative participation of genotypes and growth conditions on the residual sum of squares (SCR). The GPA consensus integrating all the available information allowed us to determine *S. cerevisiae* vineyard genotypes most affected by the evaluated growth conditions.

3. Results

3.1. Molecular genotyping and genotype selection

A total of 120 *S. cerevisiae* isolates representatives of the 94

Table 2
List of the 30 *S. cerevisiae* genotypes selected for the yeast acclimatization study.

SC	VNI		I (%)
	SA	ST	
3	G	H_10	15.4
5	G	H_10	100.0
7	G	H_10	92.9
17	S	PH	85.7
19	S	PH	100.0
23	BD	PR	100.0
26	BD	SPR	95.0
29	BK	SPR	100.0
30	BK	SPR	100.0
47	G	EV	86.7
51	G	EV	100.0
59	BK	EV	53.3
61	BK	EV	100.0
63	BK	AV	100.0
64	G	H_11	6.7
72	G	AV	100.0
76	G	AV	46.7
80	G	AV	53.3
81	S	AV	100.0
84	BK	AV	100.0
93	G	H_11	5.9
97	G	H_11	100.0
98	BK	H_11	100.0
105	G	H_11	18.7
106	G	H_11	80.0
108	S	H_11	6.7
114	BK	H_11	86.7
116	BK	H_11	100.0
118	BK	H_11	86.7
119	BK	H_11	29.4

SC: Strain Code; VNI: Vineyard Niche of Isolation; I (%): Incidence is calculated as the ratio between a genotype isolates and all the *S. cerevisiae* isolates obtained from that vineyard niche (González et al., 2020); SA: Sample; ST: Stage; G: Grapes; S: Soil; BD: Buds; BK: Barks; H10: 2010th Harvest; PH: Post-Harvest; PR: Pruning; SPR: Sprouting; EV: Early Veraison; AV: Advanced Veraison; H11: 2011th Harvest.

Interdelta-PCR patterns obtained from 13 vineyard niches (González et al., 2020), were molecular characterized by MM-PCR. The dendrogram built with the MM-PCR patterns exhibited 90 different clusters for a similarity cut-off for 80% (data not shown). Thus, MM-PCR and Interdelta-PCR typing techniques showed a similar *S. cerevisiae* strain differentiation power. The dendrogram used to select the set of 30 representative *S. cerevisiae* genotypes is shown in Fig. 1. A cut-off of 40% similarity was used to select a manageable number of genotypes with the greatest genetic differences. Some of the previous Interdelta-PCR strain identifications (González et al., 2020) were confirmed by MM-PCR, such as isolates 4, 5 and 6 or isolates 62 and 63; whereas in other cases no coincidence was observed. In some cases, isolates with different Interdelta-PCR pattern showed the same MM-PCR amplification profile (isolates 50 and 105; 57, 86 and 100; 60 and 65 or 77, 78 and 79); but also, isolates with similar Interdelta-PCR pattern showed different MM-PCR amplification profiles, like isolates 29 and 30; 49 or 50; 97 and 98 or 109 and 110 (Fig. 1). The dendrogram showed 22 different clusters, thus, to select 30 genotypes reflecting all the vineyard ecological niches studied and the isolates proportion from each niche, all the monophyletic groups were considered, and a representative genotype was designated by hand in the large clusters (Fig. 1, Table 2).

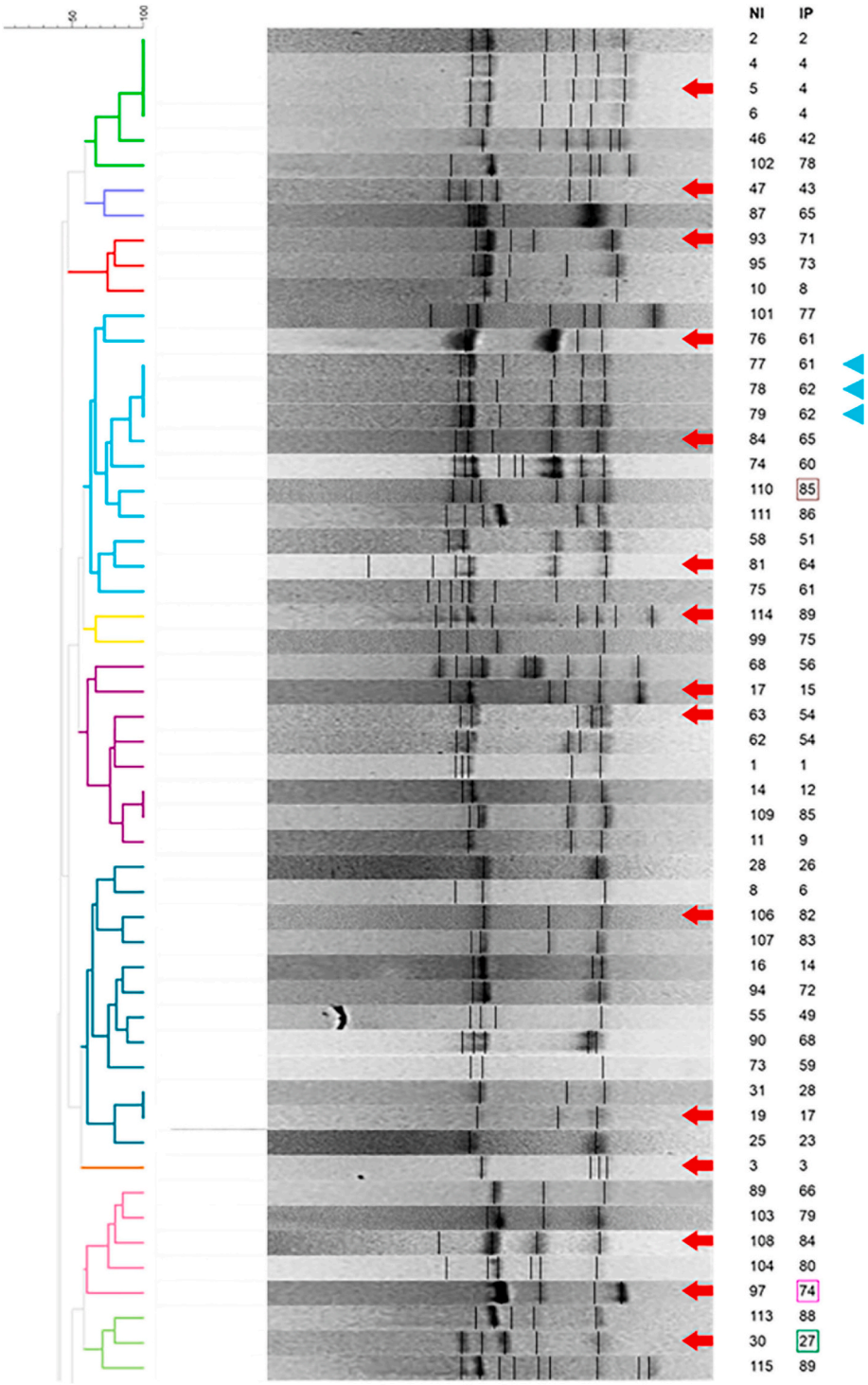

Fig. 1. Dendrogram based on Dice coefficient and UPGMA method of MM-PCR patterns corresponding to 120 *S. cerevisiae* isolates from a complete phenological cycle of a Malbec vineyard discriminated at 40% similarity. The identification of isolates on the right side includes: Number of isolate (NI: 1 to 120) and original Interdelta-PCR pattern (IP: 1–94). Monophyletic groups are shown with different colours in the branches. Isolates with the same Interdelta-PCR and MM-PCR patterns are indicated with a blue square bracket. Isolates with the same Interdelta-PCR pattern and different MM-PCR are presented with a square of the same colour. Isolates with different Interdelta-PCR pattern but the same MM-PCR amplification profile are indicated with light blue triangles The 30 *S. cerevisiae* strains selected are indicated with a red arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

