New malic acid producer strains of *Saccharomyces cerevisiae* for preserving wine acidity during alcoholic fermentation

Vion Charlotte[#1,2], Muro Maitena[#1,2], Bernard Margaux[1,2], Bruce Richard [2], Valentine Fautre [2], Nadine Yeramian[3], Isabelle Masneuf-Pomarède[2], Tempère Sophie[2], Marullo Philippe[1,2]*.

[1]Biolaffort, Bordeaux, FRANCE

[2] UMR 1366 Œnologie, Université de Bordeaux, INRAE, Bordeaux INP, BSA, ISVV

[3] Microbiology Division, Department of Biotechnology and Food Science, Faculty of Science-University of Burgos

#Authors contributes to the same level to this publication

*Corresponding author: philippe.marullo@u-bordeaux.fr

Permanent adress : UMR 1366 Œnologie, Université de Bordeaux, INRAE, Bordeaux INP, BSA, ISVV 210 chemin de Leysotte. 33882, Villenave d'Ornon

Figures can be shown in color online only

1 Abstract:

In the context of climate change, the chemical composition of wines is characterized by a massive drop of malic acid concentration in grape berries. Then wine professionals have to find out physical and/or microbiological solutions to manage wine acidity. The aim of this study is to develop wine *Saccharomyces cerevisiae* strains able to produce significant amount of malic acid during the alcoholic fermentation. By applying a large phenotypic survey in small scale fermentations, the production level of malic acid in seven grape juices confirmed the importance of the grape juice in the production of malic acid during the alcoholic fermentation. Beside the grape juice effect, our results demonstrated that extreme individuals able to produce up to 3 g/L of malic acid can be selected by crossing together appropriate parental strains. A multivariate analysis of the dataset generated illustrate that the initial the amount of malic acid produced by yeast is a determining exogenous factor for controlling the final pH of wine. Interestingly most of the acidifying strains selected are particularly enriched in alleles that have been previously reported for increasing the level of malic acid at the end of the alcoholic fermentation. A small set of acidifying strains were compared with strains able to consume a large amount of malic acid previously selected. The total acidity of resulting wines was statistically different and a panelist of 28 judges was able to discriminate the two groups of strains during a free sorting task analysis.

2 Key words

pH, malic acid, breeding, acidity perception, wine yeast

3 Highlights

- The initial amount of malic acid of grape juices is a major factor affecting the metabolism of malic acid by *Saccharomyces cerevisiae* strains
- By implementing a breeding program several strains producing more than 3 g/L of malic acid have been selected paving a new avenue for managing wine acidity during the alcoholic fermentation.
- The impact of some QTLs linked to malic acid has been validated in high producer strains
- Strains that produce or consume high amount of malic acid during the alcoholic fermentation differently impact the acidity perception of the resulting wines

4 Introduction

Climate change is a direct consequence of the global warming and accentuates the greenhouse effect with a drastic impact for many agricultural productions including vine (IPCC 2020; van Leeuwen and Darriet 2016). It is well documented that temperature increase modifies vine phenology (van Leeuwen and Darriet 2016) and affects grape juice composition by increasing sugar concentration (Coombe 1987; Poudel et al. 2009; Nistor et al. 2018), and decreasing titratable acidity (Arrizabalaga et al., 2018; Coombe, 1987; Mira de Orduña, 2010; Nistor et al., 2018; Poudel et al., 2009; van Leeuwen and Darriet, 2016) due to a drastic drop of malic acid concentrations. In turn, the resulting wines have a greater alcohol content (Mira de Orduña 2010; van Leeuwen and Darriet 2016) and are also affected in their aroma composition and their sensorial properties (Bureau et al., 2000).

These combined modifications constitute a great challenge for the wine industry that have to adapt winemaking processes and enological products for maintaining wine quality and stability despite the drastic changes of grape juice composition. Concerning the correction of acidity drop, the addition of chemical products (tartaric or malic acids) is efficient but represents an expensive solution. In addition, the context of a new green deal promotes microbiological and biotechnological solutions as a good alternative for wine acidification.

Alcoholic and malolactic fermentations modify the equilibrium of organic acids by the metabolic activity of wine microorganisms as reported by many authors (Redzepovic et al., 2003; Volschenk et al., 2006, Su et al., 2014). However, wine microorganisms mostly reduce the wine acidity by consuming malic acid through malo-alcoholic (Volschenk et al., 1997) and malolactic fermentation (Lonvaud-Funel, 1994). Quite recently, the species *Lachancea thermotolerans* has been proposed for correcting wine acidity due to its ability to produce significant amount of L-lactic acid during the alcoholic fermentation (Hranilovic et al., 2021, 2018). This non-conventional yeast species can be mixed with *Saccharomyces cerevisiae* starters and constitutes a promising solution for managing wine acidity. However, the development of mixed culture in winemaking conditions requires an accurate management of alcoholic fermentation which is sometime not compatible with a routine application in the cellar.

Beside *L. thermotolerans,* studies reporting a microbiological acidification of wine matrix without an elevation of acetic acid are quite rare. Within the *Saccharomyces* genus the species *Saccharomyces uvarum* has been described for its acidification properties (Castellari et al., 1994; Coloretti et al., 2002) that was related to the psychrophilic property of this species. This feature is mostly shared by hybrids between *S. cerevisiae* and *S. uvarum* (Origone et al., 2018) that have been proposed as a solution for coping both acidity drop and high sugar levels in grape juices (da

Silva et al., 2015; Origone et al., 2018). Concerning *S. cerevisiae*, former studies described the contribution of strains on malic acid production.

Malic acid plays a central role in yeast metabolism being a key molecule of glyoxylate shunt (Regev-rudzki et al., 2009) and tricarboxylic acid cycle as extensively reviewed by (Saayman and Viljoen-Bloom, 2006). Schwartz and Radler deeply investigated the physicochemical factors influencing the production of malic acid by *S. cerevisiae* reporting production levels of nearly 1 g/L in enological compatible conditions (Schwartz and Radler, 1988). A large screening of natural isolate reported that few strains isolated in Spain or in China are able to produce up to 1 g/L of malic acid (Yéramian et al., 2007) and to increase titratable acidity (Chen et al., 2022). However, this interesting feature has not been deeply exploited.

Recently, the identification of QTLs (Quantitative Trait Loci) influencing malic acid metabolism of *S. cerevisiae* during the alcoholic fermentation (Peltier et al., 2021) paves new avenues for the control of acidity using the main species used in alcoholic fermentation. Recently, we demonstrated that such QTLs can be efficiently used for enhancing the consumption of malic acid during the alcoholic fermentation. Starting from two F1 hybrids obtained from 4 enological strains we drastically increased the percentage of malic acid consumed by enological strain in two rounds of selection (Vion et al., 2021). This strategy could be useful for shortening malolactic fermentation by reducing the amount of malic acid to be degraded by lactic bacteria. However, the use of high malic consumer strains reduces wine acidity which is not suitable in the context of global climate change.

In the present study, we applied a phenotype driven breeding program aiming to select *S. cerevisiae* strains able to preserve the wine acidity. Starting from the parental strains used by Vion et al. (2021) new strains able to produce up to 3.2 g/L of malic acid at the end of the alcoholic fermentation were obtained. These high malic producer strains are statistically enriched in QTL's alleles that preserve wine acidity constituting a new genetic resource for managing acidity in wine. A preliminary comparison of malic acid producer and consumer strains was carried out, the resulting wines can be clearly discriminated on the basis of the acidity perception in a *Sauvignon blanc* matrix.

5 Methods

5.1 Yeast strains, conservation, and propagation methods used

Yeast (*S. cerevisiae*) were propagated on YPD 2% (1% peptone, 1% yeast extract, 2% glucose) at 28°C in both liquid and plate cultures (2% agar). Long term storage at -80°C was achieved by adding one volume of glycerol to YPD 2% overnight cultures. All the strains are homozygous for the *HO* locus and are therefore diploids. The main yeast strains used were described in the Table 1 and are derived from the two F1-hybrids (M2×F15 and SB×GN) used in previous QTL mapping studies (Huang et al., 2014; Peltier et al., 2018b). Meiotic spore clones of SB×GN (GS-56B, GS-13C, GS-8B) and of M2×F15 (FM-8B, FM-1C, FM-3C) were used as parental candidates for achieving a breeding program aiming to increase the production of malic acid during the alcoholic fermentation. These strains have been previously characterized for their ability to produce small amounts of malic acid (Vion et al., 2021). A first F1-hybrid was obtained by crossing the strains GS-8b and FM-1C. A second hybridization cycle was achieved using the monosporic clones AC1-13, AC1-46, AC1-191, AC1-217. Finally, three *S. cerevisiae* strains C1-4, M2-9, and M6-7 previously described to produce mild amount of malic acid during the alcoholic fermentation (Yéramian et al., 2007) were used as control.

Table 1. *S. cerevisiae* strains used

Strain	Description	Reference
ZF15	Zymaflore F15	Laffort, FRANCE
ZF10	Zymaflore Fx10	Laffort, FRANCE
M2	parental strain, a meiotic spore clone from Enoferm M2 (Lallemand, Canada)	(Huang et al., 2014)
F15	parental strain, a meiotic spore clone from Zymaflore F15 (Laffort, France)	(Huang et al., 2014)
GN	parental strain, a meiotic spore clone from Zymaflore VL1 (Laffort, France)	(Peltier et al., 2018)
SB	parental strain, a meiotic spore clone from Zymaflore BO213 (Laffort, France)	(Peltier et al., 2018)
M2xF15	F1-hybrid (M2 × F15)	(Huang et al., 2014)
SBxGN	F1-hybrid (SB × GN)	(Peltier et al., 2018)
C4-2	Yeast strain isolated from Cordoba region, mild malic acid producer	(Yéramian et al., 2007)
M2-9	Yeast strain isolated from Madrid region, mild malic acid producer	(Yéramian et al., 2007)
M6-7	Yeast strain isolated from Madrid region, mild malic acid producer	(Yéramian et al., 2007)
GS-56B	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
GS-13C	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
GS-8B	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
FM-8B	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
FM-1C	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
FM-3C	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
AC1	F1-hybrid (GS-8b x FM-1C)	this work
AC1-191	meiotic clone of AC1, malic acid producer strain	this work
AC1-13	meiotic clone of AC1, high malic acid producer	this work
AC1-217	meiotic clone of AC1, high malic acid producer	this work
AC1-46	meiotic clone of AC1, high malic acid producer	this work
AC2	F1-hybrid (AC1-191 × AC1-46)	this work
AC3	F1-hybrid (AC1-13 × AC1-217)	this work
AC2-1	meiotic clone of AC2, high malic acid producer	this work
AC2-467	meiotic clone of AC2, high malic acid producer	this work
AC3-22	meiotic clone of AC3, high malic acid producer	this work
FMGS_889	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS_71	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS_263	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS2_107	meiotic clone of FMGS2, high malic consumer	(Vion et al., 2021)
FMGS2_714	meiotic clone of FMGS2, high malic consumer	(Vion et al., 2021)

5.2 Spore mating and isolation

F1-hybrids (AC1, AC2 and AC3) were obtained by spore to spore mating on YPD 2% using the method previously described (Marullo et al., 2009). After few hours, zygotes (trilobed shaped) were observed and manually isolated; their genotype was confirmed by a SNP genotyping (see below). Each F1-hybrids was sporulated by cultivating 10^8 cells in 5-ml of potassium acetate 1%

(ACK) during three days at 24°C. After a digestion by cytohelicase (2 mg/L) (Sigma, France) spores were isolated by micromanipulation or by spore purification according to the procedure previously described (Vion et al., 2021). As the yeast strains of this study are homozygous for the *HO* gene, colonies derived form a spore are considered as diploids and fully homozygous.

5.3 Cell culture and DNA extraction in microplates

Strains were cultivated in 96-Well microplate containing 200 µL of YPD coated with gaz-permeable sheets allowing CO_2 production. Genomic DNA was extract by using a Li-Ac SDS protocol previously adapted for microplate handling (Chernova et al., 2018). Broadly, 5×10^6 cells (200µl of overnight cultures) were centrifuged and incubated with 50 µL of 200mM LiAc/1% SDS at 70°C for 5 min. Genomic DNA was extracted by mixing cell lysates with 150µL of pure ethanol and vortexed for 15 s. After a briefly spin (2 min, 4000 rpm) the pellet was washed with 70% ethanol and was the solubilized in 200 µL of milliQ water at 60°C for 5 min.

5.4 Mass ARRAY genotyping of selected clones

According to a recent publication (Peltier et al., 2021), QTLs that possibly enhance the malic acid production in the SB×GN background were used. These QTLs have the same localization than those used by Vion *et al* for enhancing the consumption of malic acid (Vion et al., 2021). For optimizing the genotyping procedure, the allelic form tracked in the present study were specific to a malic consumption feature. In addition, for most QTLs a pair of SNPs narrowing the region was used in order to increase the quality of the genotyping. The genotypes of each strain founder (M2, F15, SB, GN) and of the parental strains of hybrids AC1, AC2, and AC3 are given in the Table S1. The genotyping of these SNP was carried out by using Mass ARRAY technology, which allows large multiplex SNP detection (Gabriel et al., 2009). Less than 15ng of DNA were used for genotyping the strain. Primers were design using the tool MassARRAY® Assay Design (version 4.0.0.2) and amplified fragment of less than 120 bases having a mass difference between 4545.0 and 8633.7 Da. The genotype of selected progenies of hybrids AC1 (37), AC2 (28) and AC3 (29) was carried out in the same multiplex. All the spore clones genotyped have a fully homozygous genotype consistent with their meiotic progeny nature (Table S2).

5.5 Alcoholic fermentation assays

5.5.1 Grape juices

The grape juices Merlot 2013 and Merlot 2018 (M13 and M18) and Cabernet Sauvignon 2013, 2014, 2015, 2017 and 2019 (CS13, CS14, CS15, CS27 and CS19) used were provided by Vignobles Ducourt (Ladaux, France) and stored at -20˚C. Red grape juices have been thermically treated by the cellar and only the liquid phase was used. Grape juices were sterilized by membrane filtration and their nitrogen content were adjusted to 200 mg/L N in order to provide a sufficient nitrogen nutrition. The composition of grape juice in terms of fermenting sugars, nitrogen sources and malic acid content as well their pH are listed in Table S3 for original and corrected musts.

5.5.2 Small scale fermentation monitoring

Small-volume alcoholic fermentations were implemented in screwed vials fermentations according to the general procedure described in (Peltier et al., 2018a). Briefly, 20 mL-screwed vials (Fisher Scientific, Hampton, New Hampshire, USA) were filled with 12.0 ml of filtered grape must were tightly closed with screw cap-magnetic (Fisher Scientific, Hampton, New Hampshire, USA) perforated by hypodermic needles for allowing the CO_2 release. Vessel was inoculated by 2×10^6 viable cell.mL^{-1} precultured in liquid YPD for 24h. Cellular concentration and viability was estimated by flow cytometry using a Cytoflex apparatus (Beckmann Coulter, Indianapolis, USA). Fermentation took place at 24°C and was each vial shaken at 175 rpm by an orbital shaker (SSL1, Stuart, Vernon Hills, Illinois, USA). The fermentation kinetics was estimated by monitoring manually (2–3 times per day) the weight loss caused by CO_2 release using a precision balance with automatic weight recording (Mettler Toledo, Greifensee, Switzerland). The amount of CO_2 released according to time was modeled by local polynomial regression fit allowing the estimation of parameters related to fermentation (Peltier et al., 2018a) : the maximal amount of CO_2 released (CO_2max in g.L^{-1}), the lag phase (h), the time to release 35 and 80 g/L of CO_2 after subtracting the lag phase (*t35* and *t80* in h). The CO_2max value was divided by the amount of sugar consumed for computing the *yield* of the fermentation.

5.6 Enzymatic assay of wine

At the end of the alcoholic fermentation, a sample volume of 800 µL was manually transferred in Micronics tubes (Novazine, Lyon, France, ref: MP32033L) and stored at -20˚C. The concentrations of the following organic metabolites were measured: acetic acid, glycerol, malic acid, pyruvate, acetaldehyde and total SO_2 using the respective enzymatic kits: K-ACETGK, K-GCROLGK, K-LMAL-116A, K-PYRUV, K-ACHYD, K-TSULPH, K-SUCCI (Megazyme, Bray, Ireland) following the instructions of the manufacturer. Glucose and fructose were assayed by using the enzymatic method previously described. All the enzymatic assays were performed by a robotic platform using the Bordeaux metabolomics facilities (http://metabolome. cgfb.u-bordeaux.fr/). The Malic Acid Consumption (MAC%) is the ratio of malic acid consumed and was computed according to the following formula:

$$MAC\% = \frac{\left([\mathrm{L-malic\ acid}]_{initial} - [\mathrm{L-malic\ acid}]_{final}\right)}{[\mathrm{L-malic\ acid}]_{initial}} \times 100$$

5.7 Sensory analysis

5.7.1 Wines preparation

Twelves strains were used for assessing the sensorial impact of malic acid production/consumption on a Sauvignon Blanc wine. The wine was produced at the laboratory scale from a Sauvignon blanc grape juice harvested in 2020 in the Bordeaux area (Vignobles Ducourt, Ladaux, FRANCE) containing 35 mg/L of total SO_2. The composition of this grape juice is given in Table S3. Fermentations were carried out in 1.2L bioreactors initially incubated at 18°C. At the fifth day of fermentation, 30 mg N/L of nitrogen (Ammonium sulfate) were added for enhancing the fermentation kinetics. At the seventh day, the temperature was rise up 22°C for ensuring a rapid achievement of the fermentation. Bioreactors were shaken daily for 60 sec with a magnetic stirrer in order to resuspend yeast cells and the CO_2 production rate was followed by manual weighting. At the end of the fermentation (between 11 and 13 days), wines were settled for a week at 4°C and yeast lees were resuspended each 48 hours for preserving wines from oxidation. A week before tasting wines were sulfited (50 mg/L) and transferred in tapped bottles and stored at 12°C.

5.7.2 Sensory analysis conditions

Sample assessments were undertaken in a temperature-controlled room, using covered, white ISO glasses (ISO 3591:1977) containing about 25 mL of liquid, coded with random three-digit numbers. Sessions lasted approximately 15 min. Judges were selected on the basis of availability and interest. A total of 28 volunteers belonging to the Laffort and ISVV staff and showed different levels of expertise in sensory analysis but representing different domains or expertise of the wine industry. The ages of the panelists ranged from 25 to 50 years old, and the panel was constituted by 55% of women.

5.7.3 Preliminary control of acidity perception by the panel

A preliminary session was carried out using a commercial *Sauvignon blanc* wine (Mas de L'oncle 2019, Languedoc Roussillon) with spiked concentrations of malic acid. The aim of this preliminary session was to control that all the judges correctly perceive malic acid contribution in a Sauvignon blanc wine. The panelists performed a ranking test (ISO 8587:2007), sorting four samples according to their acidity from least to most intense. The sums of ranks for increasing concentrations of malic acid (1.0, 1.7, 2.4 and 3.0 g/L) were 43, 60, 66, and 81, respectively. A Page test (Conover, 1999) assuming the increasing concentration of malic acid in wine confirmed that judges have a correct perception of acidity.

5.7.4 Free sorting task

Two days after the control session a descriptive sorting task (Chollet et al., 2014) was used to characterize the olfactory and gustatory differences between 12 Sauvignon Blanc wines produce in the laboratory. The protocol of the sorting focused on both a full evaluation (olfactory and gustatory). The panelists (n=28) were asked to constitute groups of wines according to their acidity. Judges must constitute at least two groups of wines and each group must contain at least two wines.

5.8 Use of former datasets for figures

Two previously published datasets were used for illustrating the efficiency of this breeding programs. In such dataset phenotypes were measured in a Merlot 2015 grape juice in the same conditions and in the same grape juices allowing to compare the data. Malic acid consumption of 35 enological strains and 193 progeny clones of M2×F15 or SB×GN hybrids were extracted form (Emilien Peltier, Bernard, et al. 2018) and (Vion et al., 2021). These distributions were presented in the Figure S1.

5.9 Statistical analyses

All the statistical and graphical analyses were done using R software (R Core Team, 2018). Median differences were assayed using the non-parametric Kruskal test (*agricolae package*). When required, multiple comparisons were achieved using a post-hoc test using the criterium Fisher's least significant difference with Benjamini-Hochberg corrected p values (alpha=0.05). The effect of grape juice and strains on MAC% was estimated by a two-way analysis of variance (*car* package) using the data collected for the strains SB, GN, AC2 and AC3 in the grape juices CS14 and CS15. The linear model used is expressed in the following formula

$$MAC\% = \mu + Strain_i * Must_j + \varepsilon$$

Where µ is the average value observed in the dataset, *Strain* is the nature of the strain taking four levels *i*, *Must* is the nature of the grape juice taking two levels *j* and ε is the residual error of the model. The analysis of variance was carried out after checking the normal distribution of the residues of the model (Shapiro test) and the homoscedasticity of the variance (Levene test).

To evaluate the contribution of different metabolites on final pH of wine a multiple linear regression model was carried out. The initial model computed is expressed in the following formula.

$$pH_{wine} \sim pH_{must} + [malic\ acid]_{wine} + [acetic\ acid]_{wine} + [succinic\ acid]_{wine} + [residual\ sugar]_{wine} + \varepsilon$$

Where ε represents the error of the model. A subsequent analysis of variance of the model was applied in order to estimate which variables have a significative impact on the total variance observed.