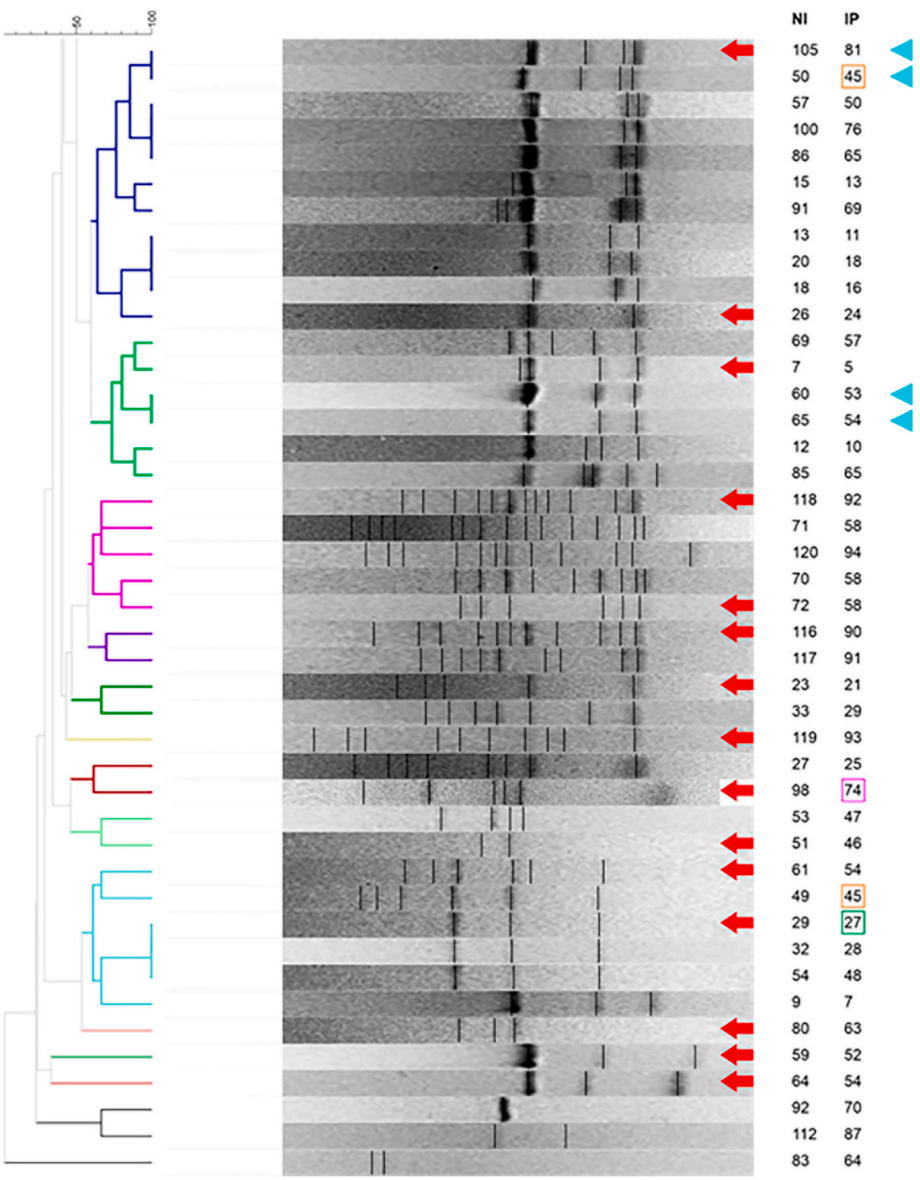

Fig. 1. (*continued*).

3.2. Growth response under sublethal stress conditions

Growth kinetics of the 30 representative *S. cerevisiae* vineyard genotypes was evaluated in 10 treatments (Table 1; Table S1). The average effect of the treatments over the maximum specific growth rate (μ_{max}) and the lag phase (λ) regardless of the genotypes are shown in Fig. 2. All treatments produced a statistical reduction of μ_{max} compared to the control condition (Fig. 2 A). The lowest μ_{max} was observed in GB, being this reduction greater than that registered at 7 °C (TB). No statistical differences in μ_{max} values were observed between NA and NB, which were also comparable to that of CU24 treatment. All *S. cerevisiae* genotypes showed λ values significantly lower than the control in the treatments TA, NB, and NA (Fig. 2 B). Contrary to the effect observed on μ_{max}, GB did not affect the latency time. The same occurred with the lag phases in PHA and PHB treatments. In contrast, TB, GA and CU24 significantly increased λ values compared to the control condition (Fig. 2 B).

To gain insight in the influence of genotypes on the growth parameters in the treatments, statistical approach by the GPA was carried out. This analysis uses appropriate transformations to provide optimal data comparability when matching individual matrices to construct a consensus where Procrustes is a measure of its similarities. The most efficient GPA transformation (lowest p-value) was scaled followed by translation (Table S2). Once the GPA consensus configuration was verified as significant (evidenced by the Rc value obtained by permutation, data not shown), the variability of overall genotypes response to the growing conditions was analysed in the PANOVA residuals by factors (Fig. 3). As a lower residual value was produced by a strain behaviour closest to the consensus, the control condition, NA, NB and PHA treatments did not significantly affect the yeasts' growth, which displayed little differentiation between their responses (Fig. 3). On the contrary, CU24 and TB produced the highest residual values, i.e., generated diverse behaviour, resulting in a variation between the *S. cerevisiae* genotypes on their copper resistance and their aptitude to grow at low temperature. Both glucose concentrations (GA and GB) presented intermediate residual level, such as TA. pH did not have an important influence on yeasts growth since the residues were minimally reduced when going from a basic (PHB) to an acid pH (PHA), although all the *S. cerevisiae* genotypes responded more homogeneously in PHA than in PHB (Fig. 3).

Later, the GPA consensus was submitted to a PCA analysis which results are summarized in Tables S3 and S5 (Eigenvalues for the 3 main

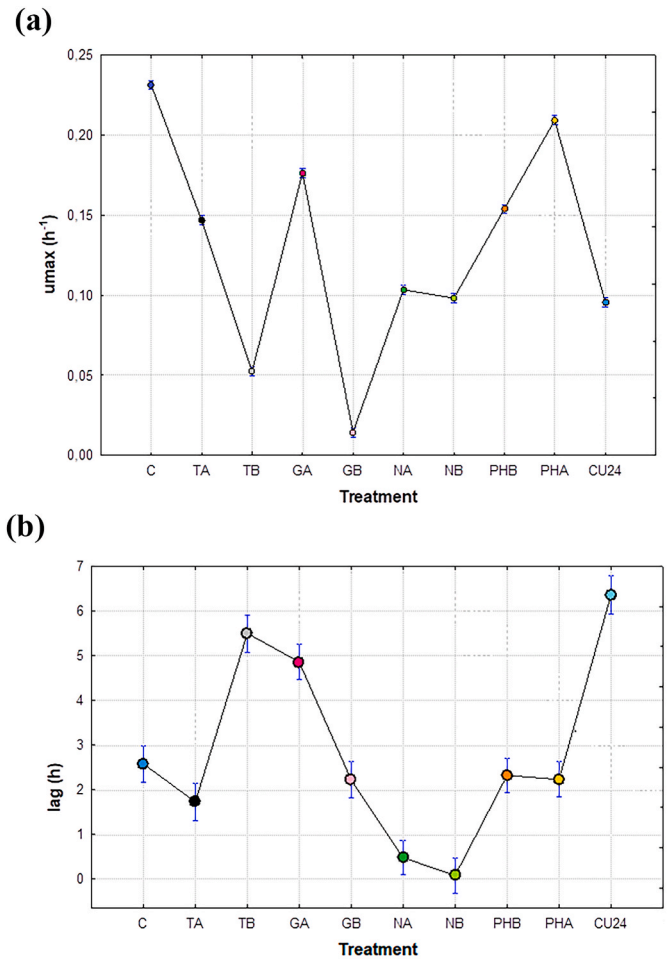

Fig. 2. Average effect of the treatments assayed regardless of genotype: A) effect over μmax (maximum specific growth rate); B) effect over λ (lag phase). C: control condition; TA: high temperature; TB: low temperature; GA: high glucose concentration; GB: low glucose concentration; NA: high nitrogen concentration; NB: low nitrogen concentration; PHB: alkali pH medium; PHA: acid pH medium; CU24: presence of copper organic fungicide.

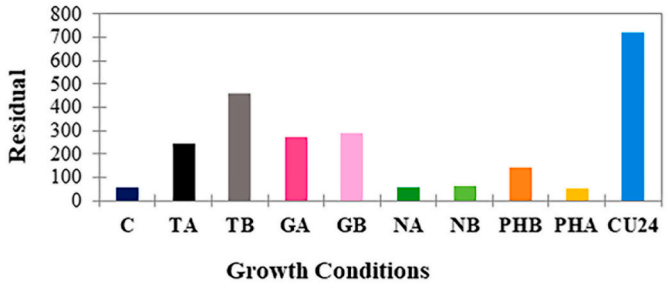

Fig. 3. GPA ANOVA residuals by growth conditions (factors). C: control condition; TA: high temperature; TB: low temperature; GA: high glucose concentration; GB: low glucose concentration; NA: high nitrogen concentration; NB: low nitrogen concentration; PHB: alkali pH medium; PHA: acid pH medium; CU24: presence of copper organic fungicide.

PCA components and Variance by configuration (strain) and PCA components, respectively). The PCA biplot (Fig. 4) shows the relation between the GPA consensus configuration (average values of variables *A*, μ_{max} and λ) and the growth conditions (factors). The first two components accounted 99% of the variability. The biplot also illustrates the relationship between growth parameters and how they are modified by growth condition. The GPA analysis confirmed the previous observations on μ_{max} and λ and showed also that GB decreased the maximum population growth (*A*) (Fig. 4). The close relationship between μ_{max} and *A* was evidenced in the reduced angle between them. The λ vector showed an orthogonal arrangement with these two variables suggesting a slight relationship with them (Fig. 4).

3.3. Changes in biological growth parameters due to sublethal stress conditions

In the previous section, we evaluated how the sublethal treatments affected growth parameters of 30 *S. cerevisiae* genotypes, considering them as a whole group. However, the analysis of a diverse growth response for the individual genotypes is also important since it would indicate their aptitude to survive, acclimatize or tolerate the vineyard stressing conditions. A MANOVA analysis was performed in search of significant differences among the fitted biological growth parameters λ and μ_{max} obtained for the 30 *S. cerevisiae* genotypes in the growth conditions evaluated (the asymptotic maximum A was not considered since it is irrelevant for ANOVA). The results shown in Table 3 evidenced that λ and μ_{max} were significantly affected by the *S. cerevisiae* genotype, the factors (10 treatments), and the interaction between them. The Fisher-LSD *post-hoc* comparison test for the λ means showed significant differences ($p \leq 0.05$) in only four cases (Table S4) which included genotypes with an extended lag phase, over 14 h, such as isolate 108 in PHB and isolates 7, 23 and 93 in the CU24 condition. Conversely, the *post-hoc* comparison for μ_{max} showed a high differentiation between the *S. cerevisiae* genotypes. It formed 119 statistically homogeneous groups (Fig. 5), which were differentiated in a colour scale from 1 (lowest value in red) to 119 (greatest value, in dark green), with intermediate values between 40 and 50 (orange) and between 60 and 70 (yellow). In Fig. 5, the genotypes are shown in rows (SC), the treatments in columns and different numbers in the cell represent statistical difference ($p \leq 0.05$) between the μ_{max} means groups indicated with other numbers and colours. This allowed us to compare simultaneously how each genotype modified its μ_{max} in every treatment, its performance with respect to the other genotypes, and additionally, a uniform colour in a column evidenced that all the genotypes responded homogeneously to that treatment. Therefore, it is observed that the control condition had the highest μ_{max} values showing all rows coloured in dark green. In contrast, GB and TB were the most restrictive treatments for all genotypes, as all their

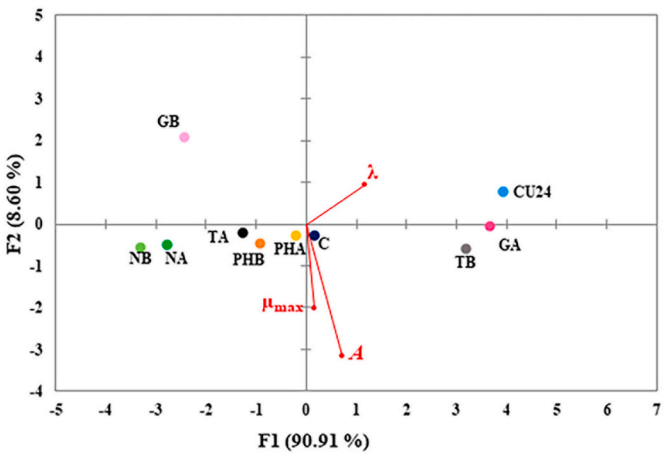

Fig. 4. PCA biplot showing the relationship between the GPA consensus configuration of A, μmax and λ (biological growth parameters) and yeast growth conditions. F1 and F2 axes accumulate 99.51% of the variability. C: control condition; TA: high temperature; TB: low temperature; GA: high glucose concentration; GB: low glucose concentration; NA: high nitrogen concentration; NB: low nitrogen concentration; PHB: alkali pH medium; PHA: acid pH medium; CU24: presence of copper organic fungicide.

Table 3
ANOVA analyses for latency times (λ) and maximum specific growth rates (μ_{max}) comparison of the 30 *S. cerevisiae* vineyard genotypes selected in 10 growing conditions (factor).