Page test was carried out for controlling the correct perception of acid malic in a spiked Sauvignon blanc. The L value obtained for 28 judges was 685 with four wines presented. The threshold L' value was equal to 4.15 allowing to reject the null hypothesis. L' is computed assuming the normal distribution of the samples according to the following formula:

$$L' = \frac{12 \times L - 3 \times J \times P(P+1)^2}{P(P+1) \times \sqrt{J \times (P-1)}}$$

Where J is the number of judges (n=28) and P is the number of wines presented (n=4). Multidimensional scaling was assed using the R package *smacof* (de Leeuw and Mair, 2009) Dissimilarities between samples were analyzed using the cooccurrence of wines belonging to the same group as proposed by (Cox and Cox, 2001).

6 Results

6.1 Phenotypic characterization of parental candidates

The aim of the present work was to increase the production level of malic acid by *S. cerevisiae* strains in order to prevent de drastic drop of acidity caused by climatic change.

Six monosporic clones (GS-56B, GS-13C, GS-8B, FM-8B, FM-1C, FM-3C) constitutes the starting genetic material of this study. They were obtained from the F1-hybrids M2×F15 and SB×GN. Their MAC% in a Merlot grape juice of 2015 was previously reported (Vion et al., 2021). These strains outcompeted a panel of 35 commercial starters and some of them had a weak production of malic acid at the end of the alcoholic fermentation (Figure S1). In order to better characterized such strains, the amount of malic acid at the end of the alcoholic fermentation of seven red grape juices characterized by a wide range of initial malic acid content (1.45 to 4.40 g/L) was measured. The fermentation characteristics of such strains were compared to some reference ones including two commercial starters (Zymaflore Fx10 and Zymaflore F15), three malic acid producer strains (C1-4, M2-9 and M6-7) (Yéramian et al., 2007) and the strain FMGS_889 which is a high malic acid consumer strains selected by (Vion et al., 2021). Fermentations were carried out in duplicate, and traits collected are given in the Table S4.

In order to compare all the conditions, the MAC% was computed as described in material and methods. Average MAC% values measured in the seven grape juices was significantly different according to the yeast strain (Kruskal test, pval=3.7×10^{-9}). A Post-hoc test using the criterium Fisher's LSD revealed that FM-8B and FMGS-889 were statistically different to two commercial Fx10 and F15 (Figure 1A). However, most of the acidifier strains cannot be statistically differentiated due to a strong variability of MAC% observed within the grape juice fermented. This significative effect of grape juice is confirmed by Kruskal test, p= 9.3×10^{-14} and subsequent post hoc analysis (Figure 1B). The impact of grape juice was clearly correlated to the initial concentration of malic acid (Spearman's correlation test: rho=0.60, pval<2.2×10^{-16}). Interestingly, for low initial malic acid concentrations (1.41 g/L in M-2018 and 2.02 in CS-2014) average MAC% reaches negative values (malic acid production) suggesting that such matrices are relevant for the selection of acid malic producing strains.

In order to better characterize the parental candidates, we narrowed our analysis on these two grape juices. The Table 2 provides the average phenotypic values measured for 13 quantitative traits in M-2018 and CS-2014 with the attribution of statistical groups (Kruskal test with *post hoc* group attribution. The average amount of malic acid at the end of the alcoholic fermentation ranged between 1 g/L and 2.6 g/L for the extreme strains FMGS-889 and GS-8B. Significative differences between strains were also observed for other organic acids such as acetate but were not correlated to the production of malic acid (Figure S2).

Table 2. Average phenotypic values of initial acidifying strains.

Strain	MAC%	pH wine	Acetic acid (g.L-1)	Malic acid (g.L-1)	Glycerol (g.L-1)	SO2 (mg.L-1)	Succinic acid (g.L-1)	CO_2 max (g/L)	lp (h)	V50-80 (g.h-1.L-1)	t35g (h)	t50g (h)	t80g (h)
GS-8B	-52.1 ^{f}	3.6	0.10 ^{d}	2.6 ^{a}	9.2 ^{a}	8.6	1.1^{abc}	107.4	8.0	0.91 ^{de}	38.5 ^{bc}	55.3 ^{abc}	109.8 ^{ab}
FM-1C	-42.2 ^{f}	3.6	0.20 ^{abcd}	2.5 ^{ab}	8.9 ^{ab}	7.2	1.5^{a}	110.1	6.5	0.93 ^{de}	38.8 ^{bc}	55.3 ^{bc}	106.8 ^{ab}
FM-8B	-41.5 ^{f}	3.6	0.20^{abcd}	2.4 ^{ab}	8.2 ^{abcd}	6.1	1.1^{abc}	110.8	7.3	1.13 ^{bc}	38.0 ^{bc}	53.0 ^{bcd}	96.0 ^{bc}
C1-4	-30.7 ^{ef}	3.7	0.13 ^{bcd}	2.3 ^{abc}	6.5 ^{d}	6.8	1.1^{abc}	110.0	6.8	1.05 ^{cd}	34.8 ^{cd}	50.3 ^{cde}	96.8 ^{b}
M6-7	-24.1 ^{def}	3.7	0.18 ^{bcd}	2.1 ^{abc}	6.8 ^{cd}	4.8	1.4^{ab}	110.9	6.3	0.81 ^{e}	45.3 ^{a}	68.0 ^{a}	132.8 ^{a}
GS-56B	-14.9 ^{bcde}	3.7	0.14 ^{abc}	2.0 ^{bcd}	7.3 ^{bcd}	8.9	0.8^{c}	110.4	7.0	0.85 ^{e}	41.8 ^{ab}	59.0 ^{ab}	116.5 ^{a}
M2-9	-13.0 ^{cde}	3.7	0.23 ^{ab}	2.0 ^{abcd}	7.3 ^{abcd}	7.5	1.0^{abc}	110.2	6.3	0.85 ^{e}	40.8 ^{ab}	58.8 ^{abc}	116.5 ^{a}
FM-C3	-4.7 ^{bcd}	3.7	0.27 ^{cd}	1.8 ^{cd}	8.7 ^{abc}	10.9	1.4^{ab}	111.3	7.0	1.32 ^{ab}	32.5 ^{d}	45.5 ^{def}	80.8 ^{cd}
GS-13C	-1.3 ^{abc}	3.7	0.12 a	1.7 ^{cde}	6.1 ^{d}	5.2	0.9^{bc}	112.0	7.3	0.95 ^{cde}	39.3 ^{bc}	57.0 ^{abc}	108.5 ^{ab}
Zymaflore FX10	0.6 ^{abc}	3.8	0.31 ^{abc}	1.7 ^{cde}	7.3^{abcd}	8.6	0.8^{bc}	111.8	8.8	1.48 ^{a}	29.3 ^{d}	41.0 ^{f}	73.8 ^{d}
Zymaflore F15	5.7 ^{ab}	3.8	0.23 ^{a}	1.6 ^{de}	6.1 ^{abcd}	5.7	1.3^{abc}	112.0	8.0	1.46^{a}	31.3 ^{d}	44.3 ^{ef}	77.0 ^{d}
FMGS-889	45.0 ^{a}	4.0	0.19 ^{abcd}	1.0 ^{e}	8.0 ^{abcd}	8.8	1.2^{abc}	113.7	9.0	1.55 ^{a}	32.0 ^{d}	45.3 ^{def}	77.8 ^{d}

6.2 Phenotypic optimization of malic acid production by a breeding program

The two best malic acid producer strains GS-8B and FM-1C were crossed by pairing their spores using a micromanipulator and the F1-hybrid AC1 was isolated. Forty-one progeny clones of the AC1 hybrid were recovered and analyzed for their MAC% in the CS14 grape juice that positively promotes malic acid production (Figure 1B). The AC1 hybrid showed a negative MAC% value similar to those of parental strains (-74.2 %) (Figure 2A). In this grape juice this MAC% value represented a final malic acid production of 1.5 g/L. The hybrid AC1 was sporulated and 41 progeny clones were isolated. All of them reached negative MAC% ranging between -23.7% and -146.8 %. Four strains AC1-13, AC1-191, AC1-46 and AC1-217 were selected for achieving a second round of phenotypic optimization. The hybrid AC2 (AC1-191 × AC1-46) and AC3 (AC1-13 × AC1-217) were isolated by using the micromanipulator. The MAC% of these new F1-hybrids was clearly improved respect to AC1 with values of -130.7% and -149% for AC2 and AC3, respectively. To our knowledge, such levels of malic acid production (up to 3.0 g/L) were never reported in an enological context and constitute a technological rupture compared to the phenotypic variability of industrial starters that was illustrated by the four founder strains of this study (SB, GN, M2 and F15) (Figure 2A).

The phenotypic segregation of MAC% of AC2 and AC3 hybrids was measured for a small set of progenies (AC2=14, AC3=19) and compared to the 41 AC1 progenies (Figure 2B). Both populations showed an average MAC% significantly lower than the AC1 population (Wilcoxon test, alpha= 0.05). The phenotypic variance of each progeny was statistically similar (Variance F-test, alpha= 0.05). For AC1 progeny, 70% of clones had a MAC% value higher than the F1-hybrid while for AC2 and AC3 the distribution was mostly centered around the respective F1-hybrid value. This second segregation step allowed the selection of strains with a -160% lower MAC% representing a production of malic acid of 3.2 g/L.

In order to confirm these findings, MAC% was assayed in a second grape juice (CS15) using additional progenies of AC2 (n=26) and AC3 (n=22). In this grape juice, AC2 and AC3 hybrids as well as the control strains GN and SB produced less malic acid since their MAC% was significantly higher than in CS14 grape juice. This effect was quantified by two-way ANOVA (Figure S3) underlining the impact of the grape juice discussed above. Despite this environmental effect a similar phenotypic segregation was observed for both hybrids and some progenies produced more malic acid than their respective hybrids (Figure 2C).

6.3 Multivariate analysis of acidic strains behavior

The large dataset of 484 fermentations characterized by 15 quantitative variables was generated (Table S5) offering the opportunity to investigate traits linked to the strong production of malic acid of acidic strains. This represents the phenotypic values of 127 strains phenotyped in CS14 and/or in CS15 at least in triplicate. The phenotypic variability of this dataset was figured out in a PCA analysis (Figure 3A). The first two components represent 60% of the total inertia and clearly discriminate the hybrid background and the grape juice origin. Correlation circles indicates that the content of malic acid drives the inertia in the same direction that t50 and t80 but was mostly decoupled to succinic acid, glycerol, CO_2, and acetic acid production (Figure 3B). Correlation analysis (Figure S4) confirmed the strong positive correlation between kinetics parameters and malic acid content suggesting that acidifying strains have some difficulties to ferment efficiently.

This was also corroborated by the strong correlation between malic acid production and residual sugars (rho= 0.45, pvalue= 2×10^{-26}) that are highlighted by the 123 stuck fermentations (open symbol) shown on the Figure 3A. The distribution of malic acid produced in completed and stuck fermentations was figured out FigureS5. Stuck fermentations mostly occurred in the CS15 grape juice which is characterized by a lower CO_2 yield than observed in CS14 (0.427 vs 0.456 g/g, Wilcoxon test, alpha=0.05). In CS15, the production level of succinic acid also significantly higher than observed in CS14 (1.61 vs 0.52 g/L, Wilcoxon test, alpha=0.05) contributing to separate the two grape juices on the PCA. As expected, the pH value of the resulting wine was negatively correlated with malic acid content in both grape juice (rho= -0.40 and -0.69 for CS14 and CS15, respectively, alpha=0.001) (Figure 3C). This demonstrates that the selection of strains controlling malic acid concentration was quite efficient for modulating the pH. On average, the dynamic range of final pH of wine was 3.41-3.82 for CS14 and 3.52-4.01 for CS15.

Since other organic acids than malate would influence the wine acidity, we applied a linear model for predicting the quantitative factors statistically linked to the final pH. Five quantitative parameters were used for building the model: the initial pH of the matrix, the organic acids produced by yeast (malic, acetic, succinic) as well as the residual sugar in the grape juice were interrogated. Except succinic acid, all the parameters significantly contributed to the multiple linear regression of the final pH according to the following equation.

$$pH = 0.982 - 0.0058\, x\, [Malic\ acid]p + 0.1287\, x\, [Acetic\ acid]p + 0.678\, x\, pHi - 0.0013\, x\, [residual\ sugar]$$

The adjusted R square of the model is 0.392 (pvalue $<2\times10^{-16}$), suggesting that a large part of the explaining factors was not identified as illustrated Figure 3D. A variance analysis of this linear model indicated that an important part of final pH variation was explained by initial pH of grape juice and the malic acid produced by yeast (25 and 13%, respectively). In contrast, acetic acid, and residual sugars accounted for a very minor part of the model.

6.4 Malic acid producer strains are enriched in ACIDIC alleles

In a previous study (Vion et al., 2021), we applied a marker assisted selection using eleven QTLs related to MAC% and mapped on the SB×GN genome (Peltier et al., 2021). The penetrance of such QTLs was only partial due to complex genetic interactions. Since SB and GN genome constitute 50% or the AC's hybrids we hypothesized that the most malic acid producer strains should be enriched in alleles conferring low MAC% values. The QTLs linked to MAC% were given in Table S1 and were tracked with 19 mass array markers suitable to interrogate all the genotypes in the same assay. Most of the QTLs were narrowed by a pair of markers in order to increase the robustness of genotyping.

The Figure 4A shows the relative position of such markers on yeast chromosomes and specify the contribution of SB and GN alleles respect to the MAC%. Alleles promoting malic consumption were named DEMALIC while alleles promoting malic acid production were named ACIDIC. The Figure 4B indicated the SNP call of both DEMALIC and ACIDIC alleles that are represented by green and red, respectively. Among the 19 ACIDIC alleles, 14 are derived from the parental strain GN encompassing nine of the 11 QTLs tracked. The allele inheritances for the four parental strains and the hybrids AC1 to AC3 are also detailed in the Figure 4C. Homozygous alleles are represented by green or red shaded area and can be considered as fixed in the respective hybrid's progeny. For instance, the acidic allele of the QTLs XI_382 corresponding the gene *MAE1* is present in all the AC's hybrids of this study. Conversely, for the loci VII_425 and VI_426, the GN allele was lost indicating that the acidifying copy of *PNC1* is not present in the progeny analyzed (Figure 4C). Among the 118 progeny strains phenotyped in this work, 94 were randomly genotyped for their inheritance for these 19 markers using a mass array strategy (see methods). The genotype of each strain is given in the Table S2.

As shown in the Figure 5A, the number of ACIDIC alleles was negatively correlated with MAC% values. However, this number was quite different according to the progeny genotyped. Indeed, the number of segregating markers was different according to the hybrid. In the AC1 progeny the number of ACIDIC alleles ranged between 2 and 13 while AC2 and AC3 progenies had fixed a greater number of ACIDIC alleles (between 10 and 14) (Figure 4C). Two QTLs (IV_28 and VII_480) segregated in all the progenies. Their effect on MAC% was confirmed in each AC's hybrid as illustrated in the Figure 5 B and C. Interestingly, this effect was much higher in the AC2 and AC3 backgrounds where numerous other acidic alleles were present. This result illustrated modifications of QTL penetrance according to the genetic background.

6.5 Malic acid consumer and producer strains drastically impact the acidity perception of wine

The organoleptic impact of strains able to modulate the amount of malic acid at the end of the alcoholic fermentation was evaluated by the sensory analysis of a Sauvignon blanc wine. In order to have a representative image of the technological potential of such strains, five high malic acid producers and five high malic acid consumers were selected. The producer strains are progeny clones of hybrids AC1, AC2 and AC3 while the consumer strains are progeny clones of FMGS and FMGS2 hybrids previously described by Vion *et al.* 2021. Two commercial starters (BO213 and RB4, Laffort, France) were added to complete this panel. Those commercial starters showed the highest difference in MAC% among 32 commercial starters tested (Peltier et al., 2018a). Each strain was fermented in a single bioreactor of 1.2L according to standard enological procedure, control of temperature, nitrogen management and preservation to oxidation (see methods). The final composition of wines is presented in the Table 3.

Table 3 Main characteristics of wine evaluated by sensory analysis

Strain	Group	Ethanol (%, v/v)	Total acidity (g/L H_2SO_4)	pH	Malic acid (g/L)	acetic acid (g/L)	Glycerol (g/L)	Residual sugar (g/L)
AC1_191	high producer	12.27	4.23	3.29	3.71	0.13	8.50	<2
AC2-1A	high producer	12.25	4.40	3.27	3.90	0.12	8.56	<2
AC3-22	high producer	12.39	3.71	3.34	3.34	0.29	6.75	<2
AC1-217	high producer	12.22	4.29	3.29	3.57	0.26	8.81	<2
AC2-467	high producer	12.16	4.74	3.24	4.01	0.11	8.81	<2
FMGS2-714	high consumer	12.34	3.51	3.4	2.30	0.74	8.63	<2
FMGS-889	high consumer	12.42	3.02	3.44	2.00	0.27	8.37	<2
FMGS-71	high consumer	12.28	3.22	3.42	2.23	0.24	8.63	<2
FMGS2-107	high consumer	12.28	3.21	3.44	2.04	0.49	8.66	<2
FMGS-263	high consumer	11.57	3.94	3.35	3.38	0.36	6.79	>5
BO213	starter	12.40	3.68	3.33	3.36	0.29	6.69	<2
RB4	starter	12.36	3.61	3.37	2.98	0.40	7.22	<2

As expected, high producer and consumer strains showed significative differences in malic acid content with a direct impact on pH and total acidity (Figure 6A) which is a good indicator of the overall acidity of wines (Plane et al., 1980). Other traits such as residual sugar, acetic acid, glycerol, or ethanol production were not significantly different between groups underlining a relative variability of strains belonging to the same group.

The resulting wines were presented to a panel of 28 tasters previously trained to appreciate the acidity in a Sauvignon Blanc wine. Dissimilarities between samples were analyzed using non-metric multi-dimensional scaling (MDS) allowing to figure out the perceptive proximities between wines on a map. The average stress value of the analysis was 0.051 which is significantly lower than explained by chance for 28 tasters and 12 wines (FDR of 5% 0.064). The two-dimensional MDS configuration reveals (Figure 6B) that the wines were grouped according to the type of the strain and discriminates more specifically the malic acid consumers and producers. Such approach demonstrated that wines had different acid profiles that were clearly perceived by the tasters.

7 Discussion

7.1 The nature of the grape juice and yeast strains are determining factors to modulate the malic acid level and final pH of wines.

Our findings shed light on the impact of the grape juice on the production of malic acid by *S. cerevisiae* strains corroborating previous studies (Delcourt et al., 1995; Vilanova et al., 2007). As previously suggested, the MAC% parameter is useful for comparing the behavior of various strains in different grape juices (Vion et al., 2021). By measuring this trait for 12 *S. cerevisiae* strains in seven grape juices we observed a strong effect of the grape juice origin (Figure 1B). This variability is partially related to the initial content of malic acid in the grape juice. The lowest the initial malic acid concentration, the highest the malic acid production by yeast. Since there is an obvious relation between malic acid concentration and pH of grape juice, it is not clear if the amount of malic acid of grape itself determines its production by yeast. Indeed, previous studies reported that malic acid production would be related to the pH of the medium with a similar concentration of malic acid (Schwartz and Radler, 1988). Malic acid is a diacid with two *pKa* of 3.46 and 5.10, respectively. Since the pH of grape juice ranges between 3.2 and 4.0, malic acid is mostly found in its undissociated and monodissociated form (H_2M and HM). Once it enters into the cell, malic acid mostly takes its deprotonated configuration (M) since the intracellular *pH* of the yeast is around 5-6. A proton efflux ensured by active pumps maintains the intracellular pH between 5 and 6. Malic acid entrance in the cell has been described as happening by facilitated diffusion (Salmon, 1987) as *S. cerevisiae* does not possess any known diacid transporter. In such case, only the protonated configuration of malic acid (H_2M) can enter into the cell which represent only 50% of the total malic acid available at pH= 3.46. Since low pH values enhance the H_2M/HM ratio, more di-protonated form is consumed triggering a higher deacidification of the media. This explains why high MAC% values are generally reached in more acid grape juices. In contrast, mechanisms triggering the expulsion of malic acid outside the cell much are less documented. Salmon (1987) provides a preliminary evidence of a malic acid efflux dependent on glucose in resting cells suggesting the existence of an active transporter. However such transporter was never characterized (Casal et al., 2008) despite several efforts. In addition, other abiotic factors such as nitrogen composition, sugar content or vitamins concentrations would also impact the production and consumption of malic acid during the alcoholic fermentation (Delcourt et al., 1995; Vilanova et al., 2007; Schwartz and Radler, 1988). Beside the composition of grape juice, our findings demonstrate that yeast strain has a definitive impact on wine malic acid content. Although the range of MAC% in the population of commercial starters is not very large (0 to 45%), the selection of strains metabolically biased toward production or consumption of malic acid allows the modification of wine pH in a surprising manner. Indeed, the dynamic range of pH for the same initial grape juice was higher than 0.4 units which is highly relevant in an enological context. Indeed, such range of pH variation drastically affect the antimicrobial activity of SO_2 (Divol et al., 2012), as well as the anthocyanins stability with a great influence on the color of red wines (Ribéreau-Gayon et al., 2006). The composition of 484 wines obtained during the breeding program allows the investigation of the main factors influencing the final pH of wine by a multiple linear regression approach. The main two contributors detected by the model and confirmed by an analysis of variance were the pH of grape juice and the malic acid produced by yeast. Although significative, the quite low adjusted R^2 (0.392) of the model suggested that many other compounds present in wines and/or produced by yeast should influence the final pH of wines. Among them potassium salt and tartaric acid concentrations could likely modulate the variability observed. The role of yeast strains regarding these factors remains to be explored.