Parameter	Effect	Effect	Degrees of freedom	Sum of Squares	Mean Square	F
λ	Intercept	11055.98	1	11055.98	86.24541	<0.001
	Factor	12842.07	9	1426.90	11.13093	<0.001
	Strain	13199.42	29	455.15	3.55055	<0.001
	Factor*Strain	97005.66	261	371.67	2.89932	<0.001
	Error	70633.83	551	128.19		
μ_{max}	Intercept	13.63342	1	13.63342	82004.75	<0.001
	Factor	3.40332	9	0.37815	2274.54	<0.001
	Strain	0.13158	29	0.00454	27.29	<0.001
	Factor*Strain	0.93134	261	0.00357	21.46	<0.001
	Error	0.09160	551	0.00017		

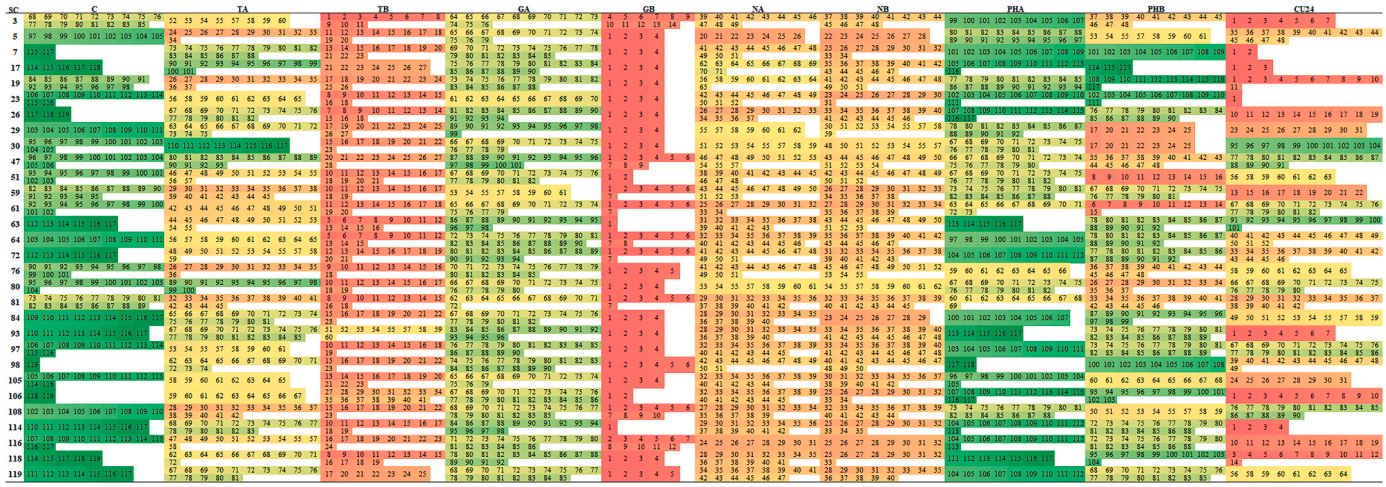

Fig. 5. Homogeneous groups obtained with the Fisher-LSD post-hoc test for the multiple comparison of μ_{max} values from the set of 30 *S. cerevisiae* strains in different growth conditions. The 119 groups are presented with a colour scale that ranges from 1 (lowest value in red) to 119 (highest value in dark green), passing through orange (values between 40 and 50) and yellow (values between 60 and 70) in the middle. Different numbers in/between the rows (strains) or columns (growth conditions) mean significant statistical differences in μ_{max} values ($p \leq 0.05$). Mean μ_{max} values were calculated from triplicate experiments in all conditions (Homogenous Groups, $\alpha = 0.05$. Error: Between MS = 0.00017, df = 551.00). Media compositions and culture conditions are described in Table 1. SC reference of the 30 selected *S. cerevisiae* genotypes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

groups are coloured in dark red and orange, respectively. On the contrary, the presence of copper (CU24) generated a varied response among the genotypes, suggested by the colour range observed for this treatment. The PHB and TA treatments also showed variation in yeast's responses, indicating differences in their ability to grow in slightly alkaline pH and high temperature assayed. This behaviour was not observed at PHA, where the μ_{max} were homogeneous and close to the control. Finally, NA and NB conditions produced a similar decrease in the yeasts growth rate (Fig. 5).

The 30 *S. cerevisiae* genotypes were classified using the scaling factor obtained in the GPA transformation (Table 4). A GPA factor below 1 indicates that the genotype presents highly variable responses to the treatments, modifying its growth parameters depending on each condition. On the contrary, a GPA factor above 1 represents a genotype less affected by the diverse culture conditions. Hence, the higher the GPA factor value, the narrower the variability of the genotypes in the treatments. Therefore, a group of 10 genotypes was considered as "very resistant" since they showed values of extremely narrow variability (GPA factor values ≥ 2) (Table 4). As the culture conditions assayed simulated changes in the substrate and stressful environmental conditions of the vine growth cycle, it is important to remark that a third of the 30 *S. cerevisiae* genotypes displayed a less variable behaviour (i.e., better acclimatized) to the different treatments.

4. Discussion

Previous results concerning *Saccharomyces* populations in Malbec vineyards of the ZARM viticultural region allowed us to identify and characterize the extremely diverse populations of *S. cerevisiae* in this important wine region (González et al., 2020; Mercado, Dalcero, Masuelli & Combina, 2007; Mercado et al., 2011). To accurately select a set of *S. cerevisiae* genotypes representative of the wide genetic variation found in 13 vineyard niches during the grapevine growth cycle, another molecular typing was used, in addition to the Interdelta-PCR formerly used. Thus, MM-PCR (Vaudano et al., 2019) was applied on a representative group of 120 previously obtained *S. cerevisiae* isolates (González et al., 2020). In general, MM-PCR amplification patterns largely matched the previous Interdelta-PCR strain discrimination. Nevertheless, we also found cases in which no coincidence was observed between Interdelta-PCR and MM-PCR patterns. In natural isolates, changes in ORFs amplification of *Ty* elements could be related to adaptive mechanisms to environmental conditions and SNPs could be also related to mechanisms involved in the generation of intra-strain phenotypic variability (Franco-Duarte et al., 2015). As all the genotypes evaluated in this study proceed from soil and vine samples, which evolved through an annual cycle renewing its vegetative tissues and experimenting strong seasonal environmental fluctuations, the mechanism suggested by these authors could explain the differences in strain

Table 4
Classification of the *S. cerevisiae* vineyard genotypes characterized according to their GPA isotropic factor (response to stress conditions).

GPA Factor RANGE	GPA Factor	SC	VNI		TI (%)
			S	ST	
1 <	0.245	108	S	H_11	0.1
	0.332	7	G	H_10	1.2
	0.405	93	G	H_11	0.1
	0.426	51	G	EV	1.4
	0.757	17	S	PH	1.1
	0.789	5	G	H_10	2.9
	0.851	105	G	H_11	0.3
	0.872	23	BD	PR	1.6
	0.970	116	BK	H_11	1.4
1–2	1.025	3	G	H_10	0.2
	1.027	119	BK	H_11	0.5
	1.047	26	BD	SPR	1.7
	1.130	59	BK	EV	0.7
	1.245	29	BK	SPR	3.4
	1.594	114	BK	H_11	1.2
	1.642	106	G	H_11	1.1
	1.769	84	BK	AV	5.1
	1.814	118	BK	H11	1.2
	1.832	61	BK	EV	1.7
> 2	2.052	98	BK	H_11	2.9
	2.068	47	G	EV	1.2
	2.161	97	G	H_11	2.9
	2.164	30	BK	SPR	3.4
	2.223	81	S	AV	2.5
	2.332	80	G	AV	0.7
	2.346	64	G	H_11	5.9
	2.529	63	BK	AV	5.9
	2.632	72	G	AV	3.6
	2.887	**76***	**G**	**AV**	**3.1**

GPA Factor: Genotype isotropic factor calculated in the General Procrustes Analysis; SC: Genotype identification number; VNI: Vineyard Niche of Isolation; S: Sample; ST: Stage; TI (%): Total Incidence is defined as the ratio between the number of *S. cerevisiae* isolates with the same molecular pattern in the niche and the 1047 *S. cerevisiae* vineyard isolates obtained during the complete vine annual growth cycle studied (González et al., 2020). Genotypes with a GPA Factor ≥2 present a narrow variability. The genotype with the narrowest variability is in bold and marked with a *. Interdelta-PCR molecular patterns shared between different vineyard niches are shaded. G: Grapes; S: Soil; BD: Buds; BK: Barks; H10: 2010th Harvest; PH: Post-Harvest; PR: Winter Pruning; SPR: Sprouting; EV: Early Veraison; AV: Advanced Veraison; H11: 2011th Harvest.

typing between some MM-PCR and Interdelta-PCR patterns. Subsequently, a set of 30 *S. cerevisiae* genotypes with the greatest genetic differences was selected reflecting the 13 vineyard's ecological niches assayed and the proportion of isolates obtained from each one. To study a plausible vineyard acclimatization of *S. cerevisiae*, yeasts were forced to grow under different stressful conditions simulating variations in the vine substrate and temperature, the main seasonal environmental factor causing *S. cerevisiae* population differentiation during the annual cycle in the ZARM region (González et al., 2020).

Overall, the 30 genotypes were able to grow in the stressful conditions, by modifying both biological growth parameters, μ_{max} and lambda, according to the stressor. Consequently, some treatments produced a homogeneous response in all the group and others evidenced the diverse strain ability to overcome stresses. The specific differences among the genotypes responses were more evident in the variation of the maximum specific growth rate rather than in the lag phase parameter.

Temperature represents the most frequent environmental stress that microorganisms must cope with (García-Ríos et al., 2014; Paget, Schwartz, & Delneri, 2014). The *S. cerevisiae* strains included in the present study were isolated along the 2010–2011 annual cycle where environmental temperature was identified as the main force driving population differentiation (González et al., 2020). In vineyard ecological niches, temperature fluctuations can occur daily, seasonally, or unexpectedly, depending on the region and climate, and consequently, the natural isolates of *S. cerevisiae* are expected to be acclimated to the gradual temperature variations of their natural environment (Aguilera, Randez-Gil, & Prieto, 2007; Guyot et al., 2015). As expected, both temperatures assayed directly influenced the maximum specific growth rate and the lag phase. A reduction of μ_{max} was observed with both treatments, low temperature produced a more diverse response between the genotypes since it was more restrictive over the growth rate, and it also caused a lag phase extension. The genotypes response at 40 °C (TA) was more homogeneous as the PANOVA residues decreased when the temperature increased, whilst at TB they showed different degrees of response, thus presenting diverse aptitude to growth at low temperature. Schade, Jansen, Whiteway, Entian, and Thomas (2004) evaluated *S. cerevisiae* response to an abrupt decrease from 30 to 10 °C and found that yeasts maintained a normal growth curve exhibiting a μ_{max} reduction and a lag phase extension. Therefore, even when 7 °C (TB) represented a real challenge for *S. cerevisiae*, results showed that the 30 *S. cerevisiae* vineyard genotypes characterized were able to survive and grow at this temperature. In Mendoza (Argentina), desert conditions result in monthly T_{max} (maximum average temperature) with larger variance than that seen for T_{min} (minimum average temperature) (i.e., 2° and 1.4 °C, respectively), due to the greater sensitivity to cold-air intrusions during winter (June–August) (Agosta, Canziani, & Cavagnaro, 2012). Thus, *S. cerevisiae* populations in the ZARM vineyards are subjected to a highly challenging conditions in the winter season. Population heterogeneity has been proposed as survival strategy for organisms that cannot control their external environment, because even if a less heat or cool-tolerant proportion of the population loses viability, other cells are still able to survive and resume growth (Guyot et al., 2015). Despite the differences in growth of the *S. cerevisiae* genotypes, mainly associated to diverse diminution of their maximum growth rate, all the vineyard genotypes were able to grow both at winter and summer temperatures found in the ZARM region, suggesting that *S. cerevisiae* could survive and be acclimatized to the vineyard seasonal environmental conditions.

Our study also included a copper treatment since it is a component of the most currently used fungicides in the vineyard, even in organic viticulture (Cavazza, Guzzon, Malacarne, & Larcher, 2013; Crosato et al., 2018; Grangeteau et al., 2017; Sun et al., 2019). This treatment generated the most diverse response in the 30 genotypes evaluated, principally affecting lag phase. After a delay on the growth onset, most strains were able to grow showing differences in μ_{max} values, although in some cases equally or slightly lower than the control condition. Thus, the most sensitive strains required a longer period of adaptation to overcome this stress but later they were able to grow. Some previous studies showed that despite the detrimental effect of copper on yeast viability during early stages of alcoholic fermentation, this inhibition is not permanent, and most strains can overcome copper toxicity (Cavazza et al., 2013; Crosato et al., 2020; Sun et al., 2019). In addition, other authors have found that *S. cerevisiae* strains isolated from the same vineyard differed in their tolerance to copper (Capece, Romaniello, Scrano, Siesto, & Romano, 2018), supporting that just as the vineyard isolation environment can exert selective pressure on the natural microbiota, a strain genetic basis for natural traits variation should be considered.

Among the other treatments evaluated, the effect of factors associated with the plant substrate were homogeneous on the strain's growth. pH had little effect on the yeasts' general growth. The genotype's behaviour was the closest to the control condition at pH 2.5 (PHA), while it showed some variability in μ_{max} values at pH 8.0 (PHB). *S. cerevisiae* vegetative growth occurs at a relatively wide range of external pH, being more vigorous at acidic than at neutral or alkaline pH. In fact, most strains can hardly proliferate in a medium buffered above pH 8.0–8.2 and what is more, external alkaline pH promotes invasive haploid growth and sporulation in *S. cerevisiae*, features inhibited at low pH (Ariño, 2010). Regarding nitrogen content,