7.2 Phenotypic characterization of wine yeast strains according to their malic acid production level.

The phenotypic characterization of progeny clones derived from three newly made F1-hybrids illustrates the efficiency of a breeding program for enhancing a complex trait. This result has been likely obtained because we crossed together extreme strains having distinct genetic origins. As previously documented for other quantitative traits of enological interest the phenotypic variability of the parental strains of this study (FM-1C and GS-8B) are mostly determined by distinct allelic pools (Peltier et al., 2018b). Therefore, their comparable MAC% (-75%) results from the combination of different alleles controlling different metabolic targets. Consequently, merging such allelic pools in a new hybrid background (AC1) allows the emergence of a wide genetic and phenotypic variability with some strains showing high transgressive values. Interestingly, the distribution of MAC% in AC1 progeny (Figure 2B) suggested that the inheritance of this trait is mostly non additive since only few progenies produce more malic acid than their F1-hybrid. However, once alleles controlling a high malic acid production have been fixed by crossing tail distribution progenies, it was possible to get many strains with very high acidifying properties. Thus, nearly 50% of the AC2 and AC3 progenies showed very low MAC% values offering the possibility to select a palette of malic acid producer strains that could segregates for many other traits not related to the malic acid production. For instance, the multivariate phenotypic analysis of 127 strains presented in Figure 3 showed that the three main secondary metabolites produced by yeast (acetic acid malic acid and glycerol) are not statistically correlated each togethers (Figure S4). In contrast, a weak positive correlation (rho=0.37) was found between malic and succinic acids suggesting than the production of both acids might be partially coupled which is consistent with their metabolic relationships (Camarasa et al., 2003).

This analysis also suggested a possible phenotypic trade-off between fermentation completion and malic acid production. In fact, the most acidifying strains have the slowest fermentation kinetics (high values of t35 and t80) and some of them did not complete the alcoholic fermentation (residual sugars > 2 g/L). Therefore, the production of high concentrations of malic acid during the alcoholic fermentation could result in a loss of fitness for *S. cerevisiae* explaining why most of the commercial starters empirically selected are malic acid consumers. Complementary studies with more phenotypes and more conditions will be necessary for confirming these preliminary conclusions.

7.3 Despite a partial penetrance QTLs previously mapped are statistically linked to the MAC% of malic producer strains

From a genetic point of view, the hybrids AC1, AC2, and AC3 and their relative progenies can be considered as mosaic strains derived from two parental strains (FM-1C and GS-8B). Such parental strains provided a large set of allelic variations enhancing the production of malic acid during the alcoholic fermentation that have been inherited from the four founder strains M2, GN, F15 and SB that well represent the genetic diversity of commercial starter population (Peltier et al, 2018). A fraction of this allelic pool has been previously mapped by a QTL analysis defining ACIDIC and DEMALIC alleles that modulate the production or consumption of malic acid, respectively. In order to evaluate what is the contribution of such alleles in the AC-hybrids, we randomly genotyped 94 progenies to find out statistical link between the inheritance of these alleles and the phenotype of the strains. The small number of progenies genotyped, and the high number of QTL tracked does not allow to test individually the effect of each QTL. However, some relations between genotype and phenotype have been elucidated. First, we found a negative correlation between the number of ACIDIC alleles and the MAC% whatever the background indicating that higher is the number of ACIDIC alleles stronger is the production of malic acid. Second, the global effect of two QTLs (VII_480 and IV_34) that played an important role in the MAC% determinism (Vion et al., 2021) was confirmed in new genetic backgrounds. Interestingly, such QTLs have a stronger effect in the AC2 and AC3 background which have already fixed many ACIDIC alleles respectively to AC1. This finding illustrates that major QTLs may have different level of penetrance due to the presence of other segregating alleles commonly called “modifiers” as review by several authors (Hou et al., 2016; Peltier et al., 2019; Yadav et al., 2016). The fixation of these buffering alleles in the AC2 and AC3 backgrounds allows a better expressivity of the QLTs VII_480 and VI_28 as shown in the Figure 5 B and C.

7.4 Selection of a new type of *S. cerevisiae* strains for coping the drastic drop of acidity in wines

Despite numerous stuck fermentations of high malic acid producers, this study allows to isolate eight strains that completed the alcoholic fermentation and produced more than 3 g/L of malic acid (Figure S5). This selection proposes a set of strains with a very contrasted phenotype respectively to those previously selected by Vion et al. 2021 enhancing considerably the technological palette of resulting wines by modulating the wine acidity during the alcoholic fermentation. This original feature is particularly relevant in an enological context for coping with drastic drops of acidity level observed in grape juices. In order to test the enological relevance of such strains, five malic producer strains were compared to five malic consumers strains as well as two commercial starters. Small scale vinifications were carried out on a Sauvignon blanc matrix that was evaluated by a sensory panel using a sorting task approach. After a preliminary test of the aptitude of the panel to discriminate wine acidity, the twelves wines were evaluated and the strains showing contrasted malic acid metabolism were well separated. This very preliminary sensory characterization agrees with the chemical analyses of wines tasted that statistically differs in their total acidity, their pHs and their malic acid content.

7.5 Concluding remarks

The climate change has drastic consequences of the acidity of grape juice and constitutes a pregnant problem for wine making. The use of selected microorganisms during the alcoholic fermentation is a promising solution to moderate this drop of acidity and preserve wine quality. In order to propose a reliable alternative to the use of non-conventional yeast species such as *L. thermotolerans* we applied a selection program aiming to obtained *S. cerevisiae* strains able to produce up to 3g/L of malic acid during the alcoholic fermentation. This result has been obtained by applying a classical breeding strategy narrowing the ability of strain to produce malic acid in different grape juices with a significant impact on wine pH and titratable acidity. This result has been obtained by the accumulation of acidifying alleles of several QTLs that have been previously identified. The large number of alcoholic fermentation achieved allowed the discrimination of some biotic and abiotic factors that control the final pH of wine in *S. cerevisiae* pure cultures. Beside a strong effect of the grape juice origin, the production of malic acid by yeast strongly impacted wine pH which was not the case of succinic or acetic acid production. However, high concentrations of malic acid reached had negative consequences of fermentation kinetics due to the strong acidification of the fermenting matrix. From a sensory view point we demonstrated that acidifying strains could be clearly discriminated from the high malic acid consuming ones. This work demonstrated that breeding programs based on QTLs detection is an efficient lever for selecting new starters able to manage wine acidity.

8 Author contributions

Conceptualization CV and PM; Formal analysis CV and PM; Funding acquisition IM, PM; Investigation CV, MM, MB, VF, BR; Methodology MM, ST; Resources NY, IM, PM; Software CV, PM; Supervision PM; Writing - original draft CV, NY, PM; Writing - review & editing ST, CV PM.

9 Conflict of interest

PM, CV, MB, and MM reports a relationship with Biolaffort company that includes full time employment.

10 References

Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J.J., Hilbert, G., Pascual, I., 2018. Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. Plant Sci. 267, 74–83. https://doi.org/10.1016/j.plantsci.2017.11.009

Bureau, S.M., Razungles, A.J., Baumes, R.L., 2000. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. J. Sci. Food Agric. https://doi.org/10.1002/1097-0010

Camarasa, C., Grivet, J.P., Dequin, S., 2003. Investigation by 13C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways of succinate formation in Saccharomyces cerevisiae during anaerobic fermentation. Microbiology 149, 2669–2678. https://doi.org/10.1099/mic.0.26007-0

Casal, M., Paiva, S., Queirós, O., Soares-Silva, I., 2008. Transport of carboxylic acids in yeasts. FEMS Microbiol. Rev. 32, 974–994. https://doi.org/10.1111/j.1574-6976.2008.00128.x

Castellari, L., Ferruzzi, M., Magrini, A., Giudici, P., Passarelli, P., Zambonelli, C., 1994. Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant Saccharomyces strains. Vitis. ISSN 00427500

Chen, Y., Jiang, J., Song, Yaoyao, Zang, X., Wang, G., Pei, Y., Song, Yuyang, Qin, Y., Liu, Y., 2022. Yeast diversity during spontaneous fermentations and oenological characterisation of indigenous Saccharomyces cerevisiae for potential as wine starter cultures. Microorganisms 10, 1455.

Chernova, M., Albertin, W., Durrens, P., Guichoux, E., Sherman, D.J., Masneuf-Pomarede, I., Marullo, P., 2018. Many interspecific chromosomal introgressions are highly prevalent in Holarctic Saccharomyces uvarum strains found in human-related fermentations. Yeast 35, 141–156. https://doi.org/10.1002/yea.3248

Chollet, S., Valentin, D., Abdi, H., 2014. Free Sorting Task, in: Tomasco, P., Ares, G. (Eds.), Novel Techniques in Sensory Characterization and Consumer Profiling. Boca Raton, pp. 207–227. ISBN 9781138034273

Coloretti, F., Zambonelli, C., Castellari, L., Tini, V., Rainieri, S., 2002. The effect of DL-malic acid on the metabolism of L-malic acid during wine alcoholic fermentation. Food Technol. Biotechnol. 40, 317–320. ISSN 1330-9862

Conover, 1999. Counts and categories, in: Conover (Ed.), “Practical Nonparametric Statistics,” , Wiley, 1999, Pp. 165-195. Wiley, pp. 380–383.

Coombe, B.G., 1987. Influence of Temperature on Composition and Quality of Grapes. Acta

Hortic. "Symposium on grapevine canopy and vigor management, Kliewer, W.M. (California Univ., Davis, CA (USA). Dept. of Viticulture and Enology).- Wageningen (Netherlands): ISHS, 1987.- ISBN 90-6605-442-5. p. 23-35

Cox, T., Cox, M., 2001. Multidimensional Scaling, 2nd editio. ed. New York. ISBN 978-3-540-33037-0

da Silva, T., Albertin, W., Dillmann, C., Bely, M., la Guerche, S., Giraud, C., Huet, S., Sicard, D., Masneuf-Pomarede, I., De Vienne, D., Marullo, P., 2015. Hybridization within Saccharomyces Genus Results in Homoeostasis and Phenotypic Novelty in Winemaking Conditions. PLoS One 10, e0123834. https://doi.org/10.1371/journal.pone.0123834

de Leeuw, J., Mair, P., 2009. Multidimensional Scaling Using Majorization: SMACOF in R. J. Stat. Softw. 31, 1–30. https://doi.org/10.18637/jss.v031.i03

Delcourt, F., Taillandier, P., Vidal, F., Strehaiano, P., 1995. Influence of pH, malic acid and glucose concentrations on malic acid consumption by Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 43, 321–324. https://doi.org/10.1007/BF00172832

Divol, B., Du Toit, M., Duckitt, E., 2012. Surviving in the presence of sulphur dioxide: Strategies developed by wine yeasts. Appl. Microbiol. Biotechnol. 95, 601–613. https://doi.org/10.1007/s00253-012-4186-x

Dubourdieu, D., 2004. Influence of climate, soil, and cultivar on Terroir Influence of Climate, Soil, and Cultivar on Terroir. Am. J. Enol. Vitic 55, 3. https://www.ajevonline.org/content/55/3/207

Duchêne, E., Schneider, C., 2005. Grapevine and Climatic changes: a glance at the situation in Alsace. Agron. Sustain. Dev 93–99. https://doi.org/10.1051/agro:2004057

Gabriel, S., Ziaugra, L., Tabbaa, D., 2009. SNP genotyping using the sequenom massARRAY iPLEX Platform. Curr. Protoc. Hum. Genet. https://doi.org/10.1002/0471142905.hg0212s60

Hou, J., Sigwalt, A., Fournier, T., Pflieger, D., Peter, J., de Montigny, J., Dunham, M.J., Schacherer, J., 2016. The Hidden Complexity of Mendelian Traits across Natural Yeast Populations. Cell Rep. 16, 1106–1114. https://doi.org/10.1016/j.celrep.2016.06.048

Hranilovic, A., Albertin, W., Capone, D.L., Gallo, A., Grbin, P.R., Danner, L., Bastian, S.E.P., Masneuf-Pomarede, I., Coulon, J., Bely, M., Jiranek, V., 2021. Impact of Lachancea thermotolerans on chemical composition and sensory profiles of Merlot wines. Food Chem. 349, 129015. https://doi.org/10.1016/j.foodchem.2021.129015

Hranilovic, A., Gambetta, J.M., Schmidtke, L., Boss, P.K., Grbin, P.R., Masneuf-Pomarede, I., Bely, M., Albertin, W., Jiranek, V., 2018. Oenological traits of Lachancea thermotolerans show signs of domestication and allopatric differentiation. Sci. Rep. 8, 14812. https://doi.org/10.1038/s41598-018-33105-7

Huang, C., Roncoroni, M., Gardner, R.C., 2014. MET2 affects production of hydrogen sulfide during wine fermentation. Appl. Microbiol. Biotechnol. 98, 7125–7135. https://doi.org/10.1007/s00253-014-5789-1

Hung, G.C., Brown, C.R., Wolfe, A.B., Liu, J., Chiang, H.L., 2004. Degradation of the ghiconeogenic enzymes fructose-1,6-bisphosphatase and malate dehydrogenase is mediated by distinct proteolytic pathways and signaling events. J. Biol. Chem. 279, 49138–49150. https://doi.org/10.1074/jbc.M404544200

IPCC, 2020. Climate Change and Land, International Encyclopedia of Geography: People, the Earth, Environment and Technology. https://doi.org/10.1002/9781118786352.wbieg0538

Jones, G. V, 2007. Climate Change: Observations, Projections, and General Implications for Viticulture and Wine Production. Jones. https://chaireunesco-vinetculture.u-bourgogne.fr/colloques/actes_clima/Actes/Article_Pdf/Jones.pdf

Lonvaud-Funel, A., 1994. La Desacidification Biologique des Vins; Etat de la question, perspectives d'avenir. J. Int. des Sci. la Vigne du Vin 28, 161–170. https://doi.org/10.20870/oeno-one.1994.28.2.1149

Marullo, P., Durrens, P., Peltier, E., Bernard, M., Mansour, C., Dubourdieu, D., 2019. Natural allelic variations of Saccharomyces cerevisiae impact stuck fermentation due to the combined effect of ethanol and temperature; a QTL-mapping study. BMC Genomics 20, 680. https://doi.org/10.1186/s12864-019-5959-8

Marullo, P., Mansour, C., Dufour, M., Albertin, W., Sicard, D., Bely, M., Dubourdieu, D., 2009. Genetic improvement of thermo-tolerance in wine Saccharomyces cerevisiae strains by a backcross approach. FEMS Yeast Res. 9, 1148–1160. https://doi.org/10.1111/j.1567-1364.2009.00550.x

Mira de Orduña, R., 2010. Climate change associated effects on grape and wine quality and production. Food Res. Int. 43, 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Nistor, E., Dobrei, A.G., Dobrei, A., Camen, D., 2018. Growing season climate variability and its influence on sauvignon Blanc and Pinot Gris Berries and Wine Quality: Study case in Romania (2005-2015). South African J. Enol. Vitic. 39, 196–207. https://doi.org/10.21548/39-2-2730

Origone, A.C., Rodríguez, M.E., Oteiza, J.M., Querol, A., Lopes, C.A., 2018. Saccharomyces cerevisiae × Saccharomyces uvarum hybrids generated under different conditions share similar winemaking features. Yeast 35, 157–171.

https://doi.org/https://doi.org/10.1002/yea.3295

Peltier, E., Bernard, M., Trujillo, M., Prodhomme, D.D., Barbe, J.-C., Gibon, Y., Marullo, P., 2018a. Wine yeast phenomics: a standardized fermentation method for assessing quantitative traits of Saccharomyces cerevisiae strains in enological conditions. PLoS One 13, 191353. https://doi.org/10.1101/191353

Peltier, E., Friedrich, A., Schacherer, J., Marullo, P., 2019. Quantitative Trait Nucleotides Impacting the Technological Performances of Industrial Saccharomyces cerevisiae Strains. Front. Genet. 10, 683. https://doi.org/10.3389/fgene.2019.00683

Peltier, E., Sharma, V., Raga, M.M., Roncoroni, M., Bernard, M., Yves Gibon, Marullo, P., Jiranek, V., Gibon, Y., Marullo, P., 2018b. Dissection of the molecular bases of genotype x environment interactions: a study of phenotypic plasticity of Saccharomyces cerevisiae in grape juices. BMC Genomics 19, 772. https://doi.org/10.1186/s12864-018-5145-4

Peltier, E., Vion, C., Abou Saada, O., Friedrich, A., Schacherer, J., Marullo, P., 2021. Flor Yeasts Rewire the Central Carbon Metabolism During Wine Alcoholic Fermentation. Front. Fungal Biol. 2. https://doi.org/10.3389/ffunb.2021.733513

Plane, R., Mattick, L., Weirs, L., 1980. An acidity index for the taste of wines. Am. J. Enol. Vitic. 31, 258–265. ISSN : 0002-9254

Poudel, P.R., Mochioka, R., Beppu, K., Kataoka, I., 2009. Influence of temperature on berry composition of interspecific hybrid wine grape “Kadainou R-1” (Vitis ficifolia var. ganebu × V. vinifera ’Muscat of Alexandria’). J. Japanese Soc. Hortic. Sci. 78, 169–174. https://doi.org/10.2503/jjshs1.78.169

R Core Team, 2018. R: A language and environmentfor statistical computing. R Found. Stat. Comput. Vienna, Austria. URLhttps//www.R-project.org/.

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., Viljoen-Bloom, M., 2003. Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation. Int. J. Food Microbiol. 83, 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3

Regev-rudzki, N., Battat, E., Goldberg, I., Pines, O., 2009. Dual localization of fumarase is dependent on the integrity of the glyoxylate shunt. Mol. Microbiol. 72, 297–306. https://doi.org/10.1111/j.1365-2958.2009.06659.x

Ribérau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006. Handbook of enology Volume 2 The chemistry of wine stabilization and treatments. ISBN-13: 978-0-470-01037-2

Saayman, M., Viljoen-Bloom, M., 2006. The Biochemistry of Malic Acid Metabolism by Wine Yeasts – A Review. South African J. Enol. Vitic. 27, 113–122.

https://doi.org/10.21548/27-2-1612

Salmon, J., 1987. l-Malic-acid permeation in resting cells of anaerobically grown Saccharomyces cerevisiae. Biochim. Biophys. Acta - Biomembr. 901, 30–34. https://doi.org/10.1016/0005-2736(87)90253-7

Schwartz, H., Radler, F., 1988. Formation of l(-)malate by Saccharomyces cerevisiae during fermentation. Appl. Microbiol. Biotechnol. 27, 553–560. https://doi.org/10.1007/BF00451631

Su, J., Wang, T., Wang, Y., Li, Y.Y., Li, H., 2014. The use of lactic acid-producing, malic acid-producing, or malic acid-degrading yeast strains for acidity adjustment in the wine industry. Appl. Microbiol. Biotechnol. 98, 2395–2413. https://doi.org/10.1007/s00253-014-5508-y

van Leeuwen, C., Darriet, P., 2016. The Impact of Climate Change on Viticulture and Wine Quality. J. Wine Econ. 11, 150–167. https://doi.org/10.1017/jwe.2015.21

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I.S., Henschke, P.A., 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wine yeasts. Appl. Microbiol. Biotechnol. 77, 145–157. https://doi.org/10.1007/s00253-007-1145-z

Vion, C., Peltier, E., Bernard, M., Muro, M., Marullo, P., 2021. Marker Assisted Selection of malic-consuming Saccharomyces cerevisiae strains for winemaking. Efficiency and limits of a QTL's driven breeding program. J. Fungi 17–24. https://doi.org/10.20944/preprints202103.0132.v1

Volschenk, H., van Vuuren, H.J.J., Viljoen-Bloom, M., 2006. Malic Acid in Wine: Origin, Function and Metabolism during Vinification. South African J. Enol. Vitic. 27. https://doi.org/10.21548/27-2-1613

Volschenk, H., Viljoen, M., Grobler, J., Bauer, F., Lonvaud-Funel, A., Denayrolles, M., Subden, R.E., Van Vuuren, H.J.J., 1997. Malolactic Fermentation in Grape Musts by a Genetically Engineered Strain of Saccharomyces cerevisiae. Am. J. Enol. Vitic. 48. ISSN : 0002-9254

Yadav, A., Dhole, K., Sinha, H., 2016. Genetic Regulation of Phenotypic Plasticity and Canalisation in Yeast Growth. PLoS One 11, e0162326. https://doi.org/10.1371/journal.pone.0162326

Yéramian, N., Chaya, C., Suárez Lepe, J.A., 2007. L-(-)-malic acid production by Saccharomyces spp. during the alcoholic fermentation of wine. J. Agric. Food Chem. 55, 912–919. https://doi.org/10.1021/jf061990w

11 Supplementary material

Table S1. SNPs detected by mass array

Table S2. MASS array genotyping of AC's hybrid progenies

Table S3. Grape juices compositions

Table S4. Phenotypic characterization of parental candidates in seven grape juices

Table S5. Phenotypic characterization of AC's hybrid progenies in two grape juices.

Figure S1 Relative position of acidifying strains in the SBxGN and M2xF15 progenies

Figure S2 Correlation analysis of data presented in Table 2

Figure S3 Estimation of the strain and grape juice effects by ANOVA

Figure S4 Correlation analysis of data presented in Table S5

Figure S5 Distribution of malic acid production in completed and stuck fermentations of data presented in Table S5

12 Figures caption

Figure 1 Phenotypic characterization of acidifying strains in seven red grape juices.