genotypes growth was more homogeneous under different nitrogen concentrations (NA and NB conditions). Both nitrogen levels produced a similar decrease in the maximum specific growth rate and the small differences between the treatments could be explained mostly by the specific requirement of each strain rather than by the concentrations themselves. Likewise, all *S. cerevisiae* vineyard genotypes responded similarly to both deficiency and excess of glucose. At 3 g/L (GB), lag phase of the genotypes was similar to the control condition, while at 300 g/L (GA) a significantly larger latency period was observed for all genotypes. In the case of μ_{max}, the lowest values were observed in GB, while GA only caused a slightly decrease in the maximum growth rate. *S. cerevisiae* seem to be adapted to fluctuant conditions of glucose and nitrogen, since our results showed that all *S. cerevisiae* vineyard genotypes responded equally to both glucose and nitrogen deficiency and excess. Previous studies using *S. cerevisiae* strains from commercial wine yeasts (Gutiérrez et al., 2012), natural environments (oak bark), other industrial processes (Crépin, Sanchez, Nidelet, Dequin, & Camarasa, 2014) and some grape strains (Lemos Junior et al., 2017) have shown that above certain concentrations of ammonium (≥90 mg/L), nitrogen does not represent a limiting factor for cell growth. Moreover, if a natural environment exposes a population to the same variations and selective pressures, this long-term selection will often lead to an optimized performance during the multiple life stages of the organisms. Thus, selection for the optimal and individual use of nitrogen sources can be expected to be strong in natural yeast isolates (Ibstedt et al., 2015). As no differences were found in growth parameters of the 30 *S. cerevisiae* vineyard genotypes in both ammonium concentrations, a low nitrogen demand could be attributed to the set of genotypes characterized, adding more evidence to the suggested hypothesis of *S. cerevisiae* acclimatization to the vineyard ecosystem. Regarding glucose deficiency and excess, our results are in line with previous studies showing that *S. cerevisiae* delayed the onset of growth and significantly decreased its average growth rate when glucose concentration increased (Arroyo-López et al., 2009; Spor et al., 2009; Spor, Wang, Dillmann, de Vienne, & Sicard, 2008). Boender et al. (2011) proved that *S. cerevisiae* can drastically decrease its ATP renewal rate under glucose limitation. Moreover, as *S. cerevisiae* was able to survive up to 20 days of glucose starvation displaying a progressive loss of cell viability in the population, this ability could represent an evolutionary mechanism to maximize the life span of survivors (Boender et al., 2011). Likewise, *S. cerevisiae* might also employ other strategies to survive and compete during starvation periods, e.g., an extended low sugar availability could induce sporulation in diploid or polyploid strains (Boender, de Hulster, van Maris, Daran-Lapujade, & Pronk, 2009; Knight & Goddard, 2016). Furthermore, it was recently shown that absence or extremely low glucose concentrations promotes cell adhesion in *S. cerevisiae*, and therefore, biofilm formation which could contribute to the yeast's permanence and survival in harsh environments such as the vineyard (Van Nguyen, Plocek, Váchová, & Palková, 2020).

Finally, GPA results showed that 10 *S. cerevisiae* genotypes presented a less variable behaviour under the 10 treatments assayed, suggesting that they would be able to adapt to the changing conditions of the vine annual cycle. Interestingly, this group of strains represented the Interdelta-PCR patterns more repeatedly recovered and shared between grapes and bark samples during the last three stages (early veraison, advanced veraison and harvest) of the vine annual growth cycle previously studied (González et al., 2020). Indeed, the genotypes 63, 64, 76, 97 and 98 accounted for 48.1% of the grape isolates in the 2011th harvest (González et al., 2020). All together this evidence strengthen the notion of the vine bark as a reservoir where *S. cerevisiae* genotypes can resist the vineyard conditions and may later colonise grapes, eventually participating in the wine alcoholic fermentation (Cordero-Bueso et al., 2011c; Morrison-Whittle & Goddard, 2018; Vitulo et al., 2019).

5. Conclusions

To summarize, the present study shows that the biological growth parameters maximum population (*A*), lag phase (λ), and maximum specific growth rate (μ_{max}) are genotype dependent and they are affected by the stressor (type and degree) and the interaction between both genotype and stressor. The different *S. cerevisiae* genotypes displayed homogeneous behaviors to some treatments (NA, NB and PHA) while showed diverse responses to others (CU24 and TB), indicating that some situations do not represent limiting factors, whilst others require a specific response. Our results also show that the 30 *S. cerevisiae* genotypes, representatives of the genetic and ecological diversity found in the phenological vine cycle, present a phenotypic diversity, which could allow them to acclimatize and survive the seasonal changes in the vineyard ecosystem during the annual vine cycle. Indeed, 10 *S. cerevisiae* genotypes exhibited a remarkable narrow variability in their growth parameters, i.e., they were less affected by the growing conditions imposed, and this group was as well the genotypes most repeatedly isolated from the different vineyard niches studied. Therefore, this study provides valuable evidence supporting the hypothesis of *S. cerevisiae* vineyard niche acclimatization and how this fitness ability could enable the strains to survive in other plant tissues when fruit season is over in the vine growth cycle. Furthermore, this data is highly important for the development of *S. cerevisiae* native inoculums that highlight the biodiversity and the *terroir* in Malbec wines from the ZARM region.

CRediT authorship contribution statement

Magalí Lucía González: Investigation, Formal analysis, Visualization, Writing – original draft. **Eva Valero:** Resources, Methodology, Investigation, Writing – review & editing. **Selva Valeria Chimeno:** Investigation. **Antonio Garrido-Fernández:** Formal analysis. **Francisco Rodríguez-Gómez:** Formal analysis. **María Cecilia Rojo:** Investigation. **Marcos Paolinelli:** Investigation. **Francisco Noé Arroyo-López:** Resources, Methodology, Investigation, Writing – review & editing. **Mariana Combina:** Conceptualization, Writing – review & editing, Funding acquisition. **Laura Analía Mercado:** Conceptualization, Investigation, Writing – review & editing, Project administration, Funding acquisition.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113157.

References

Agosta, E., Canziani, P., & Cavagnaro, M. (2012). Regional climate variability impacts on the annual grape yield in Mendoza, Argentina. *Journal of Applied Meteorology and Climatology, 51*, 993–1009. https://doi.org/10.1175/JAMC-D-11-0165.1

Aguilera, J., Randez-Gil, F., & Prieto, J. A. (2007). Cold response in *Saccharomyces cerevisiae*: New functions for old mechanisms. *FEMS Microbiology Reviews, 31*, 327–341. https://doi.org/10.1111/j.1574-6976.2007.00066.x

Alsammar, H., & Delneri, D. (2020). An update on the diversity, ecology, and biogeography of the *Saccharomyces* genus. *FEMS Yeast Research, 20*, 3. https://doi.org/10.1093/femsyr/foaa013. foaa013.

Ariño, J. (2010). Integrative responses to high pH stress in *S. cerevisiae*. *OMICS, 14*(5), 517–523. https://doi.org/10.1089/omi.2010.0044

Arroyo-López, F. N., Orlić, S., Querol, A., & Barrio, E. (2009). Effects of temperature, pH, and sugar concentration on the growth parameters of *Saccharomyces cerevisiae, S. kudriavzevii* and their interspecific hybrid. *International Journal of Food Microbiology, 131*(2–3), 120–127. https://doi.org/10.1016/j.ijfoodmicro.2009.01.035

Belda, I., Zarraonaindia, I., Perisin, M., Palacios, A., & Acedo, A. (2017). From vineyard soil to wine fermentation: Microbiome approximations to explain the "terroir". *Concept. Front. Microbiol., 8*, 821.

Boender, L. G. M., Almering, M. J. H., Dijk, M., van Maris, A. J. A., de Winde, J. H., Pronk, J. T., et al. (2011). Extreme calorie restriction and energy source starvation in *Saccharomyces cerevisiae* represent distinct physiological states. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1813*, 2133–2144. https://doi.org/10.1016/j.bbamcr.2011.07.008

Boender, L. G. M., de Hulster, E. A. F., van Maris, A. J. A., Daran-Lapujade, P. A. S., & Pronk, J. T. (2009). Quantitative physiology of *Saccharomyces cerevisiae* at near-zero specific growth rates. *Applied and Environmental Microbiology, 75*, 5607–5614. https://doi.org/10.1128/AEM.00429-09

Bokulich, N. A., Thorngated, J. H., Richardsone, P. M., & Mills, D. A. (2014). Microbial biogeography of wine grapes is conditioned by cultivar, vintage, and climate. *Proceedings of the National Academy of Sciences of the United States of America, 111*, 139–148.

Buser, C. C., Newcomb, R. D., Gaskett, A. C., & Goddard, M. R. (2014). Niche construction initiates the evolution of mutualistic interactions. *Ecology Letters, 17*, 1257–1264. https://doi.org/10.1111/ele.12331

Capece, A., Romaniello, R., Scrano, L., Siesto, G., & Romano, P. (2018). Yeast starter as a biotechnological tool for reducing copper content in wine. *Frontiers in Microbiology, 8*, 2632. https://doi.org/10.3389/fmicb.2017.02632

Cavazza, A., Guzzon, R., Malacarne, M., & Larcher, R. (2013). The influence of the copper content in grape must on alcoholic fermentation kinetics and wine quality. A survey on the performance of 50 commercial Active Dry Yeasts. *Vitis, 52*(3), 149–155. https://doi.org/10.5073/vitis.2013.52.149-155

Cordero-Bueso, G., Arroyo, T., Serrano, A., Tello, J., Aporta, I., Vélez, M. D., et al. (2011a). Influence of the farming system and vine variety on yeast communities associated with grape-berries. *International Journal of Food Microbiology, 145*(1), 132–139. https://doi.org/10.1016/j.ijfoodmicro.2010.11.040

Cordero-Bueso, G., Arroyo, T., Serrano, A., & Valero, E. (2011b). Influence of different floor management strategies of the vineyard on the natural yeast population associated with grape berries. *International Journal of Food Microbiology, 148*(1), 23–29. https://doi.org/10.1016/j.ijfoodmicro.2011.04.021

Cordero-Bueso, G., Arroyo, T., Serrano, A., & Valero, E. (2011c). Remanence and survival of commercial yeast in different ecological niches of the vineyard. *FEMS Microbiology Ecology, 77*, 429–437. https://doi.org/10.1111/j.1574-6941.2011.01124.x

Crépin, L., Sanchez, I., Nidelet, T., Dequin, S., & Camarasa, C. (2014). Efficient ammonium uptake and mobilization of vacuolar arginine by *Saccharomyces cerevisiae* wine strains during wine fermentation. *Microbial Cell Factories, 13*, 109. https://doi.org/10.1186/s12934-014-0109-0

Crosato, G., Carlot, M., De Iseppi, A., Garavaglia, J., Pinto, L. M. N., Ziegler, D. R., et al. (2018). Genetic variability and physiological traits of *Saccharomyces cerevisiae* strains isolated from "Vale dos Vinhedos" vineyards reflect agricultural practices and history of this Brazilian wet subtropical area. *World Journal of Microbiology and Biotechnology, 34*(105), 1–14. https://doi.org/10.1007/s11274-018-2490-z

Crosato, G., Nadai, C., Carlot, M., Garavaglia, J., Righetto Zieglerc, D., Cassanta Rossi, R., et al. (2020). The impact of *CUP1* gene copy-number and XVI-VIII/XV-XVI translocations on copper and sulfite tolerance in vineyard *Saccharomyces cerevisiae* strain populations. *FEMS Yeast Research, 20*(4), foaa028. https://doi.org/10.1093/femsyr/foaa028

Fleet, G. H. (2008). Wine yeasts for the future. *FEMS Yeast Research, 8*, 979–995. https://doi.org/10.1111/j.1567-1364.2008.00427.x

Francesca, N., Canale, D. E., Settann, L., & Moschetti, G. (2012). Dissemination of wine-related yeasts by migratory birds. *Environ Microbiol Report, 4*(1), 105–112. https://doi.org/10.1111/j.1758-2229.2011.00310.x

Franco-Duarte, R., Bigey, F., Carreto, L., Mendes, I., Dequin, S., AS Santos, M., et al. (2015). Intrastrain genomic and phenotypic variability of the commercial *Saccharomyces cerevisiae* strain Zymaflore VL1 reveals microevolutionary adaptation to vineyard environments. *FEMS Yeast Research, 15*, fov063. https://doi.org/10.1093/femsyr/fov063

García-Ríos, E., López-Malo, M., & Guillamón, J. M. (2014). Global phenotypic and genomic comparison of two *Saccharomyces cerevisiae* wine strains reveals a novel role of the sulfur assimilation pathway in adaptation at low temperature fermentations. *BMC Genomics, 15*, 1059. https://doi.org/10.1186/1471-2164-15-1059

Goddard, M. R., Anfang, N., Tang, R., Gardner, R. C., & Jun, C. (2010). A distinct population of *Saccharomyces cerevisiae* in New Zealand: Evidence for local dispersal by insects and human-aided global dispersal in oak barrels. *Environmental Microbiology, 12*(1), 63–73.

González, M. L., Sturm, M. E., Lerena, M. C., Rojo, M. C., Chimeno, S. V., Combina, M., et al. (2020). Persistence and reservoirs of *Saccharomyces cerevisiae* biodiversity in different vineyard niches. *Food Microbiology, 86*, 1–12. https://doi.org/10.1016/j.fm.2019.103328

Gower, J. C. (1975). Generalized Procrustes analysis. *Psychometrika, 40*, 33–51. https://doi.org/10.1007/BF02291478

Grangeteau, C., David, V., Hervé, A., Guilloux-Benatier, M., & Rousseaux, S. (2017). The sensitivity of yeasts and yeasts-like fungi to copper and sulfur could explain lower yeast biodiversity in organic vineyards. *FEMS Yeast Research, 17*(8), 1–7. https://doi.org/10.1093/femsyr/fox092

Gutiérrez, A., Chiva, R., Sancho, M., Beltran, G., Arroyo-López, F. N., & Guillamón, J. M. (2012). Nitrogen requirements of commercial wine yeast strains during fermentation of a synthetic grape must. *Food Microbiology, 31*, 25–32. https://doi.org/10.1016/j.fm.2012.02.012

Guyot, S., Gervais, P., Young, M., Winckler, P., Dumont, J., & Davey, H. M. (2015). Surviving the heat: Heterogeneity of response in *Saccharomyces cerevisiae* provides insight into thermal damage to the membrane. *Environmental Microbiology, 17*(8), 1462–2912. https://doi.org/10.1111/1462-2920.12866

Ibstedt, S., Stenberg, S., Bagés, S., Gjuvsland, A. B., Salinas, F., Kourtchenko, O., et al. (2015). Concerted evolution of life stage performances signals recent selection on yeast nitrogen use. *Molecular Biology and Evolution, 32*(1), 153–161. https://doi.org/10.1093/molbev/msu285

Knight, S., & Goddard, M. (2015). Quantifying separation and similarity in a Saccharomyces cerevisiae metapopulation. *The ISME Journal, 9*, 361–370. https://doi.org/10.1038/ismej.2014.132

Knight, S. J., & Goddard, M. R. (2016). Sporulation in soil as an overwinter survival strategy in. *Saccharomyces cerevisiae. FEMS Yeast Res, 16*, fov102. https://doi.org/10.1093/femsyr/fov102

Lavee, S. (2000). Grapevine (vitis vinifera) growth and performance in warm climates. In A. Erez (Ed.), *Temperate fruit crops in warm climates*. Dordrecht: Springer.

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. *Molecular Biology and Evolution, 35*(7), 1712–1727. https://doi.org/10.1093/molbev/msy066, 1.