Panel A. The dot plot indicates the distribution of MAC% value measured for 12 strains in seven grape juices (2 replicates per grape juice). Strains were colored according to their origin. Green= industrial starters; light blue= malic producer strains isolated in Spain, orange M2xF15 progenies, purple= SBxGN progenies, red = malic consumer strain. The letters below the dot plot indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Panel B. The dot plot indicates the distribution of MAC% value measured for 12 strains in seven grape juice (2 replicates per grape juice). Grape juices were sorted according to their initial pH values. Negative MAC% values (indicating a malic acid production) were obtained in only two grape must colored in burgundy color. The letters below the dot plot indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Figure 2 Breeding program for enhancing malic acid production

Panel A. Box plot indicates the optimization of MAC% values by a phenotype driven breeding program. The purple color indicates the MAC% distribution of founder strains GN, SB, M2 and

F15 and parental strains selected GS-8B and FM-1C. The resulting F1 hybrid AC1 and its four optimal progeny clones are colored in red. The second-generation hybrids AC2 and AC3 are respectively colored in red and green; each F1-hybrid is narrowed by its respective parents. The letters below the box plots indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Panel B. Distribution of MAC% values in AC1 AC2 and AC3 progenies. Data presented are the average values of triplicates and were measured in the CS_14 grape juice. The large black dot indicates the average of values per background. The p value above the box plot indicates a statistical difference of MAC% according to the hybrid background (Kruskal test).

Panel C. Distribution of MAC% values in AC2 and AC3 progenies. Data presented are the average values of triplicates and were measured in the CS_15 grape juice. The large black dot indicates the average of values per background. The p value above the box plot indicates a statistical difference of MAC% according to the hybrid background (Kruskal test).

Figure 3 Multivariate analysis

Panel A. The phenotypic behavior of 127 strains for 12 quantitative traits was figured out in the first two dimension of a Principal Component Analysis (encompassing 60.1% of the total inertia). A total of 484 fermentations were carried out in two grape juices (CS_14 and CS15). The color of the dots indicated the yeast background (AC1, AC2, AC3 and control strains) while the shape indicated the grape must fermented. Open and full symbols indicates if the fermentations were stuck or completed, respectively.

Panel B. Circle of correlation of the 12 variables used for the PCA.

Panel C. Correlation between the final malic acid content and the final pH of the wine in both CS_14 and CS_15 grape juices, the dot key is the same than for panel A.

Panel D. Real and predicted values of the final pH according to following linear model regression $pH = 0.0631 - 0.0058\ x\ [Malic\ acid] + 0.1282\ x\ [Acetic\ acid] + 0.9495\ x\ pHi - 0.0014\ x\ [residual\ sugar]$. Open and full symbols indicates if the fermentations were stuck or complete, respectively.

Figure 4 QTLs genotyped by Mass Array in AC's hybrids

Panel A. Schematic localization of QTLs tracked on the genomic map of SBxGN hybrid. alleles conferring a malic acid production were colored in purple and blue for SB and GN, respectively.

Panel B. SNP call detected by mass array genotyping for each locus tracked in this study. The DEMALIC alleles (in green) enhance the consumption of malic acid while the ACIDIC alleles enhance the production of malic acid (in red).

Panel C. Genotypes of the four founders of the study (GN, SB, M2 and F15), the two parental strains GS-8B, FM-1C and the three hybrids AC1, AC2 and AC3. For each locus, the green and red colored areas indicated if the DEMALIC or ACIDIC allele is fixed in the hybrid background.

Figure 5 QTL effect

Panel A. Linear regression of the MAC% according to the number of ACIDIC alleles carried by the progeny clones of the hybrids AC1, AC2 and AC3 that are colored in red, blue and green respectively.

Panel B. The boxplot shows the MAC% values of strains carrying the DEMALIC (C) or ACIDIC (G) allele of the locus VII_480 according to the hybrid background. Colored numbers indicated the MAC% differences between the genotype (same key than panel A). The p value above the box plot indicates a statistical difference of MAC% according to the genotype in indicated (Wilcoxon test).

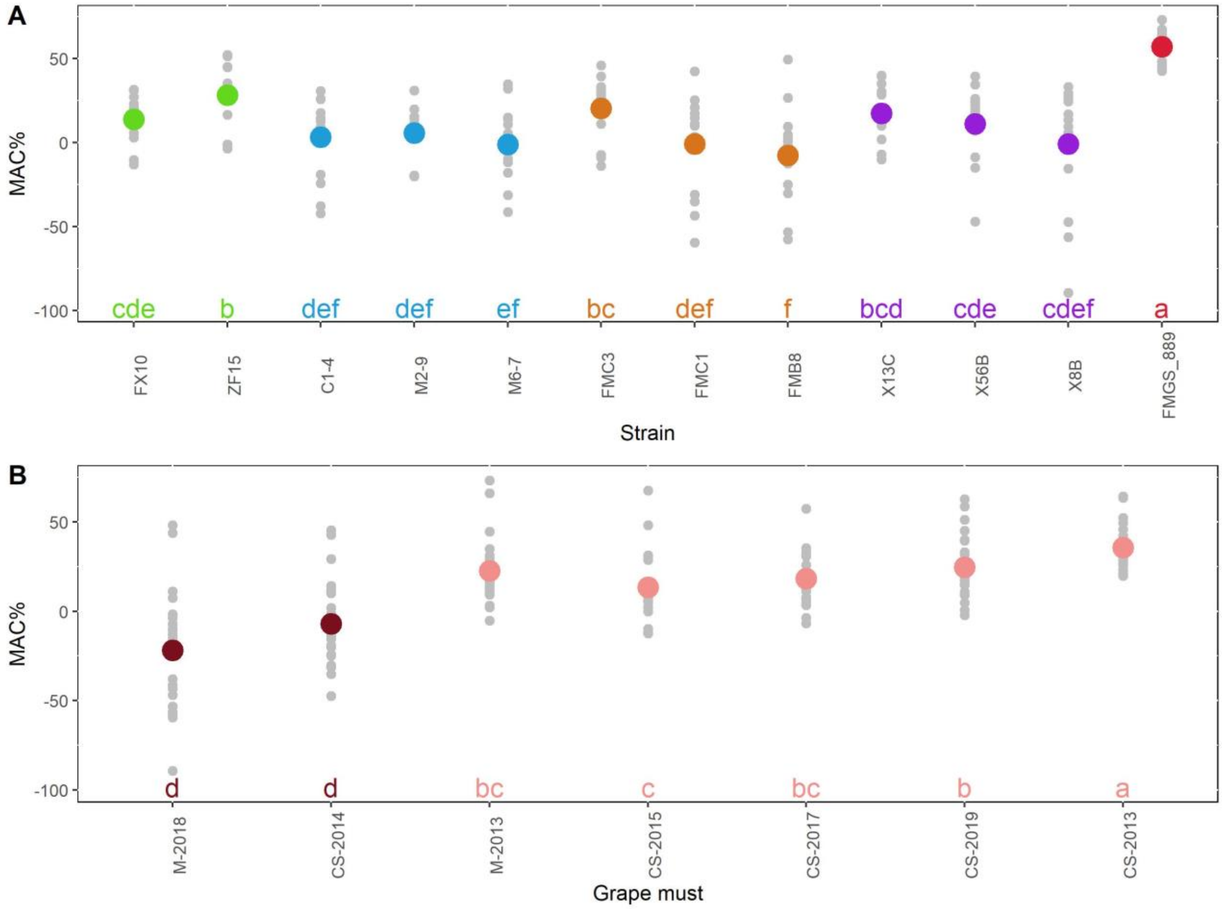
Panel C. The boxplot shows the MAC% values of strains carrying the DEMALIC (A) or ACIDIC (G) allele of the locus IV_28 according to the hybrid background. Colored numbers indicated the MAC% differences between the genotype (same key than panel A). The p value above the box plot indicates a statistical difference of MAC% according to the genotype in indicated (Wilcoxon test).

Figure 6 Acidity perception estimated by sensory analysis

Panel A. The boxplot figures out the distribution total acidity for each groups of strains. The significative difference between malic acid consumers and producers is confirmed by a Wilcoxon test (alpha<0.05).

Panel B. The relative position of the wines tasted are figured out on the first two dimensions of a MDS analysis. Wines are represented by points that are positioned such that the distances between the pairs of points reflect distances between the pairs of wines. The average stress value of the analysis was 0.051 which is significantly lower than explained by chance for 28 tasters and 12 wines (FDR of 5% 0.064). The area of each point is proportional to the individual stress indicating the confidence of the projection. The colors indicate the group of the strain, starter, malic acid consumers or producers.

Figure 1.



A
MAC%
50
0
-50
-100
cde
b
def
def
ef
bc
def
f
bcd
cde
cdef
a
FX10
ZF15
C1-4
M2-9
M6-7
FMC3
FMC1
FMB8
X13C
X56B
X8B
FMGS_889
Strain
B
MAC%
50
0
-50
-100
d
d
bc
c
bc
b
a
M-2018
CS-2014
M-2013
CS-2015
CS-2017
CS-2019
CS-2013
Grape must

Figure 2

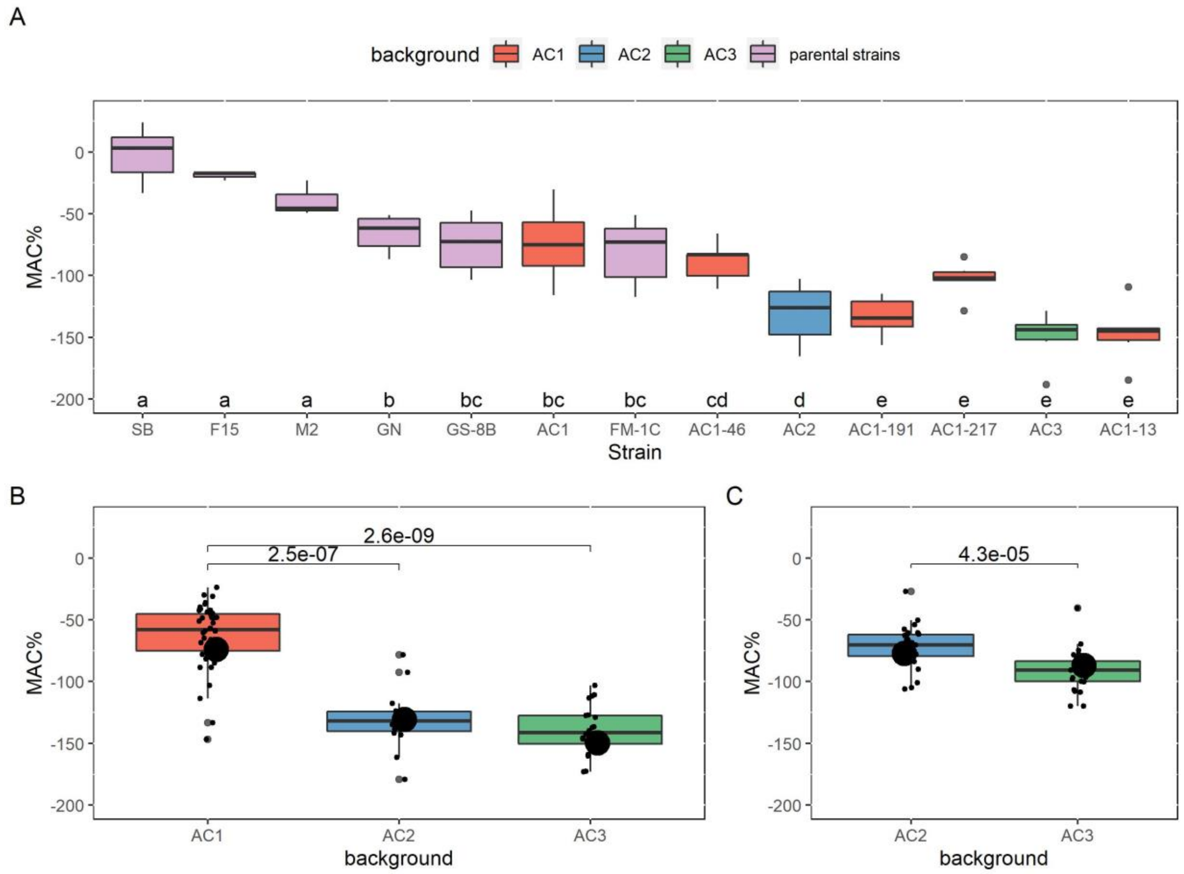

A
background AC1 AC2 AC3 parental strains
MAC%
0
-50
-100
-150
-200
a a a b bc bc bc cd d e e e e
SB F15 M2 GN GS-8B AC1 FM-1C AC1-46 AC2 AC1-191 AC1-217 AC3 AC1-13
Strain
B
2.6e-09
2.5e-07
AC1 AC2 AC3
background
C
4.3e-05
AC2 AC3
background

Figure 3

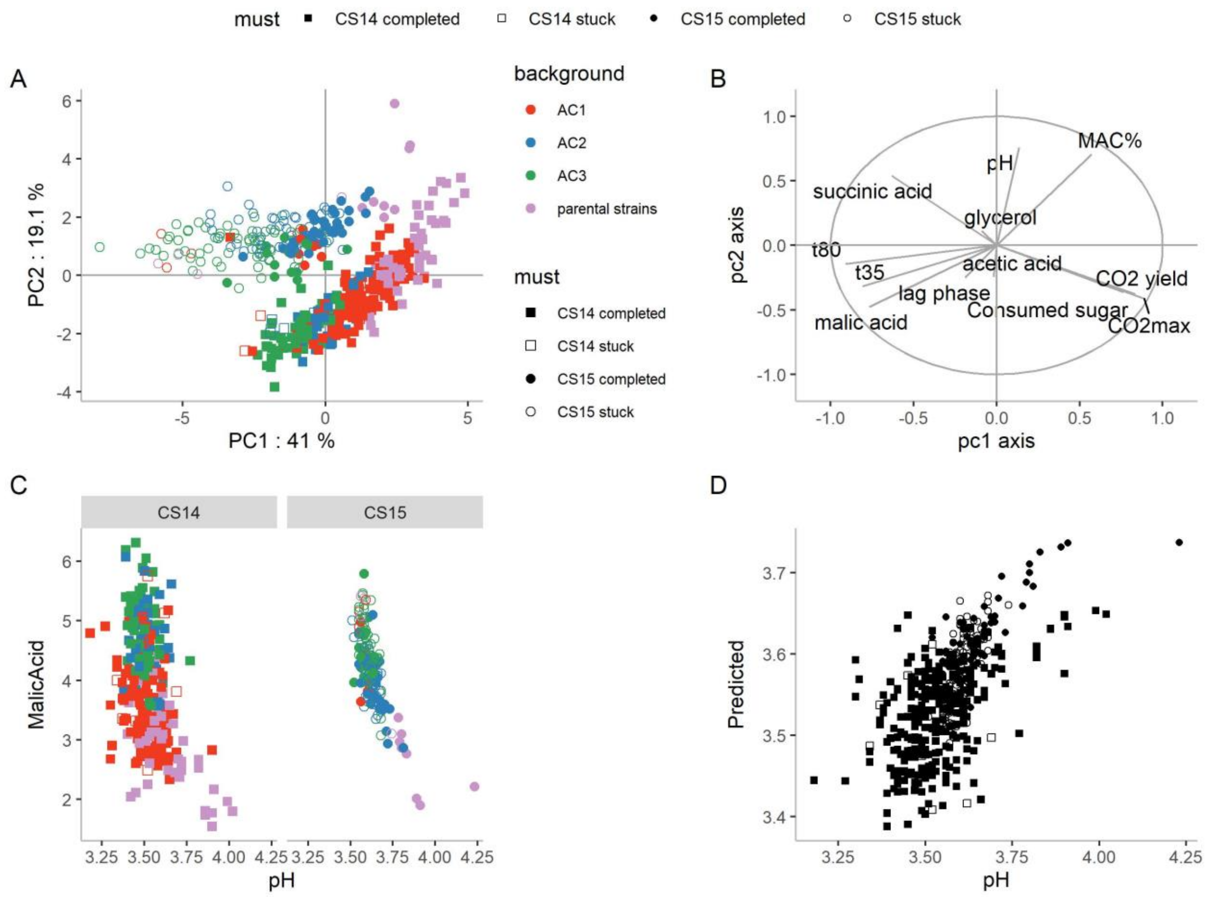
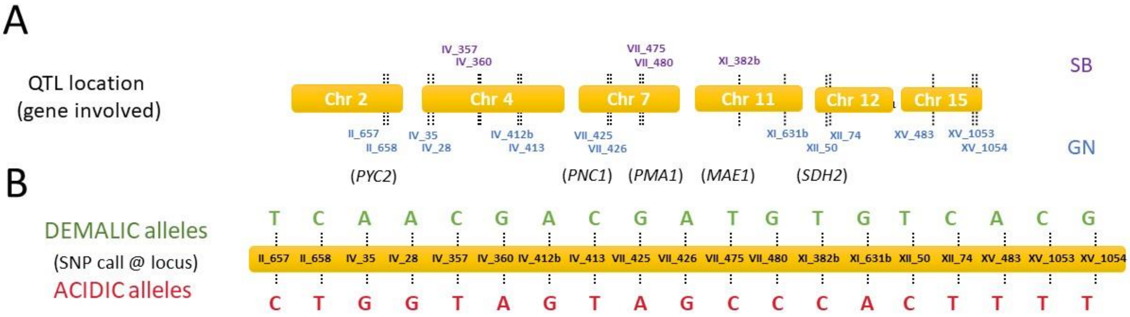

must
CS14 completed
CS14 stuck
CS15 completed
CS15 stuck
A
PC2 : 19.1 %
PC1 : 41 %
background
AC1
AC2
AC3
parental strains
must
CS14 completed
CS14 stuck
CS15 completed
CS15 stuck
B
pc2 axis
pc1 axis
MAC%
pH
succinic acid
glycerol
t80
t35
acetic acid
CO2 yield
lag phase
Consumed sugar
malic acid
CO2max
C
CS14
CS15
MalicAcid
pH
D
Predicted
pH

Figure 4



C

	II_657	II_658	IV_28	IV_35	IV_357	IV_360	IV_412b	IV_413	VII_425	VII_426	VII_475	VII_480	XI_382	XI_631	XII_50	XII_74	XV_1053	XV_1054	XV_483
GN	C	T	G	G	C	G	G	T	A	G	T	G	C	A	C	C	T	T	T
SB	T	C	A	A	T	A	A	C	G	A	C	C	T	G	T	C	A	C	G
M2	T	C	A	A	C	G	A	C	G	A	T	G	T	A	T	T	A	C	T
F15	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	C	A	C	G
GS-8B	C	T	G	G	T	A	G	T	G	A	C	C	C	A	T	C	A	C	G
FM-1C	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	T	A	C	T
AC1	T/C	C/T	A/G	G/A	C/T	A/G	A/G	T/C	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/C	A/A	C/C	G/T
AC2	C/C	T/T	A/G	G/G	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T
AC3	C/C	T/T	A/G	G/A	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T

Figure 5

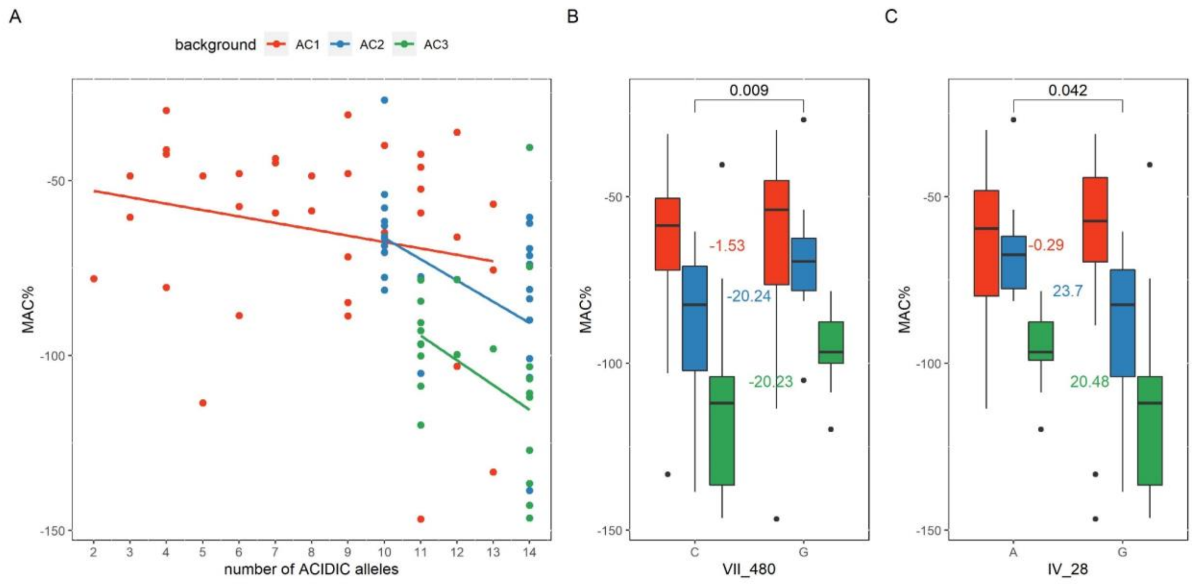

A
B
C
background
AC1
AC2
AC3
MAC%
-50
-100
-150
2
3
4
5
6
7
8
9
10
11
12
13
14
number of ACIDIC alleles
0.009
-1.53
-20.24
-20.23
C
G
VII_480
0.042
-0.29
23.7
20.48
A
G
IV_28

Figure 6

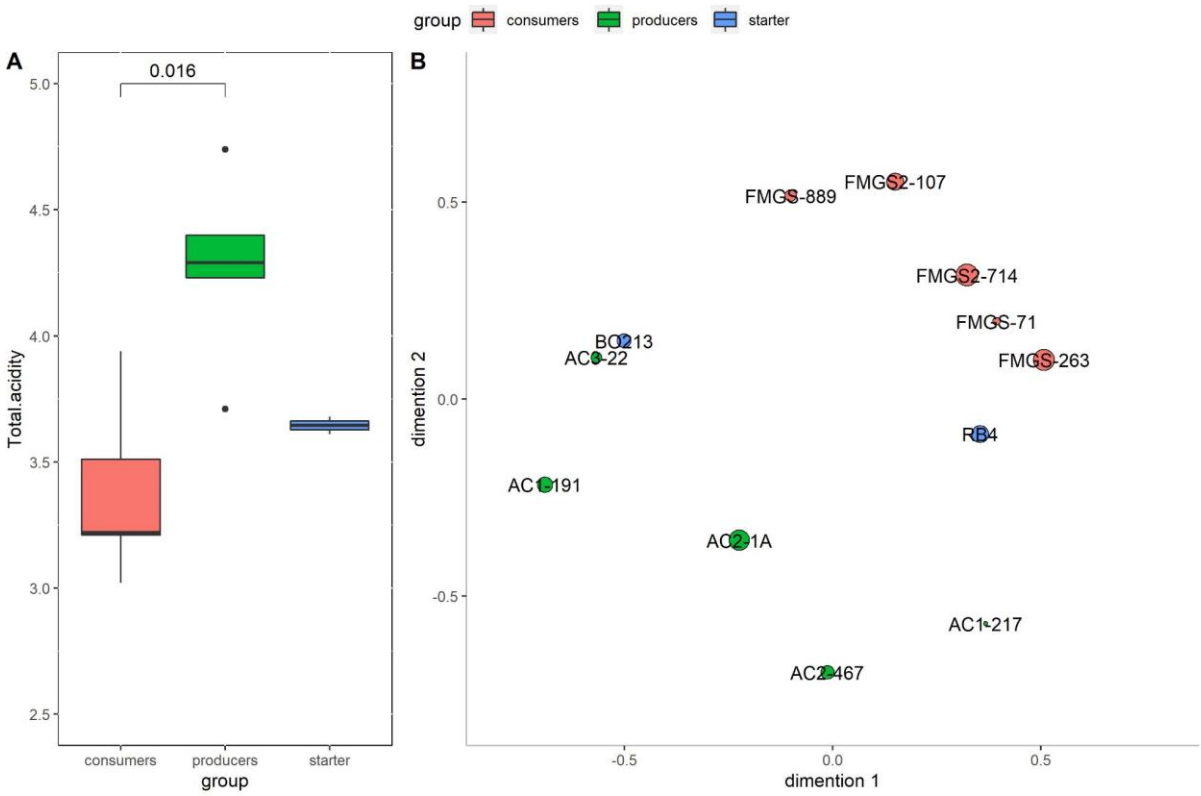

group consumers producers starter
A
0.016
Total.acidity
5.0
4.5
4.0
3.5
3.0
2.5
consumers
producers
starter
group
B
dimention 2
0.5
0.0
-0.5
dimention 1
FMGS2-107
FMGS-889
FMGS2-714
FMGS-71
BO213
AC3-22
FMGS-263
RB4
AC1-191
AC2-1A
AC1-217
AC2-467

Figure S1

Caption: Distribution of MAC% for starters (green), M2xF15 (orange) and SBxGN (purple) progenies and relative position of candidate parental strains (black dots), all the data were obtained in M15_5ml conditions; data obtained from Peltier et al. 2018 (starters) and Vion et al 2021 (M2xF15 and SBxGN progenies).

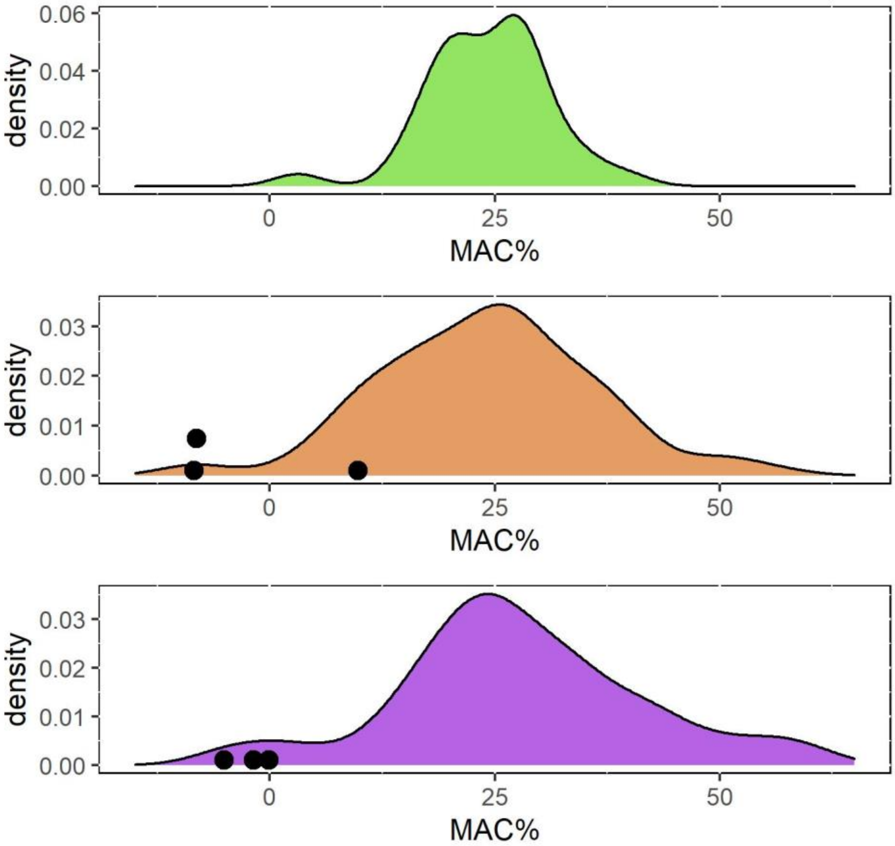


Figure S2

Caption: Correlation matrix between kinetics and metabolic traits measured in seven grape musts and 12 strains, only significative correlations were figured out. The intensity and the size of the color reflects the value of rho parameter (Spearman test with Bonferroni multiple corrections, alpha =0.001). Positive correlations are depicted in blue while negative correlations are depicted in red.

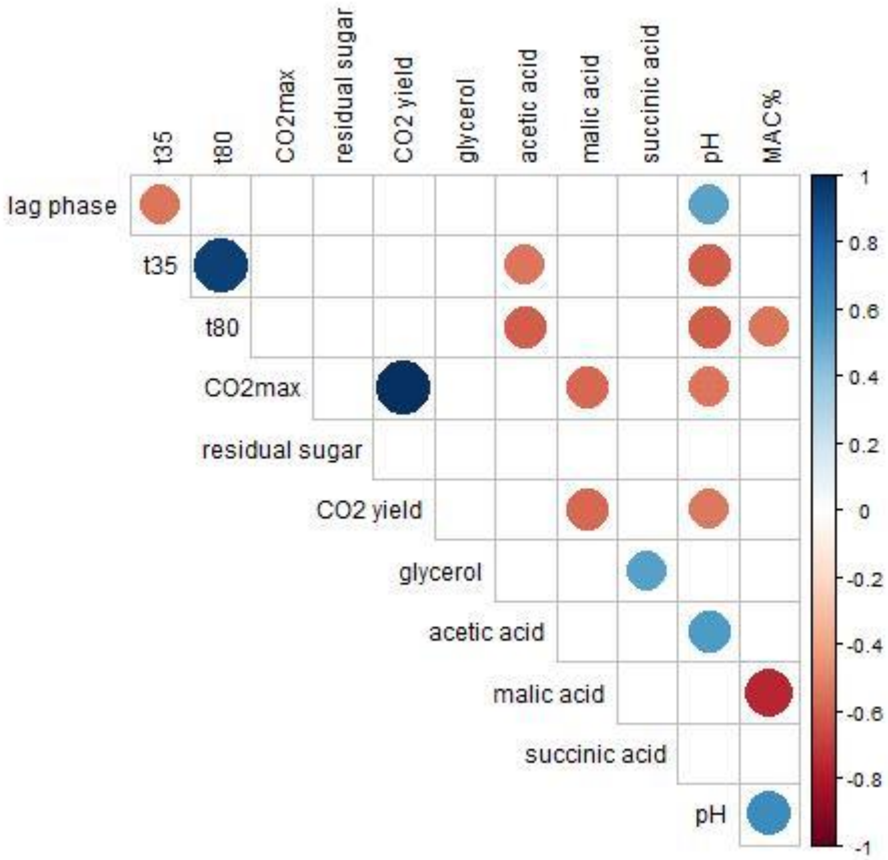


Figure S3

Caption: Panel A the box plot shows the distribution of MAC% values of four strains fermented in two different grape must CS14 and CS15 colored in blue and red, respectively. Panel B part of variance explained by strain and nature origin and their interaction according to a two-way analysis of variance. The number of stars above the bars indicates the significative threshold levels : * <0.05, ***<0.001.

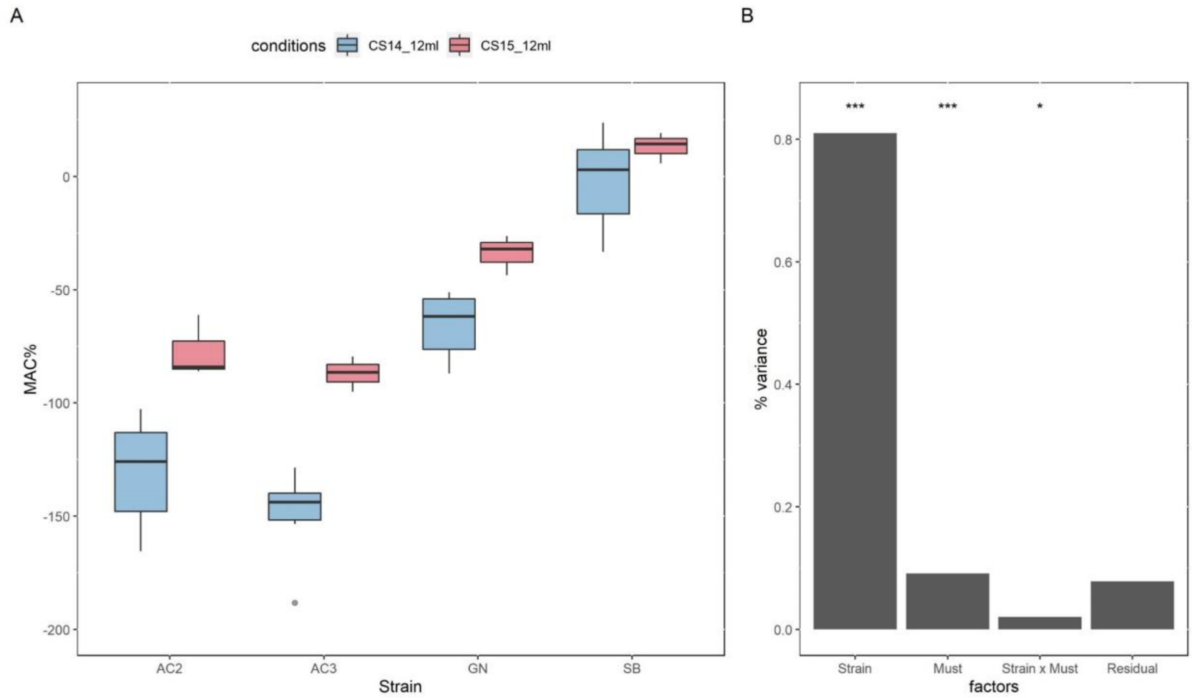


Figure S4

Caption: Correlation matrix between kinetics and metabolic traits measured in two grape musts and 127 strains, only significative correlations were figured out. The intensity and the size of the color reflects the value of rho parameter (Spearman test with Bonferroni multiple corrections, alpha =0.001). Positive correlations are depicted in blue while negative correlations are depicted in red.

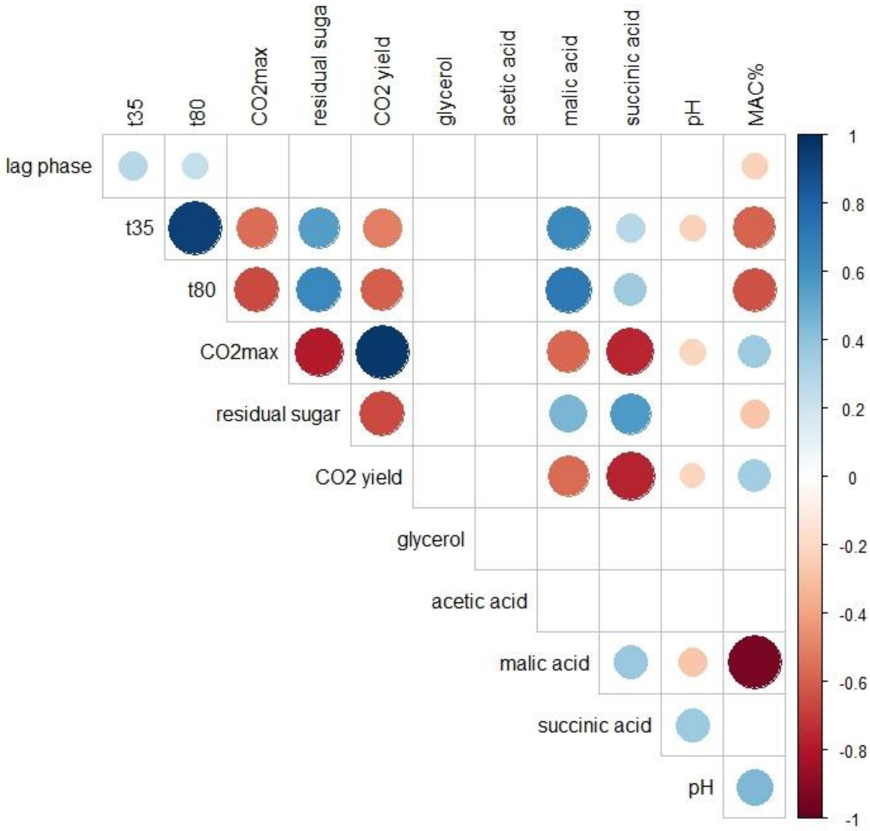
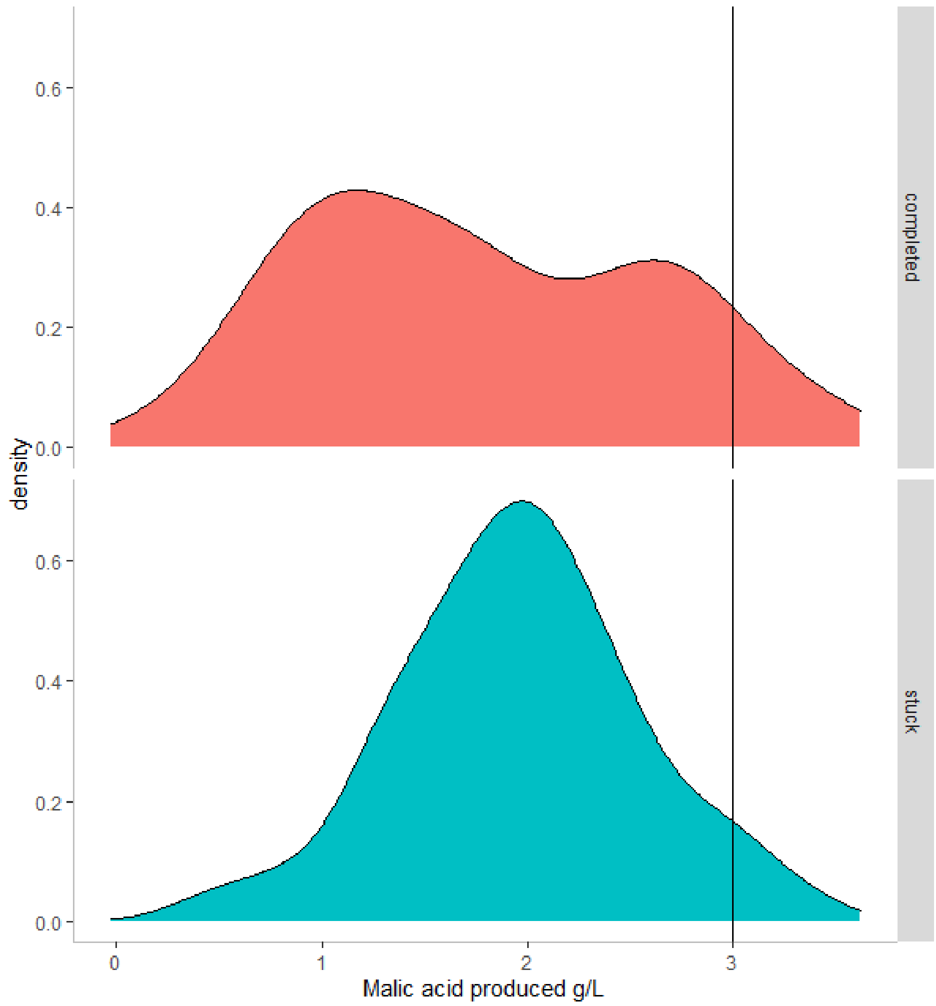

t35
t80
CO2max
residual suga
CO2 yield
glycerol
acetic acid
malic acid
succinic acid
pH
MAC%
lag phase
residual sugar
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure S5

Caption: Distribution of the average malic acid produced expressed in g/L according to the status of the fermentation (completed or stuck). The vertical line indicates the proportion of strains that have produced more than 3 g/L of malic acid (in addition to the initial quantity present in the grape must).



New malic acid producer strains of *Saccharomyces cerevisiae* for preserving wine acidity during alcoholic fermentation

Vion Charlotte[#1,2], Muro Maitena[#1,2], Bernard Margaux[1,2], Bruce Richard [2], Valentine Fautre [2], Nadine Yeramian[3], Isabelle Masneuf-Pomarède[2], Tempère Sophie[2], Marullo Philippe[1,2]*.

[1]Biolaffort, Bordeaux, FRANCE
[2] UMR 1366 Œnologie, Université de Bordeaux, INRAE, Bordeaux INP, BSA, ISVV
[3] Microbiology Division, Department of Biotechnology and Food Science, Faculty of Science-University of Burgos

#Authors contributes to the same level to this publication

*Corresponding author: philippe.marullo@u-bordeaux.fr
Permanent adress : UMR 1366 Œnologie, Université de Bordeaux, INRAE, Bordeaux INP, BSA, ISVV 210 chemin de Leysotte. 33882, Villenave d'Ornon

Figures can be shown in color online only

1 Abstract:

In the context of climate change, the chemical composition of wines is characterized by a massive drop of malic acid concentration in grape berries. Then wine professionals have to find out physical and/or microbiological solutions to manage wine acidity. The aim of this study is to develop wine *Saccharomyces cerevisiae* strains able to produce significant amount of malic acid during the alcoholic fermentation. By applying a large phenotypic survey in small scale fermentations, the production level of malic acid in seven grape juices confirmed the importance of the grape juice in the production of malic acid during the alcoholic fermentation. Beside the grape juice effect, our results demonstrated that extreme individuals able to produce up to 3 g/L of malic acid can be selected by crossing together appropriate parental strains. A multivariate analysis of the dataset generated illustrate that the initial the amount of malic acid produced by yeast is a determining exogenous factor for controlling the final pH of wine. Interestingly most of the acidifying strains selected are particularly enriched in alleles that have been previously reported for increasing the level of malic acid at the end of the alcoholic fermentation. A small

set of acidifying strains were compared with strains able to consume a large amount of malic acid previously selected. The total acidity of resulting wines was statistically different and a panelist of 28 judges was able to discriminate the two groups of strains during a free sorting task analysis.

2 Key words

pH, malic acid, breeding, acidity perception, wine yeast

3 Highlights

- The initial amount of malic acid of grape juices is a major factor affecting the metabolism of malic acid by *Saccharomyces cerevisiae* strains
- By implementing a breeding program several strains producing more than 3 g/L of malic acid have been selected paving a new avenue for managing wine acidity during the alcoholic fermentation.
- The impact of some QTLs linked to malic acid has been validated in high producer strains
- Strains that produce or consume high amount of malic acid during the alcoholic fermentation differently impact the acidity perception of the resulting wines

4 Introduction

Climate change is a direct consequence of the global warming and accentuates the greenhouse effect with a drastic impact for many agricultural productions including vine (IPCC 2020; van Leeuwen and Darriet 2016). It is well documented that temperature increase modifies vine phenology (van Leeuwen and Darriet 2016) and affects grape juice composition by increasing sugar concentration (Coombe 1987; Poudel et al. 2009; Nistor et al. 2018), and decreasing titratable acidity (Arrizabalaga et al., 2018; Coombe, 1987; Mira de Orduña, 2010; Nistor et al., 2018; Poudel et al., 2009; van Leeuwen and Darriet, 2016) due to a drastic drop of malic acid concentrations. In turn, the resulting wines have a greater alcohol content (Mira de Orduña 2010; van Leeuwen and Darriet 2016) and are also affected in their aroma composition and their sensorial properties (Bureau et al., 2000).

These combined modifications constitute a great challenge for the wine industry that have to adapt winemaking processes and enological products for maintaining wine quality and stability despite the drastic changes of grape juice composition. Concerning the correction of acidity drop, the addition of chemical products (tartaric or malic acids) is efficient but represents an expensive solution. In addition, the context of a new green deal promotes microbiological and biotechnological solutions as a good alternative for wine acidification.