Lemos Junior, W. J. F., Viel, A., Bovo, B., Carlot, M., Giacomini, A., & Corich, V. (2017). *Saccharomyces cerevisiae* vineyard strains have different nitrogen requirements that affect their fermentation performances. *Letters in Applied Microbiology, 65*(5), 381–387. https://doi.org/10.1111/lam.12798

Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., & Weber, E. (1995). Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*) - codes and descriptions according to the extended BBCH scale. *Australian Journal of Grape and Wine Research, 1*, 100–110. https://doi.org/10.1111/j.1755-0238.1995.tb00085.x

Mercado, L., Dalcerob, A., Masuelli, R. W., & Combina, M. (2007). Diversity of *Saccharomyces* strains on grapes and winery surfaces: Analysis of their contribution to fermentative flora of Malbec wine from Mendoza (Argentina) during two consecutive years. *Food Microbiology, 24*, 403–412.

Mercado, L., Sturm, M. E., Rojo, M. C., Ciklic, I., Martínez, C., & Combina, M. (2011). Biodiversity of *Saccharomyces cerevisiae* populations in Malbec vineyards from the "Zona Alta del Río Mendoza" region in Argentina. *International Journal of Food Microbiology, 151*, 319–326. https://doi.org/10.1016/j.ijfoodmicro.2011.09.026

Morrison-Whittle, P., & Goddard, M. R. (2018). From vineyard to winery: A source map of microbial diversity driving wine fermentation. *Environmental Microbiology, 20*(1), 75–84. https://doi.org/10.1111/1462-2920.13960

Paget, C. M., Schwartz, J. M., & Delneri, D. (2014). Environmental systems biology of cold-tolerant phenotype in *Saccharomyces* species adapted to grow at different temperatures. *Molecular Ecology, 23*, 5241–5257. https://doi.org/10.1111/mec.12930

Ramírez, M., López-Piñeiro, A., Velázquez, R., Muñoz, A., & Regodón, J. A. (2019). Analysing the vineyard soil as a natural reservoir for wine yeasts. *Food Research International, 129*, Article 108845. https://doi.org/10.1016/j.foodres.2019.108845

Ribéreau-Gayon, P., Dubordieu, D., Donèche, B., & Lombaud, A. (2006). Citology, taxonomy and ecology of grape and wine yeasts. In P. Ribéreau-Gayon, D. Dubordieu, B. Donèche, & A. Lombaud (Eds.), *Handbook of enology. The microbiology of wine and vinifications* (2nd ed., pp. 1–52). Chichester: John Wiley& Sons Ltd.

Ruiz-Barba, J. L., Maldonado-Barragán, A., & Jiménez Díaz, R. (2005). Small-scale total DNA extraction from bacteria and yeast for PCR applications. *Analytical Biochemistry, 347*, 333–335. https://doi.org/10.1016/j.ab.2005.09.028

Salvadó, Z., Arroyo-López, F. N., Guillamón, J. M., Salazar, G., Querol, A., & Barrio, E. (2011). Temperature adaptation markedly determines evolution within the genus. *Saccharomyces. Appl Environ Microbiol, 77*(7), 2292–2302. https://doi.org/10.1128/AEM.01861-10

Schade, B., Jansen, G., Whiteway, M., Entian, K. D., & Thomas, D. Y. (2004). Cold adaptation in budding yeast. *Molecular Biology of the Cell, 15*, 5492–5502. https://doi.org/10.1091/mbc.e04-03-0167

Sipiczki, M. (2016). Overwintering of vineyard yeasts: Survival of interacting yeast communities in grapes mummified on vines. *Frontiers in Microbiology, 7*, 212. https://doi.org/10.3389/fmicb.2016.00212

Spor, A., Nidelet, T., Simon, J., Bourgais, A., de Vienne, D., & Sicard, D. (2009). Niche-driven evolution of metabolic and life-history strategies in natural and domesticated populations of *Saccharomyces cerevisiae*. *BMC Evolutionary Biology, 9*, 296. https://doi.org/10.1186/1471-2148-9-296

Spor, A., Wang, S., Dillmann, C., de Vienne, D., & Sicard, D. (2008). Ant'' and "Grasshopper'' life-history strategies in *Saccharomyces cerevisiae*. *PLoS One, 3*(2), e1579. https://doi.org/10.1371/journal.pone.0001579

Stefanini, I., Dapporto, L., Legras, J.-L., Calabretta, A., Di Paola, M., De Filippo, C., et al. (2012). Role of social wasps in *Saccharomyces cerevisiae* ecology and evolution. *Proceedings of the National Academy of Sciences of the United States of America, 109* (33), 13398–13403. https://doi.org/10.1073/pnas.1208362109

Sun, X.-y., Liu, L.-l., Ma, T., Yu, J., Huang, W.-d., Fang, Y., et al. (2019). Effect of high Cu^{2+} stress on fermentation performance and copper biosorption of *Saccharomyces cerevisiae* during wine fermentation. *Food Science and Technology, 39*(1), 19–26. https://doi.org/10.1590/1678-457X.24217

Valero, E., Schuller, D., Cambon, B., Casal, M., & Dequin, S. (2005). Dissemination and survival of commercial wine yeast in the vineyard: A large-scale, three-years study. *FEMS Yeast Research, 5*, 959–969. https://doi.org/10.1016/j.femsyr.2005.04.007

Van Nguyen, P., Plocek, V., Váchová, L., & Palková, Z. (2020). Glucose, Cyc8p and Tup1p regulate biofilm formation and dispersal in wild *Saccharomyces cerevisiae*. *NPJ Biofilms Microbiomes, 6*, 7. https://doi.org/10.1038/s41522-020-0118-1

Vaudano, E., & Garcia-Moruno, E. (2008). Discrimination of *Saccharomyces cerevisiae* wine strains using microsatellite multiplex PCR and band pattern analysis. *Food Microbiology, 25*, 56–64.

Vaudano, E., Quinterno, G., Costantini, A., Pulcini, L., Enrica Pessione, E., & Garcia-Moruno, E. (2019). Yeast distribution in Grignolino grapes growing in a new vineyard in Piedmont and the technological characterization of indigenous *Saccharomyces spp*. Strains. *International Journal of Food Microbiology, 289*, 154–161. https://doi.org/10.1016/j.ijfoodmicro.2018.09.016

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. *International Journal of Food Microbiology, 258*, 1–11. https://doi.org/10.1016/j.ijfoodmicro.2017.07.006

Viel, A., Legras, J.-L., Nadai, C., Carlot, M., Lombardi, A., Crespan, M., et al. (2017). The geographic distribution of *Saccharomyces cerevisiae* isolates within three Italian neighboring winemaking regions reveals strong differences in yeast abundance, genetic diversity and industrial strain dissemination. *Frontiers in Microbiology, 8*, 1595. https://doi.org/10.3389/fmicb.2017.01595

Vitulo, N., Lemos, W. J. F., Jr., Calgaro, M., Confalone, M., Felis, G. E., Zapparoli, G., et al. (2019). Bark and grape microbiome of Vitis vinifera: Influence of geographic patterns and agronomic management on bacterial diversity. *Frontiers in Microbiology, 9*, 3203. https://doi.org/10.3389/fmicb.2018.03203

Zapata, D., Salazar-Gutierrez, M., Chaves, B., Keller, M., & Hoogenboom, G. (2017). Predicting key phenological stages for 17 grapevine cultivars (Vitis vinifera L.). *American Journal of Enology and Viticulture, 68*(1), 60–72. https://doi.org/10.5344/ajev.2016.15077

Zwietering, M. H., Jongenburger, I., Rombouts, F. M., & Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology, 56*, 1875–1881, 0099-2240/90/061875-07$02.00/0.