Alcoholic and malolactic fermentations modify the equilibrium of organic acids by the metabolic activity of wine microorganisms as reported by many authors (Redzepovic et al., 2003; Volschenk et al., 2006, Su et al., 2014). However, wine microorganisms mostly reduce the wine acidity by consuming malic acid through malo-alcoholic (Volschenk et al., 1997) and malolactic fermentation (Lonvaud-Funel, 1994). Quite recently, the species *Lachancea thermotolerans* has been proposed for correcting wine acidity due to its ability to produce significant amount of L-lactic acid during the alcoholic fermentation (Hranilovic et al., 2021, 2018). This non-conventional yeast species can be mixed with *Saccharomyces cerevisiae* starters and constitutes a promising solution for managing wine acidity. However, the development of mixed culture in winemaking conditions requires an accurate management of alcoholic fermentation which is sometime not compatible with a routine application in the cellar.

Beside *L. thermotolerans*, studies reporting a microbiological acidification of wine matrix without an elevation of acetic acid are quite rare. Within the *Saccharomyces* genus the species *Saccharomyces uvarum* has been described for its acidification properties (Castellari et al., 1994; Coloretti et al., 2002) that was related to the psychrophilic property of this species. This feature is mostly shared by hybrids between *S. cerevisiae* and *S. uvarum* (Origone et al., 2018) that have been proposed as a solution for coping both acidity drop and high sugar levels in grape juices (da

Silva et al., 2015; Origone et al., 2018). Concerning *S. cerevisiae*, former studies described the contribution of strains on malic acid production.

Malic acid plays a central role in yeast metabolism being a key molecule of glyoxylate shunt (Regev-rudzki et al., 2009) and tricarboxylic acid cycle as extensively reviewed by (Saayman and Viljoen-Bloom, 2006). Schwartz and Radler deeply investigated the physicochemical factors influencing the production of malic acid by *S. cerevisiae* reporting production levels of nearly 1 g/L in enological compatible conditions (Schwartz and Radler, 1988). A large screening of natural isolate reported that few strains isolated in Spain or in China are able to produce up to 1 g/L of malic acid (Yéramian et al., 2007) and to increase titratable acidity (Chen et al., 2022). However, this interesting feature has not been deeply exploited.

Recently, the identification of QTLs (Quantitative Trait Loci) influencing malic acid metabolism of *S. cerevisiae* during the alcoholic fermentation (Peltier et al., 2021) paves new avenues for the control of acidity using the main species used in alcoholic fermentation. Recently, we demonstrated that such QTLs can be efficiently used for enhancing the consumption of malic acid during the alcoholic fermentation. Starting from two F1 hybrids obtained from 4 enological strains we drastically increased the percentage of malic acid consumed by enological strain in two rounds of selection (Vion et al., 2021). This strategy could be useful for shortening malolactic fermentation by reducing the amount of malic acid to be degraded by lactic bacteria. However, the use of high malic consumer strains reduces wine acidity which is not suitable in the context of global climate change.

In the present study, we applied a phenotype driven breeding program aiming to select *S. cerevisiae* strains able to preserve the wine acidity. Starting from the parental strains used by ~~same genetic material than~~ Vion et al. (2021) new ~~many~~ strains able to produc~~eing~~ up to 3.2 g/L of malic acid at the end of the alcoholic fermentation were ~~selected~~obtained. These high malic producer strains are statistically enriched in QTL's alleles that preserve wine acidity constituting a new genetic resource for managing acidity in wine. A preliminary comparison of malic acid producer and consumer strains was carried out, the resulting wines can be clearly discriminated on the basis of the acidity perception in a *Sauvignon blanc* matrix.

5 Methods

5.1 Yeast strains, conservation, and propagation methods used

Yeast (*S. cerevisiae*) were propagated on YPD 2% (1% peptone, 1% yeast extract, 2% glucose) at 28°C in both liquid and plate cultures (2% agar). Long term storage at -80°C was achieved by adding one volume of glycerol to YPD 2% overnight cultures. All the strains are homozygous for the *HO* locus and are therefore diploids. The main yeast strains used were described in the Table 1 and are derived from the two F1-hybrids (M2×~~x~~F15 and SB×~~x~~GN) used in previous QTL mapping studies (Huang et al., 2014; Peltier et al., 2018b). Meiotic spore clones of SB×~~x~~GN (GS-56B, GS-13C, GS-8B) and of M2×~~x~~F15 (FM-8B, FM-1C, FM-3C) were used as parental candidates for achieving a breeding program aiming to increase the production of malic acid during the alcoholic fermentation. These strains have been previously characterized for their ability to produce small amounts of malic acid (Vion et al., 2021). A first F1-hybrid was obtained by crossing the strains GS-8b and FM-1C. A second hybridization cycle was achieved using the monosporic clones AC1-13, ~~AC1-42,~~ AC1-46, AC1-191, AC1-217. Finally, three *S. cerevisiae* strains C1-4, M2-9, and M6-7 previously described to produce mild amount of malic acid during the alcoholic fermentation (Yéramian et al., 2007) were used as control.

Table 1. *S. cerevisiae* strains used

Strain	Description	Reference
ZF15	Zymaflore F15	Laffort, FRANCE
ZF10	Zymaflore Fx10	Laffort, FRANCE
M2	parental strain, a meiotic spore clone from Enoferm M2 (Lallemand, Canada)	(Huang et al., 2014)
F15	parental strain, a meiotic spore clone from Zymaflore F15 (Laffort, France)	(Huang et al., 2014)
GN	parental strain, a meiotic spore clone from Zymaflore VL1 (Laffort, France)	(Peltier et al., 2018)
SB	parental strain, a meiotic spore clone from Zymaflore BO213 (Laffort, France)	(Peltier et al., 2018)
M2xF15	F1-hybrid (M2 × F15)	(Huang et al., 2014)
SBxGN	F1-hybrid (SB × GN)	(Peltier et al., 2018)
C4-2	Yeast strain isolated from Cordoba region, mild malic acid producer	(Yéramian et al., 2007)
M2-9	Yeast strain isolated from Madrid region, mild malic acid producer	(Yéramian et al., 2007)
M6-7	Yeast strain isolated from Madrid region, mild malic acid producer	(Yéramian et al., 2007)
GS-56B	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
GS-13C	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
GS-8B	meiotic clone of SB×GN, mild malic acid producer	(Vion et al., 2021)
FM-8B	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
FM-1C	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
FM-3C	meiotic clone of M2×F15, mild malic acid producer	(Vion et al., 2021)
AC1	F1-hybrid (GS-8b x FM-1C)	this work
AC1-191	meiotic clone of AC1, malic acid producer strain	this work
AC1-13	meiotic clone of AC1, high malic acid producer	this work
AC1-217	meiotic clone of AC1, high malic acid producer	this work
AC1-46	meiotic clone of AC1, high malic acid producer	this work
AC2	F1-hybrid (AC1-191 × AC1-46)	this work
AC3	F1-hybrid (AC1-13 × AC1-217)	this work
AC2-1	meiotic clone of AC2, high malic acid producer	this work
AC2-467	meiotic clone of AC2, high malic acid producer	this work
AC3-22	meiotic clone of AC3, high malic acid producer	this work
FMGS_889	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS_71	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS_263	meiotic clone of FMGS, high malic consumer	(Vion et al., 2021)
FMGS2_107	meiotic clone of FMGS2, high malic consumer	(Vion et al., 2021)
FMGS2_714	meiotic clone of FMGS2, high malic consumer	(Vion et al., 2021)

5.2 Spore mating and isolation

F1-hybrids (AC1, AC2 and AC3) were obtained by spore to spore mating on YPD 2% using the method previously described (Marullo et al., 2009). After few hours, zygotes (trilobed shaped) were observed and manually isolated; their genotype was confirmed by a SNP genotyping (see below). Each F1-hybrids was sporulated by cultivating 10^8 cells in 5-ml of potassium acetate 1%

(ACK) during three days at 24°C. After a digestion by cytohelicase (2 mg/L) (Sigma, France) spores were isolated by micromanipulation or by spore purification according to the procedure previously described (Vion et al., 2021). As the yeast strains of this study are homozygous for the *HO* gene, colonies derived form a spore are considered as diploids and fully homozygous.

5.3 Cell culture and DNA extraction in microplates

Strains were cultivated in 96-Well microplate containing 200 µL of YPD coated with gaz-permeable sheets allowing CO_2 production. Genomic DNA was extract by using a Li-Ac SDS protocol previously adapted for microplate handling (Chernova et al., 2018). Broadly, 5×x10^{6} cells (200µl of overnight cultures) were centrifuged and incubated with 50 µL of 200mM LiAc/1% SDS at 70°C for 5 min. Genomic DNA was extracted by mixing cell lysates with 150µL of pure ethanol and vortexed for 15 s. After a briefly spin (2 min, 4000 rpm) the pellet was washed with 70% ethanol and was the solubilized in 200 µL of milliQ water at 60°C for 5 min.

5.4 Mass ARRAY genotyping of selected clones

According to a recent publication (Peltier et al., 2021), QTLs that possibly enhance the malic acid production in the SB×xGN background were used. These QTLs have the same localization than those used by Vion *et al* for enhancing the consumption of malic acid (Vion et al., 2021). For optimizing the genotyping procedure, the allelic form tracked in the present study were specific to a malic consumption feature. In addition, for most QTLs a pair of SNPs narrowing the region was used in order to increase the quality of the genotyping. The genotypes of each strain founder (M2, F15, SB, GN) and of the parental strains of hybrids AC1, AC2, and AC3 are given in the Table S1. The genotyping of these SNP was carried out by using Mass ARRAY technology, which allows large multiplex SNP detection (Gabriel et al., 2009). Less than 15ng of DNA were used for genotyping the strain. Primers were design using the tool MassARRAY® Assay Design (version 4.0.0.2) and amplified fragment of less than 120 bases having a mass difference between 4545.0 and 8633.7 Da. The genotype of selected progenies of hybrids AC1 (37), AC2 (28) and AC3 (29) was carried out in the same multiplex. All the spore clones genotyped have a fully homozygous genotype consistent with their meiotic progeny nature (Table S2).

5.5 Alcoholic fermentation assays

5.5.1 Grape juices

The grape juices Merlot 2013 and Merlot 2018 (M13 and M18) and Cabernet Sauvignon 2013, 2014, 2015, 2017 and 2019 (CS13, CS14, CS15, CS27 and CS19) used were provided by Vignobles Ducourt (Ladaux, France) and stored at -20˚C. Red grape juices have been thermically treated by the cellar and only the liquid phase was used. Grape juices were sterilized by membrane filtration and their nitrogen content were adjusted to 200 mg/L N in order to provide a sufficient nitrogen nutrition. The composition of grape juice in terms of fermenting sugars, nitrogen sources and malic acid content as well their pH are listed in Table S3 for original and corrected musts.

5.5.2 Small scale fermentation monitoring

Small-volume alcoholic fermentations were implemented in screwed vials fermentations according to the general procedure described in (Peltier et al., 2018a). Briefly, 20 mL-screwed vials (Fisher Scientific, Hampton, New Hampshire, USA) were filled with 12.0 ml of filtered grape must were tightly closed with screw cap-magnetic (Fisher Scientific, Hampton, New Hampshire, USA) perforated by hypodermic needles for allowing the CO_2 release. Vessel was inoculated by 2××10^6 viable cell.mL^{-1} precultured in liquid YPD for 24h. Cellular concentration and viability was estimated by flow cytometry using a Cytoflex apparatus (Beckmann Coulter, Indianapolis, USA). Fermentation took place at 24°C and was each vial shaken at 175 rpm by an orbital shaker (SSL1, Stuart, Vernon Hills, Illinois, USA). The fermentation kinetics was estimated by monitoring manually (2–3 times per day) the weight loss caused by CO_2 release using a precision balance with automatic weight recording (Mettler Toledo, Greifensee, Switzerland). The amount of CO_2 released according to time was modeled by local polynomial regression fit allowing the estimation of parameters related to fermentation (Peltier et al., 2018a) : the maximal amount of CO_2 released (CO_2max in g.L^{-1}), the lag phase (h), the time to release 35 and 80 g/L of CO_2 after subtracting the lag phase (*t35* and *t80* in h). The CO_2max value was divided by the amount of sugar consumed for computing the *yield* of the fermentation.

5.6 Enzymatic assay of wine

At the end of the alcoholic fermentation, a sample volume of 800 µL was manually transferred in Micronics tubes (Novazine, Lyon, France, ref: MP32033L) and stored at -20˚C. The concentrations of the following organic metabolites were measured: acetic acid, glycerol, malic acid, pyruvate, acetaldehyde and total SO_2 using the respective enzymatic kits: K-ACETGK, K-GCROLGK, K-LMAL-116A, K-PYRUV, K-ACHYD, K-TSULPH, K-SUCCI (Megazyme, Bray, Ireland) following the instructions of the manufacturer. Glucose and fructose were assayed by using the enzymatic method previously described. All the enzymatic assays were performed by a robotic platform using the Bordeaux metabolomics facilities (http://metabolome. cgfb.u-bordeaux.fr/). The Malic Acid Consumption (MAC%) is the ratio of malic acid consumed and was computed according to the following formula:

$$MAC\% = \frac{([\text{L}-\text{malic acid}]_{initial} - [\text{L}-\text{malic acid}]_{final})}{[\text{L}-\text{malic acid}]_{initial}} \times 100$$

5.7 Sensory analysis

5.7.1 Wines preparation

Twelves strains were used for assessing the sensorial impact of malic acid production/consumption on a Sauvignon Blanc wine. The wine was produced at the laboratory scale from a Sauvignon blanc grape juice harvested in 2020 in the Bordeaux area (Vignobles Ducourt, Ladaux, FRANCE) containing 35 mg/L of total SO_2. The composition of this grape juice is given in Table S3. Fermentations were carried out in 1.2L bioreactors initially incubated at 18°C. At the fifth day of fermentation, 30 mg N/L of nitrogen (Ammonium sulfate) were added for enhancing the fermentation kinetics. At the seventh day, the temperature was rise up 22°C for ensuring a rapid achievement of the fermentation. Bioreactors were shaken daily for 60 sec with a magnetic stirrer in order to resuspend yeast cells and the CO_2 production rate was followed by manual weighting. At the end of the fermentation (between 11 and 13 days), wines were settled for a week at 4°C and yeast lees were resuspended each 48 hours for preserving wines from oxidation. A week before tasting wines were sulfited (50 mg/L) and transferred in tapped bottles and stored at 12°C.

5.7.2 Sensory analysis conditions

Sample assessments were undertaken in a temperature-controlled room, using covered, white ISO glasses (ISO 3591:1977) containing about 25 mL of liquid, coded with random three-digit numbers. Sessions lasted approximately 15 min. Judges were selected on the basis of availability and interest. A total of 28 volunteers belonging to the Laffort and ISVV staff and showed different levels of expertise in sensory analysis but representing different domains or expertise of the wine industry. The ages of the panelists ranged from 25 to 50 years old, and the panel was constituted by 55% of women.

5.7.3 Preliminary control of acidity perception by the panel

A preliminary session was carried out using a commercial *Sauvignon blanc* wine (Mas de L'oncle 2019, Languedoc Roussillon) with spiked concentrations of malic acid. The aim of this preliminary session was to control that all the judges correctly perceive malic acid contribution in a Sauvignon blanc wine. The panelists performed a ranking test (ISO 8587:2007), sorting four samples according to their acidity from least to most intense. The sums of ranks for increasing concentrations of malic acid (1.0, 1.7, 2.4 and 3.0 g/L) were 43, 60, 66, and 81, respectively. A Page test (Conover, 1999) assuming the increasing concentration of malic acid in wine confirmed that judges have a correct perception of acidity.

5.7.4 Free sorting task

Two days after the control session a descriptive sorting task (Chollet et al., 2014) was used to characterize the olfactory and gustatory differences between 12 Sauvignon Blanc wines produce in the laboratory. The protocol of the sorting focused on both a full evaluation (olfactory and gustatory). The panelists (n=28) were asked to constitute groups of wines according to their acidity. Judges must constitute at least two groups of wines and each group must contain at least two wines.

5.8 Use of former datasets for figures

Two previously published datasets were used for illustrating the efficiency of this breeding programs. In such dataset phenotypes were measured in a Merlot 2015 grape juice in the same conditions and in the same grape juices allowing to compare the data. Malic acid consumption of 35 enological strains and 193 progeny clones of M2××F15 or SB××GN hybrids were extracted form (Emilien Peltier, Bernard, et al. 2018) and (Vion et al., 2021). These distributions were presented in the Figure S1.

5.9 Statistical analyses

All the statistical and graphical analyses were done using R software (R Core Team, 2018). Median differences were assayed using the non-parametric Kruskal test (*agricolae package*). When required, multiple comparisons were achieved using a post-hoc test using the criterium Fisher's least significant difference with Benjamini-Hochberg corrected p values (alpha=0.05). The effect of grape juice and strains on MAC% was estimated by a two-way analysis of variance (*car* package) using the data collected for the strains SB, GN, AC2 and AC3 in the grape juices CS14 and CS15. The linear model used is expressed in the following formula

$$MAC\% = \mu + Strain_i * Must_j + \varepsilon$$

Where μ is the average value observed in the dataset, *Strain* is the nature of the strain taking four levels *i*, *Must* is the nature of the grape juice taking two levels *j* and ε is the residual error of the model. The analysis of variance was carried out after checking the normal distribution of the residues of the model (Shapiro test) and the homoscedasticity of the variance (Levene test).

To evaluate the contribution of different metabolites on final pH of wine a multiple linear regression model was carried out. The initial model computed is expressed in the following formula.

$$pH_{wine} \sim pH_{must} + [malic\ acid]_{wine} + [acetic\ acid]_{wine} + [succinic\ acid]_{wine} + [residual\ sugar]_{wine} + \varepsilon$$

Where ε represents the error of the model. A subsequent analysis of variance of the model was applied in order to estimate which variables have a significative impact on the total variance observed.

Page test was carried out for controlling the correct perception of acid malic in a spiked Sauvignon blanc. The L value obtained for 28 judges was 685 with four wines presented. The threshold L' value was equal to 4.15 allowing to reject the null hypothesis. L' is computed assuming the normal distribution of the samples according to the following formula:

$$L' = \frac{12 \times L - 3 \times J \times P(P+1)^2}{P(P+1) \times \sqrt{J \times (P-1)}}$$

Where *J* is the number of judges (n=28) and *P* is the number of wines presented (n=4). Multidimensional scaling was assed using the R package *smacof* (de Leeuw and Mair, 2009) Dissimilarities between samples were analyzed using the cooccurrence of wines belonging to the same group as proposed by (Cox and Cox, 2001).

6 Results

6.1 Phenotypic characterization of parental candidates

The aim of the present work was to increase the production level of malic acid by *S. cerevisiae* strains in order to prevent de drastic drop of acidity caused by climatic change.

Six monosporic clones (GS-56B, GS-13C, GS-8B, FM-8B, FM-1C, FM-3C) constitutes the starting genetic material of this study. They were obtained from the F1-hybrids M2××F15 and SB××GN. Their MAC% in a Merlot grape juice of 2015 was previously reported (Vion et al., 2021). These strains outcompeted a panel of 35 commercial starters and some of them had a weak production of malic acid at the end of the alcoholic fermentation (Figure S1). In order to better characterized such strains, the amount of malic acid at the end of the alcoholic fermentation of seven red grape juices characterized by a wide range of initial malic acid content (1.45 to 4.40 g/L) was measured. The fermentation characteristics of such strains were compared to some reference ones including two commercial starters (Zymaflore Fx10 and Zymaflore F15), three malic acid producer strains (C1-4, M2-9 and M6-7) (Yéramian et al., 2007) and the strain FMGS_889 which is a high malic acid consumer strains selected by (Vion et al., 2021). Fermentations were carried out in duplicate, and traits collected are given in the Table S4.

In order to compare all the conditions, the MAC% was computed as described in material and methods. Average MAC% values measured in the seven grape juices wasis significantly different according to the yeast strain (Kruskal test, pval=3.7×-10^{-9}). A Post-hoc test using the criterium Fisher's LSD revealeds that FM-8B and FMGS-889 wereare statistically different to two commercial Fx10 and F15 (Figure 1A). However, most of the acidifier strains cannot be statistically differentiated due to a strong variability of MAC% observed within the grape juice fermented. This significative effect of grape juice is confirmed by Kruskal test, p= 9.3×-10^{-14} and subsequent post hoc analysis (Figure 1B). The impact of grape juice wais clearly correlated to the initial concentration of malic acid (Spearman's correlation test: rho=0.60, pval<2.2×-10^{-16}). Interestingly, for low initial malic acid concentrations (1.41 g/L in M-2018 and 2.02 in CS-2014) average MAC% reaches negative values (malic acid production) suggesting that such matrices are relevant for the selection of acid malic producing strains.

In order to better characterize the parental candidates, we narrowed our analysis on these two grape juices. The Table 2 provides the average phenotypic values measured for 13 quantitative traits in M-2018 and CS-2014 with the attribution of statistical groups (Kruskal test with *post hoc* group attribution. The average amount of malic acid at the end of the alcoholic fermentation ranged between 1 g/L and 2.6 g/L for the extreme strains FMGS-889 and GS-8B. Significative differences between strains were~~are~~ also observed for other organic acids such as acetate but were~~are~~ not correlated to the production of malic acid (Figure S2).

Table 2. Average phenotypic values of initial acidifying strains.

Strain	MAC%	pH wine	Acetic acid (g.L-1)	Malic acid (g.L-1)	Glycerol (g.L-1)	SO2 (mg.L-1)	Succinic acid (g.L-1)	CO_2 max (g/L)	lp (h)	V50-80 (g.h-1.L-1)	t35g (h)	t50g (h)	t80g (h)
GS-8B	-52.1^{f}	3.6	0.10^{d}	2.6^{a}	9.2^{a}	8.6	1.1^{abc}	107.4	8.0	0.91^{de}	38.5^{bc}	55.3^{abc}	109.8^{ab}
FM-1C	-42.2^{f}	3.6	0.20^{abcd}	2.5^{ab}	8.9^{ab}	7.2	1.5^{a}	110.1	6.5	0.93^{de}	38.8^{bc}	55.3^{bc}	106.8^{ab}
FM-8B	-41.5^{f}	3.6	0.20^{abcd}	2.4^{ab}	8.2^{abcd}	6.1	1.1^{abc}	110.8	7.3	1.13^{bc}	38.0^{bc}	53.0^{bcd}	96.0^{bc}
C1-4	-30.7^{ef}	3.7	0.13^{bcd}	2.3^{abc}	6.5^{d}	6.8	1.1^{abc}	110.0	6.8	1.05^{cd}	34.8^{cd}	50.3^{cde}	96.8^{b}
M6-7	-24.1^{def}	3.7	0.18^{bcd}	2.1^{abc}	6.8^{cd}	4.8	1.4^{ab}	110.9	6.3	0.81^{e}	45.3^{a}	68.0^{a}	132.8^{a}
GS-56B	-14.9^{bcde}	3.7	0.14^{abc}	2.0^{bcd}	7.3^{bcd}	8.9	0.8^{c}	110.4	7.0	0.85^{e}	41.8^{ab}	59.0^{ab}	116.5^{a}
M2-9	-13.0^{cde}	3.7	0.23^{ab}	2.0^{abcd}	7.3^{abcd}	7.5	1.0^{abc}	110.2	6.3	0.85^{e}	40.8^{ab}	58.8^{abc}	116.5^{a}
FM-C3	-4.7^{bcd}	3.7	0.27^{cd}	1.8^{cd}	8.7^{abc}	10.9	1.4^{ab}	111.3	7.0	1.32^{ab}	32.5^{d}	45.5^{def}	80.8^{cd}
GS-13C	-1.3^{abc}	3.7	0.12 a	1.7^{cde}	6.1^{d}	5.2	0.9^{bc}	112.0	7.3	0.95^{cde}	39.3^{bc}	57.0^{abc}	108.5^{ab}
Zymaflore FX10	0.6^{abc}	3.8	0.31^{abc}	1.7^{cde}	7.3^{abcd}	8.6	0.8^{bc}	111.8	8.8	1.48^{a}	29.3^{d}	41.0^{f}	73.8^{d}
Zymaflore F15	5.7^{ab}	3.8	0.23^{a}	1.6^{de}	6.1^{abcd}	5.7	1.3^{abc}	112.0	8.0	1.46^{a}	31.3^{d}	44.3^{ef}	77.0^{d}
FMGS-889	45.0^{a}	4.0	0.19^{abcd}	1.0^{e}	8.0^{abcd}	8.8	1.2^{abc}	113.7	9.0	1.55^{a}	32.0^{d}	45.3^{def}	77.8^{d}

6.2 Phenotypic optimization of malic acid production by a breeding program

The two best malic acid producer strains GS-8B and FM-1C were crossed by pairing their spores using a micromanipulator and the F1-hybrid AC1 was isolated. Forty-one progeny clones of the AC1 hybrid were recovered and analyzed for their MAC% in the CS14 grape juice that positively promotes malic acid production (Figure 1B). The AC1 hybrid showed a negative MAC% value similar to those of parental strains (-74.2 %) (Figure 2A). In this grape juice this MAC% value represent~~s~~ed a final malic acid production of 1.5 g/L. The hybrid AC1 was sporulated and 41 progeny clones were isolated. All of them reached negative MAC% ranging between -23.7% and -146.8 %. Four strains AC1-13, AC1-191, AC1-46 and AC1-217 were selected for achieving a second round of phenotypic optimization. The hybrid AC2 (AC1-191 ×~~x~~ AC1-46) and AC3 (AC1-13 ×~~x~~ AC1-217) were isolated by using the micromanipulator~~following the same procedure than for AC1~~. The MAC% of these new F1-hybrids was clearly improved respect to AC1 with values of -130.7% and -149% for AC2 and AC3, respectively. To our knowledge, such levels of malic acid production (up to 3.0 g/L) were never reported in an enological context and constitute a technological rupture compared to the phenotypic variability of industrial starters that was~~is~~ illustrated by the four founder strains of this study (SB, GN, M2 and F15) (Figure 2A).

The phenotypic segregation of MAC% of AC2 and AC3 hybrids was measured for a small set of progenies (AC2=14, AC3=19) and compared to the 41 AC1 progenies (Figure 2B). Both populations showed an average MAC% significantly lower than the AC1 population (Wilcoxon test, alpha= 0.05). The phenotypic variance of each progeny was~~is~~ statistically similar (Variance F-test, alpha= 0.05). For AC1 progeny, 70% of clones had~~ve~~ a MAC% value higher than the F1-hybrid while for AC2 and AC3 the distribution was~~is~~ mostly centered around the respective F1-hybrid value. This second segregation step allowed the selection of strains with a -160% lower MAC% ~~lower -160%~~ representing a production of malic acid of 3.2 g/L.

In order to confirm these findings, MAC% was assayed in a second grape juice (CS15) using additional progenies of AC2 (n=26) and AC3 (n=22). In this grape juice, AC2 and AC3 hybrids as well as the control strains GN and SB produced less malic acid since their MAC% was significantly higher than in CS14 grape juice. This effect was quantified by two-way ANOVA (Figure S3) underlining the impact~~effect~~ of the grape juice discussed above. Despite this environmental effect a similar phenotypic segregation was observed for both hybrids and some progenies produced more malic acid than their respective hybrids (Figure 2C).

6.3 Multivariate analysis of acidic strains behavior

The large dataset of 484 fermentations characterized by 15 quantitative variables was generated (Table S5) offering the opportunity to investigate traits linked to the strong production of malic acid of acidic strains. This represents the phenotypic values of 127 strains phenotyped in CS14 and/or in CS15 at least in triplicate. The phenotypic variability of this dataset was figured out in a PCA analysis (Figure 3A). The first two components represent 60% of the total inertia and clearly discriminate the hybrid background and the grape juice origin. Correlation circles indicates that the content of malic acid drives the inertia in the same direction that t50 and t80 but was mostly decoupled to succinic acid, glycerol, CO_2, and acetic acid production (Figure 3B). Correlation analysis~~es~~ (Figure S4) confirmed the strong positive correlation between kinetics parameters and malic acid content suggesting that acidifying strains have some difficulties to ferment efficiently.

This was~~is~~ also corroborated by the strong correlation between malic acid production and residual sugars (rho= 0.45, pvalue= 2×~~x~~10^{-26}) that are highlighted by the 123 stuck fermentations (open symbol) shown on the Figure 3A. The distribution of malic acid produced in completed and stuck fermentations was figured out FigureS5. Stuck fermentations mostly occurred in the CS15 grape juice which is characterized by a lower CO_2 yield than observed in CS14 (0.427 vs 0.456 g/g, Wilcoxon test, alpha=0.05). In CS15, the production level of succinic acid also significantly higher than observed in CS14 (1.61 vs 0.52 g/L, Wilcoxon test, alpha=0.05) contributing to separate the two grape juices on the PCA. As expected, the pH value of the resulting wine wasis negatively correlated with malic acid content in both grape juice (rho= -0.40 and -0.69 for CS14 and CS15, respectively, alpha=0.001) (Figure 3C). This demonstrates that the selection of strains controlling malic acid concentration wasis quite efficient for modulating the pH. On average, the dynamic range of final pH of wine wasis 3.41-3.82 for CS14 and 3.52-4.01 for CS15.

Since other organic acids than malate would influence the wine acidity, we applied a linear model for predicting the quantitative factors statistically linked to the final pH. Five quantitative parameters were used for building the model: the initial pH of the matrix, the organic acids produced by yeast (malic, acetic, succinic) as well as the residual sugar in the grape juice were interrogated. Except succinic acid, all the parameters significantly contributed to the multiple linear regression of the final pH according to the following equation.

$$pH = 0.982 - 0.0058\ x\ [Malic\ acid]p + 0.1287\ x\ [Acetic\ acid]p + 0.678\ x\ pHi - 0.0013\ x\ [residual\ sugar]$$

The adjusted R square of the model is 0.392 (pvalue $<2\times x10^{-16}$), suggesting that a large part of the explaining factors was not identified as illustrated Figure 3D. A variance analysis of this linear model indicateds that an important part of final pH variation wais explained by initial pH of grape juice and the malic acid produced by yeast (25 and 13%, respectively). In contrast, acetic acid, and residual sugars accounted for a very minor part of the model.

6.4 Malic acid producer strains are enriched in ACIDIC alleles

In a previous study (Vion et al., 2021), we applied a marker assisted selection using eleven QTLs related to MAC% and mapped on the SB××GN genome (Peltier et al., 2021). The penetrance of such QTLs was only partial due to complex genetic interactions. Since SB and GN genome constitute 50% or the AC's hybrids we hypothesized that the most malic acid producer strains should be enriched in alleles conferring low MAC% values. The QTLs linked to MAC% were given in Table S1 and were tracked with 19 mass array markers suitable to interrogate all the genotypes in the same assay. Most of the QTLs wereare narrowed by a pair of markers in order to increase the robustness of genotyping.

The Figure 4A shows the relative position of such markers on yeast chromosomes and specify the contribution of SB and GN alleles respect to the MAC%. Alleles promoting malic consumption were named DEMALIC while alleles promoting malic acid production were named ACIDIC. The Figure 4B indicated the SNP call of both DEMALIC and ACIDIC alleles that are represented by green and red, respectively. Among the 19 ACIDIC alleles, 14 are derived from the parental strain GN encompassing nine of the 11 QTLs tracked. The allele inheritances for the four parental strains and the hybrids AC1 to AC3 are also detailed in the Figure 4C. Homozygous alleles are represented by green or red shaded area and can be considered as fixed in the respective hybrid's progeny. For instance, the acidic allele of the QTLs XI_382 corresponding the gene *MAE1* is present in all the AC's hybrids of this study. Conversely, for the loci VII_425 and VI_426, the GN allele was lost indicating that the acidifying copy of *PNC1* is not present in the progeny analyzed (Figure 4C). Among the 118 progeny strains phenotyped in this work, 94 were randomly genotyped for their inheritance for these 19 markers using a mass array strategy (see methods). The genotype of each strain i~~wa~~s given in the Table S2.

As shown in the Figure 5A, the number of ACIDIC alleles was negatively correlated with MAC% values. However, this number was~~is~~ quite different according to the progeny genotyped. Indeed, the number of segregating markers was~~is~~ different according to the hybrid. In the AC1 progeny the number of ACIDIC alleles ranged between 2 and 13 while AC2 and AC3 progenies had~~ve~~ fixed a greater number of ACIDIC alleles (between 10 and 14) (Figure 4C). Two QTLs (IV_28 and VII_480) segregated in all the progenies. Their effect on MAC% was confirmed in each AC's hybrid as illustrated in the Figure 5 B and C. Interestingly, this effect was~~is~~ much higher in the AC2 and AC3 backgrounds where numerous other acidic alleles were ~~are~~ present. This result illustrated~~s~~ modifications of QTL penetrance according to the genetic background.

6.5 Malic acid consumer and producer strains drastically impact the acidity perception of wine

The organoleptic impact of strains able to modulate the amount of malic acid at the end of the alcoholic fermentation was evaluated by the sensory analysis of a Sauvignon blanc wine. In order to have a representative image of the technological potential of such strains, five high malic acid producers and five high malic acid consumers were selected. The producer strains are progeny clones of hybrids AC1, AC2 and AC3 while the consumer strains are progeny clones of FMGS and FMGS2 hybrids previously described by Vion *et al.* 2021. Two commercial starters (BO213 and RB4, Laffort, France) were added to complete this panel. Those commercial starters showed the highest difference in MAC% among 32 commercial starters tested (Peltier et al., 2018a). Each strain was fermented in a single bioreactor of 1.2L according to standard enological procedure, control of temperature, nitrogen management and preservation to oxidation (see methods). The final composition of wines is presented in the Table 3.

Table 3 Main characteristics of wine evaluated by sensory analysis

Strain	Group	Ethanol (%, v/v)	Total acidity (g/L H_2SO_4)	pH	Malic acid (g/L)	acetic acid (g/L)	Glycerol (g/L)	Residual sugar (g/L)
AC1_191	high producer	12.27	4.23	3.29	3.71	0.13	8.50	<2
AC2-1A	high producer	12.25	4.40	3.27	3.90	0.12	8.56	<2
AC3-22	high producer	12.39	3.71	3.34	3.34	0.29	6.75	<2
AC1-217	high producer	12.22	4.29	3.29	3.57	0.26	8.81	<2
AC2-467	high producer	12.16	4.74	3.24	4.01	0.11	8.81	<2
FMGS2-714	high consumer	12.34	3.51	3.4	2.30	0.74	8.63	<2
FMGS-889	high consumer	12.42	3.02	3.44	2.00	0.27	8.37	<2
FMGS-71	high consumer	12.28	3.22	3.42	2.23	0.24	8.63	<2
FMGS2-107	high consumer	12.28	3.21	3.44	2.04	0.49	8.66	<2
FMGS-263	high consumer	11.57	3.94	3.35	3.38	0.36	6.79	>5
BO213	starter	12.40	3.68	3.33	3.36	0.29	6.69	<2
RB4	starter	12.36	3.61	3.37	2.98	0.40	7.22	<2

As expected, high producer and consumer strains showed significative differences in malic acid content with a direct impact on pH and total acidity (Figure 6A) which is a good indicator of the overall acidity of wines (Plane et al., 1980). Other traits such as residual sugar, acetic acid, glycerol, or ethanol production were not significantly different between groups underlining a relative variability of strains belonging to the same group.

The resulting wines were presented to a panel of 28 tasters previously trained to appreciate the acidity in a Sauvignon Blanc wine. Dissimilarities between samples were analyzed using non-metric multi-dimensional scaling (MDS) allowing to figure out the perceptive proximities between wines on a map. The average stress value of the analysis was 0.051 which is significantly lower than explained by chance for 28 tasters and 12 wines (FDR of 5% 0.064). The two-dimensional MDS configuration reveals (Figure 6B) that the wines were grouped according to the type of the strain and discriminates more specifically the malic acid consumers and producers. Such approach demonstrated~~s~~ that wines had different acid profiles that were clearly perceived by the tasters.

7 Discussion

7.1 The nature of the grape juice and yeast strains are determining factors to modulate the malic acid level and final pH of wines.

Our findings shed light on the impact of the grape juice on the production of malic acid by *S. cerevisiae* strains corroborating previous studies (Delcourt et al., 1995; Vilanova et al., 2007). As previously suggested, the MAC% parameter is useful for comparing the behavior of various strains in different grape juices (Vion et al., 2021). By measuring this trait for 12 *S. cerevisiae* strains in seven grape juices we observed a strong effect of the grape juice origin (Figure 1B). This variability is partially related to the initial content of malic acid in the grape juice. The lowest the initial malic acid concentration, the highest the malic acid production by yeast. Since there is an obvious relation between malic acid concentration and pH of grape juice, it is not clear if the amount of malic acid of grape itself determines its production by yeast. Indeed, previous studies reported that malic acid production would be related to the pH of the medium with a similar concentration of malic acid (Schwartz and Radler, 1988). Malic acid is a diacid with two *pKa* of 3.46 and 5.10, respectively. Since the pH of grape juice ranges between 3.2 and 4.0, malic acid is mostly found in its ~~its~~ undissociated and monodissociated form (H_2M and HM). Once it enters into the cell, malic acid mostly takes its deprotonated configuration (M) since the intracellular *pH* of the yeast is around 5-6. A proton efflux ensured by active pumps maintains the intracellular pH between 5 and 6. Malic acid entrance in the cell has been described as happening by facilitated diffusion (Salmon, 1987) as *S. cerevisiae* does not possess any known diacid transporter. In such case, only the protonated configuration of malic acid (H_2M) can enter into the cell which represent only 50% of the total malic acid available at pH= 3.46. Since low pH values enhance the H_2M/HM ratio, more di-protonated form is consumed triggering a higher deacidification of the media. This explains why high MAC% values are generally reached in more acid grape juices. In contrast, mechanisms triggering the expulsion of malic acid outside the cell much are less documented. Salmon~~,~~ (1987) provides a preliminary evidence of a malic acid efflux dependent on glucose in resting cells suggesting the existence of an active transporter. However such transporter was never characterized (Casal et al., 2008) despite several efforts. In addition, other abiotic factors such as nitrogen composition, sugar content or vitamins concentrations would also impact the production and consumption of malic acid during the alcoholic fermentation (Delcourt et al., 1995; Vilanova et al., 2007; Schwartz and Radler, 1988). Beside the composition of grape juice, our findings demonstrate that yeast strain has a definitive impact on wine malic acid content. Although the range of MAC% in the population of commercial starters is not very large (0 to 45%), the selection of strains metabolically biased toward production or consumption of malic acid allows the modification of wine pH in a surprising manner. Indeed, the dynamic range of pH for the same initial grape juice was higher than 0.4 units which is highly relevant in an enological context. Indeed, such range of pH

Formatted: Subscript

Formatted: Subscript

Formatted: Subscript

Field Code Changed

variation drastically affect the antimicrobial activity of SO_2 (Divol et al., 2012), as well as the anthocyanins stability with a great influence on the color of red wines (Ribéreau-Gayon et al., 2006). The composition of 484 wines obtained during the breeding program allows the investigation of the main factors influencing the final pH of wine by a multiple linear regression approach. The main two contributors detected by the model and confirmed by an analysis of variance were the pH of grape juice and the malic acid produced by yeast. Although significative, the quite low adjusted R^2 (0.392) of the model suggested that many other compounds present in wines and/or produced by yeast should influence the final pH of wines. Among them potassium salt and tartaric acid concentrations could likely modulate the variability observed. The role of yeast strains regarding these factors remains to be explored.

7.2 Phenotypic characterization of wine yeast strains according to their malic acid production level.

The phenotypic characterization of progeny clones derived from three newly made F1-hybrids illustrates the efficiency of a breeding program for enhancing a complex trait. This result has been likely obtained because we crossed together extreme strains having distinct genetic origins. As previously documented for other quantitative traits of enological interest the phenotypic variability of the parental strains of this study (FM-1C and GS-8B) are mostly determined by distinct allelic pools (Peltier et al., 2018b). Therefore, their comparable MAC% (-75%) results from the combination of different alleles controlling different metabolic targets. Consequently, merging such allelic pools in a new hybrid background (AC1) allows the emergence of a wide genetic and phenotypic variability with some strains showing high transgressive values. Interestingly, the distribution of MAC% in AC1 progeny (Figure 2B) suggested that the inheritance of this trait is mostly non additive since only few progenies produce more malic acid than their F1-hybrid. However, once alleles controlling a high malic acid production have been fixed by crossing tail distribution progenies, it was possible to get many strains with very high acidifying properties. Thus, nearly 50% of the AC2 and AC3 progenies showed very low MAC% values offering the possibility to select a palette of malic acid producer strains that could segregates for many other traits not related to the malic acid production. For instance, the multivariate phenotypic analysis of 127 strains presented in Figure 3 showed that the three main secondary metabolites produced by yeast (acetic acid malic acid and glycerol) are not statistically correlated each togethers (Figure S4). In contrast, a weak positive correlation (rho=0.37) was found between malic and succinic acids suggesting than the production of both acids might be partially coupled which is consistent with their metabolic relationships (Camarasa et al., 2003).

This analysis also suggested a possible phenotypic trade-off between fermentation completion and malic acid production. In fact, the most acidifying strains have the slowest fermentation kinetics (high values of t35 and t80) and some of them did not complete the alcoholic fermentation (residual sugars > 2 g/L). Therefore, the production of high concentrations of malic acid during the alcoholic fermentation could result in a loss of fitness for *S. cerevisiae* explaining why most of the commercial starters empirically selected are malic acid consumers. Complementary studies with more phenotypes and more conditions will be necessary for confirming these preliminary conclusions.

7.3 Despite a partial penetrance QTLs previously mapped are statistically linked to the MAC% of malic producer strains

From a genetic point of view, the hybrids AC1, AC2, and AC3 and their relative progenies can be considered as mosaic strains derived from two parental strains (FM-1C and GS-8B). Such parental strains provided a large set of allelic variations enhancing the production of malic acid during the alcoholic fermentation that have been inherited from the four founder strains M2, GN, F15 and SB that well represent the genetic diversity of commercial starter population (Peltier et al, 2018). A fraction of this allelic pool has been previously mapped by a QTL analysis defining ACIDIC and DEMALIC alleles that modulate the production or consumption of malic acid, respectively. In order to evaluate what is the contribution of such alleles in the AC-hybrids, we randomly genotyped 94 progenies to find out statistical link between the inheritance of these alleles and the phenotype of the strains. The small number of progenies genotyped, and the high number of QTL tracked does not allow to test individually the effect of each QTL. However, some relations between genotype and phenotype have been elucidated. First, we found a negative correlation between the number of ACIDIC alleles and the MAC% whatever the background indicating that higher is the number of ACIDIC alleles stronger is the production of malic acid. Second, the global effect of two QTLs (VII_480 and IV_34) that played an important role in the MAC% determinism (Vion et al., 2021) was confirmed in new genetic backgrounds. Interestingly, such QTLs have a stronger effect in the AC2 and AC3 background which have already fixed many ACIDIC alleles respectively to AC1. This finding illustrates that major QTLs may have different level of penetrance due to the presence of other segregating alleles commonly called "modifiers" as review by several authors (Hou et al., 2016; Peltier et al., 2019; Yadav et al., 2016). The fixation of these buffering alleles in the AC2 and AC3 backgrounds allows a better expressivity of the QLTs VII_480 and VI_28 as shown in the Figure 5 B and C.

7.4 Selection of a new type of *S. cerevisiae* strains for coping the drastic drop of acidity in wines

Despite numerous stuck fermentations of high malic acid producers, this study allows to isolate eight strains that completed the alcoholic fermentation and produced more than 3 g/L of malic acid (Figure S5). This selection proposes a set of strains with a very contrasted phenotype respectively to those previously selected by Vion et al. 2021 enhancing considerably the technological palette of resulting wines by modulating the wine acidity during the alcoholic fermentation. This original feature is particularly relevant in an enological context for coping with drastic drops of acidity level observed in grape juices. In order to test the enological relevance of such strains, five malic producer strains were compared to five malic consumers strains as well as two commercial starters. Small scale vinifications were carried out on a Sauvignon blanc matrix that was evaluated by a sensory panel using a sorting task approach. After a preliminary test of the aptitude of the panel to discriminate wine acidity, the twelves wines were evaluated and the strains showing contrasted malic acid metabolism were well separated. This very preliminary sensory characterization agrees with the chemical analyses of wines tasted that statistically differs in their total acidity, their pHs and their malic acid content.

7.5 Concluding remarks

The climate change has drastic consequences of the acidity of grape juice and constitutes a pregnant problem for wine making. The use of selected microorganisms during the alcoholic fermentation is a promising solution to moderate this drop of acidity and preserve wine quality. In order to propose a reliable alternative to the use of non-conventional yeast species such as *L. thermotolerans* we applied a selection program aiming to obtained *S. cerevisiae* strains able to produce up to 3g/L of malic acid during the alcoholic fermentation. This result has been obtained by applying a classical breeding strategy narrowing the ability of strain to produce malic acid in different grape juices with a significant impact on wine pH and titratable acidity. This result has been obtained by the accumulation of acidifying alleles of several QTLs that have been previously identified. The large number of alcoholic fermentation achieved allow to discriminate biotic and abiotic factors controlling the final pH of wine in *S. cerevisiae* pure cultures. Beside a strong effect of the grape juice origin, the production of malic acid by yeast resulted strongly impacted wine pH which was not the case of succinic or acetic acid production. However, high concentrations of malic acid reached had negative consequences of fermentation kinetics due to the strong acidification of the fermenting matrix. From a sensory view point we demonstrated that acidifying strains could be clearly discriminated from the high malic acid consuming ones.

Formatted: Font: Not Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Not Italic

This work demonstrated that breeding programs based on QTLs selection is an efficient lever for selecting new starters able to manage wine acidity.

8 Author contributions

Conceptualization CV and PM; Formal analysis CV and PM; Funding acquisition IM, PM; Investigation CV, MM, MB, VF, BR; Methodology MM, ST; Resources NY, IM, PM; Software CV, PM; Supervision PM; Writing - original draft CV, NY, PM; Writing - review & editing ST, CV PM.

9 Conflict of interest

PM, CV, MB, and MM reports a relationship with Biolaffort company that includes full time employment.

10 References

Arrizabalaga, M., Morales, F., Oyarzun, M., Delrot, S., Gomès, E., Irigoyen, J.J., Hilbert, G., Pascual, I., 2018. Tempranillo clones differ in the response of berry sugar and anthocyanin accumulation to elevated temperature. Plant Sci. 267, 74–83. https://doi.org/10.1016/j.plantsci.2017.11.009

Bureau, S.M., Razungles, A.J., Baumes, R.L., 2000. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. J. Sci. Food Agric. https://doi.org/10.1002/1097-0010

Camarasa, C., Grivet, J.P., Dequin, S., 2003. Investigation by 13C-NMR and tricarboxylic acid (TCA) deletion mutant analysis of pathways of succinate formation in Saccharomyces cerevisiae during anaerobic fermentation. Microbiology 149, 2669–2678. https://doi.org/10.1099/mic.0.26007-0

Casal, M., Paiva, S., Queirós, O., Soares-Silva, I., 2008. Transport of carboxylic acids in yeasts. FEMS Microbiol. Rev. 32, 974–994. https://doi.org/10.1111/j.1574-6976.2008.00128.x

Castellari, L., Ferruzzi, M., Magrini, A., Giudici, P., Passarelli, P., Zambonelli, C., 1994. Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant Saccharomyces strains. Vitis. ISSN 00427500

Chen, Y., Jiang, J., Song, Yaoyao, Zang, X., Wang, G., Pei, Y., Song, Yuyang, Qin, Y., Liu, Y., 2022. Yeast diversity during spontaneous fermentations and oenological characterisation of indigenous Saccharomyces cerevisiae for potential as wine starter cultures. Microorganisms 10, 1455.

Chernova, M., Albertin, W., Durrens, P., Guichoux, E., Sherman, D.J., Masneuf-Pomarede, I., Marullo, P., 2018. Many interspecific chromosomal introgressions are highly prevalent in Holarctic Saccharomyces uvarum strains found in human-related fermentations. Yeast 35, 141–156. https://doi.org/10.1002/yea.3248

Chollet, S., Valentin, D., Abdi, H., 2014. Free Sorting Task, in: Tomasco, P., Ares, G. (Eds.), Novel Techniques in Sensory Characterization and Consumer Profiling. Boca Raton, pp. 207–227. ISBN 9781138034273

Coloretti, F., Zambonelli, C., Castellari, L., Tini, V., Rainieri, S., 2002. The effect of DL-malic acid on the metabolism of L-malic acid during wine alcoholic fermentation. Food Technol. Biotechnol. 40, 317–320. ISSN 1330-9862

Conover, 1999. Counts and categories, in: Conover (Ed.), “Practical Nonparametric Statistics,” , Wiley, 1999, Pp. 165-195. Wiley, pp. 380–383.

Coombe, B.G., 1987. Influence of Temperature on Composition and Quality of Grapes. Acta

Formatted: English (United States)

Formatted: English (United States)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: Font: (Default) Times New Roman, 12 pt

Formatted: English (United States)

Hortic. "Symposium on grapevine canopy and vigor management, Kliewer, W.M. (California Univ., Davis, CA (USA). Dept. of Viticulture and Enology).- Wageningen (Netherlands): ISHS, 1987.- ISBN 90-6605-442-5. p. 23-35

Formatted: English (United States)

Cox, T., Cox, M., 2001. Multidimensional Scaling, 2nd editio. ed. New York. ISBN 978-3-540-33037-0

Formatted: Italian (Italy)

Formatted: Italian (Italy)

da Silva, T., Albertin, W., Dillmann, C., Bely, M., la Guerche, S., Giraud, C., Huet, S., Sicard, D., Masneuf-Pomarede, I., De Vienne, D., Marullo, P., 2015. Hybridization within Saccharomyces Genus Results in Homoeostasis and Phenotypic Novelty in Winemaking Conditions. PLoS One 10, e0123834. https://doi.org/10.1371/journal.pone.0123834

Formatted: English (United States)

de Leeuw, J., Mair, P., 2009. Multidimensional Scaling Using Majorization: SMACOF in R. J. Stat. Softw. 31, 1–30. https://doi.org/10.18637/jss.v031.i03

Delcourt, F., Taillandier, P., Vidal, F., Strehaiano, P., 1995. Influence of pH, malic acid and glucose concentrations on malic acid consumption by Saccharomyces cerevisiae. Appl. Microbiol. Biotechnol. 43, 321–324. https://doi.org/10.1007/BF00172832

Divol, B., Du Toit, M., Duckitt, E., 2012. Surviving in the presence of sulphur dioxide: Strategies developed by wine yeasts. Appl. Microbiol. Biotechnol. 95, 601–613. https://doi.org/10.1007/s00253-012-4186-x

Formatted: English (United States)

Dubourdieu, D., 2004. Influence of climate, soil, and cultivar on Terroir Influence of Climate, Soil, and Cultivar on Terroir. Am. J. Enol. Vitic 55, 3. https://www.ajevonline.org/content/55/3/207

Formatted: English (United States)

Duchêne, E., Schneider, C., 2005. Grapevine and Climatic changes: a glance at the situation in Alsace. Agron. Sustain. Dev 93–99. https://doi.org/10.1051/agro:2004057

Gabriel, S., Ziaugra, L., Tabbaa, D., 2009. SNP genotyping using the sequenom massARRAY iPLEX Platform. Curr. Protoc. Hum. Genet. https://doi.org/10.1002/0471142905.hg0212s60

Formatted: English (United States)

Hou, J., Sigwalt, A., Fournier, T., Pflieger, D., Peter, J., de Montigny, J., Dunham, M.J., Schacherer, J., 2016. The Hidden Complexity of Mendelian Traits across Natural Yeast Populations. Cell Rep. 16, 1106–1114. https://doi.org/10.1016/j.celrep.2016.06.048

Hranilovic, A., Albertin, W., Capone, D.L., Gallo, A., Grbin, P.R., Danner, L., Bastian, S.E.P., Masneuf-Pomarede, I., Coulon, J., Bely, M., Jiranek, V., 2021. Impact of Lachancea thermotolerans on chemical composition and sensory profiles of Merlot wines. Food Chem. 349, 129015. https://doi.org/10.1016/j.foodchem.2021.129015

Hranilovic, A., Gambetta, J.M., Schmidtke, L., Boss, P.K., Grbin, P.R., Masneuf-Pomarede, I., Bely, M., Albertin, W., Jiranek, V., 2018. Oenological traits of Lachancea thermotolerans show signs of domestication and allopatric differentiation. Sci. Rep. 8, 14812. https://doi.org/10.1038/s41598-018-33105-7

Huang, C., Roncoroni, M., Gardner, R.C., 2014. MET2 affects production of hydrogen sulfide during wine fermentation. Appl. Microbiol. Biotechnol. 98, 7125–7135. https://doi.org/10.1007/s00253-014-5789-1

Hung, G.C., Brown, C.R., Wolfe, A.B., Liu, J., Chiang, H.L., 2004. Degradation of the ghiconeogenic enzymes fructose-1,6-bisphosphatase and malate dehydrogenase is mediated by distinct proteolytic pathways and signaling events. J. Biol. Chem. 279, 49138–49150. https://doi.org/10.1074/jbc.M404544200

IPCC, 2020. Climate Change and Land, International Encyclopedia of Geography: People, the Earth, Environment and Technology. https://doi.org/10.1002/9781118786352.wbieg0538

Jones, G. V, 2007. Climate Change: Observations, Projections, and General Implications for Viticulture and Wine Production. Jones. https://chaireunesco-vinetculture.u-bourgogne.fr/colloques/actes_clima/Actes/Article_Pdf/Jones.pdf

Lonvaud-Funel, A., 1994. La Desacidification Biologique des Vins; Etat de la question, perspectives d'avenir. J. Int. des Sci. la Vigne du Vin 28, 161–170. https://doi.org/10.20870/oeno-one.1994.28.2.1149

Marullo, P., Durrens, P., Peltier, E., Bernard, M., Mansour, C., Dubourdieu, D., 2019. Natural allelic variations of Saccharomyces cerevisiae impact stuck fermentation due to the combined effect of ethanol and temperature; a QTL-mapping study. BMC Genomics 20, 680. https://doi.org/10.1186/s12864-019-5959-8

Marullo, P., Mansour, C., Dufour, M., Albertin, W., Sicard, D., Bely, M., Dubourdieu, D., 2009. Genetic improvement of thermo-tolerance in wine Saccharomyces cerevisiae strains by a backcross approach. FEMS Yeast Res. 9, 1148–1160. https://doi.org/10.1111/j.1567-1364.2009.00550.x

Mira de Orduña, R., 2010. Climate change associated effects on grape and wine quality and production. Food Res. Int. 43, 1844–1855. https://doi.org/10.1016/j.foodres.2010.05.001

Nistor, E., Dobrei, A.G., Dobrei, A., Camen, D., 2018. Growing season climate variability and its influence on sauvignon Blanc and Pinot Gris Berries and Wine Quality: Study case in Romania (2005-2015). South African J. Enol. Vitic. 39, 196–207. https://doi.org/10.21548/39-2-2730

Origone, A.C., Rodríguez, M.E., Oteiza, J.M., Querol, A., Lopes, C.A., 2018. Saccharomyces cerevisiae × Saccharomyces uvarum hybrids generated under different conditions share similar winemaking features. Yeast 35, 157–171.

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: English (United States)

Formatted: English (United States)

https://doi.org/https://doi.org/10.1002/yea.3295

Peltier, E., Bernard, M., Trujillo, M., Prodhomme, D.D., Barbe, J.-C., Gibon, Y., Marullo, P., 2018a. Wine yeast phenomics: a standardized fermentation method for assessing quantitative traits of Saccharomyces cerevisiae strains in enological conditions. PLoS One 13, 191353. https://doi.org/10.1101/191353

Peltier, E., Friedrich, A., Schacherer, J., Marullo, P., 2019. Quantitative Trait Nucleotides Impacting the Technological Performances of Industrial Saccharomyces cerevisiae Strains. Front. Genet. 10, 683. https://doi.org/10.3389/fgene.2019.00683

Peltier, E., Sharma, V., Raga, M.M., Roncoroni, M., Bernard, M., Yves Gibon, Marullo, P., Jiranek, V., Gibon, Y., Marullo, P., 2018b. Dissection of the molecular bases of genotype x environment interactions: a study of phenotypic plasticity of Saccharomyces cerevisiae in grape juices. BMC Genomics 19, 772. https://doi.org/10.1186/s12864-018-5145-4

Peltier, E., Vion, C., Abou Saada, O., Friedrich, A., Schacherer, J., Marullo, P., 2021. Flor Yeasts Rewire the Central Carbon Metabolism During Wine Alcoholic Fermentation. Front. Fungal Biol. 2. https://doi.org/10.3389/ffunb.2021.733513

Plane, R., Mattick, L., Weirs, L., 1980. An acidity index for the taste of wines. Am. J. Enol. Vitic. 31, 258–265. ISSN : 0002-9254

Poudel, P.R., Mochioka, R., Beppu, K., Kataoka, I., 2009. Influence of temperature on berry composition of interspecific hybrid wine grape “Kadainou R-1” (Vitis ficifolia var. ganebu × V. vinifera ’Muscat of Alexandria’). J. Japanese Soc. Hortic. Sci. 78, 169–174. https://doi.org/10.2503/jjshs1.78.169

R Core Team, 2018. R: A language and environmentfor statistical computing. R Found. Stat. Comput. Vienna, Austria. URLhttps//www.R-project.org/.

Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H., Viljoen-Bloom, M., 2003. Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation. Int. J. Food Microbiol. 83, 49–61. https://doi.org/10.1016/S0168-1605(02)00320-3

Regev-rudzki, N., Battat, E., Goldberg, I., Pines, O., 2009. Dual localization of fumarase is dependent on the integrity of the glyoxylate shunt. Mol. Microbiol. 72, 297–306. https://doi.org/10.1111/j.1365-2958.2009.06659.x

Ribéereau-Gayon, P., Glories, Y., Maujean, A., Dubourdieu, D., 2006. Handbook of enology Volume 2 The chemistry of wine stabilization and treatments. ISBN-13: 978-0-470-01037-2

Saayman, M., Viljoen-Bloom, M., 2006. The Biochemistry of Malic Acid Metabolism by Wine Yeasts – A Review. South African J. Enol. Vitic. 27, 113–122.

Formatted: English (United States)

Formatted: English (United States)

Formatted: English (United States)

https://doi.org/10.21548/27-2-1612

Salmon, J., 1987. l-Malic-acid permeation in resting cells of anaerobically grown Saccharomyces cerevisiae. Biochim. Biophys. Acta - Biomembr. 901, 30–34. https://doi.org/10.1016/0005-2736(87)90253-7

Schwartz, H., Radler, F., 1988. Formation of l(-)malate by Saccharomyces cerevisiae during fermentation. Appl. Microbiol. Biotechnol. 27, 553–560. https://doi.org/10.1007/BF00451631

Su, J., Wang, T., Wang, Y., Li, Y.Y., Li, H., 2014. The use of lactic acid-producing, malic acid-producing, or malic acid-degrading yeast strains for acidity adjustment in the wine industry. Appl. Microbiol. Biotechnol. 98, 2395–2413. https://doi.org/10.1007/s00253-014-5508-y

van Leeuwen, C., Darriet, P., 2016. The Impact of Climate Change on Viticulture and Wine Quality. J. Wine Econ. 11, 150–167. https://doi.org/10.1017/jwe.2015.21

Formatted: Italian (Italy)

Vilanova, M., Ugliano, M., Varela, C., Siebert, T., Pretorius, I.S., Henschke, P.A., 2007. Assimilable nitrogen utilisation and production of volatile and non-volatile compounds in chemically defined medium by Saccharomyces cerevisiae wine yeasts. Appl. Microbiol. Biotechnol. 77, 145–157. https://doi.org/10.1007/s00253-007-1145-z

Formatted: English (United States)

Vion, C., Peltier, E., Bernard, M., Muro, M., Marullo, P., 2021. Marker Assisted Selection of malic-consuming Saccharomyces cerevisiae strains for winemaking. Efficiency and limits of a QTL's driven breeding program. J. Fungi 17–24. https://doi.org/10.20944/preprints202103.0132.v1

Volschenk, H., van Vuuren, H.J.J., Viljoen-Bloom, M., 2006. Malic Acid in Wine: Origin, Function and Metabolism during Vinification. South African J. Enol. Vitic. 27. https://doi.org/10.21548/27-2-1613

Volschenk, H., Viljoen, M., Grobler, J., Bauer, F., Lonvaud-Funel, A., Denayrolles, M., Subden, R.E., Van Vuuren, H.J.J., 1997. Malolactic Fermentation in Grape Musts by a Genetically Engineered Strain of Saccharomyces cerevisiae. Am. J. Enol. Vitic. 48. ISSN : 0002-9254

Formatted: English (United States)

Yadav, A., Dhole, K., Sinha, H., 2016. Genetic Regulation of Phenotypic Plasticity and Canalisation in Yeast Growth. PLoS One 11, e0162326. https://doi.org/10.1371/journal.pone.0162326

Yéramian, N., Chaya, C., Suárez Lepe, J.A., 2007. L-(-)-malic acid production by Saccharomyces spp. during the alcoholic fermentation of wine. J. Agric. Food Chem. 55, 912–919. https://doi.org/10.1021/jf061990w

Formatted: English (United States)

11 Supplementary material

Table S1. SNPs detected by mass array

Table S2. MASS array genotyping of AC's hybrid progenies

Table S3. Grape juices compositions

Table S4. Phenotypic characterization of parental candidates in seven grape juices

Table S5. Phenotypic characterization of AC's hybrid progenies in two grape juices.

Figure S1 Relative position of acidifying strains in the SBxGN and M2xF15 progenies

Figure S2 Correlation analysis of data presented in Table 2

Figure S3 Estimation of the strain and grape juice effects by ANOVA

Figure S4 Correlation analysis of data presented in Table S5

Figure S5 Distribution of malic acid production in completed and stuck fermentations of data presented in Table S5

12 Figures caption

Figure 1 Phenotypic characterization of acidifying strains in seven red grape juices.

Panel A. The dot plot indicates the distribution of MAC% value measured for 12 strains in seven grape juices (2 replicates per grape juice). Strains were colored according to their origin. Green= industrial starters; light blue= malic producer strains isolated in Spain, orange M2xF15 progenies, purple= SBxGN progenies, red = malic consumer strain. The letters below the dot plot indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Panel B. The dot plot indicates the distribution of MAC% value measured for 12 strains in seven grape juice (2 replicates per grape juice). Grape juices were sorted according to their initial pH values. Negative MAC% values (indicating a malic acid production) were obtained in only two grape must colored in burgundy color. The letters below the dot plot indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Figure 2 Breeding program for enhancing malic acid production

Panel A. Box plot indicates the optimization of MAC% values by a phenotype driven breeding program. The purple color indicates the MAC% distribution of founder strains GN, SB, M2 and

F15 and parental strains selected GS-8B and FM-1C. The resulting F1 hybrid AC1 and its four optimal progeny clones are colored in red. The second-generation hybrids AC2 and AC3 are respectively colored in red and green; each F1-hybrid is narrowed by its respective parents. The letters below the box plots indicate post hoc significative differences between strains (Fisher's least significant difference). Groups that do not share a common letters can be considered as statistically different (Kruskal test, alpha=0.05).

Panel B. Distribution of MAC% values in AC1 AC2 and AC3 progenies. Data presented are the average values of triplicates and were measured in the CS_14 grape juice. The large black dot indicates the average of values per background. The p value above the box plot indicates a statistical difference of MAC% according to the hybrid background (Kruskal test).

Panel C. Distribution of MAC% values in AC2 and AC3 progenies. Data presented are the average values of triplicates and were measured in the CS_15 grape juice. The large black dot indicates the average of values per background. The p value above the box plot indicates a statistical difference of MAC% according to the hybrid background (Kruskal test).

Figure 3 Multivariate analysis

Panel A. The phenotypic behavior of 127 strains for 12 quantitative traits was figured out in the first two dimension of a Principal Component Analysis (encompassing 60.1% of the total inertia). A total of 484 fermentations were carried out in two grape juices (CS_14 and CS15). The color of the dots indicated the yeast background (AC1, AC2, AC3 and control strains) while the shape indicated the grape must fermented. Open and full symbols indicates if the fermentations were stuck or completed, respectively.

Panel B. Circle of correlation of the 12 variables used for the PCA.

Panel C. Correlation between the final malic acid content and the final pH of the wine in both CS_14 and CS_15 grape juices, the dot key is the same than for panel A.

Panel D. Real and predicted values of the final pH according to following linear model regression $pH = 0.0631 - 0.0058\ x\ [Malic\ acid] + 0.1282\ x\ [Acetic\ acid] + 0.9495\ x\ pHi - 0.0014\ x\ [residual\ sugar]$. Open and full symbols indicates if the fermentations were stuck or complete, respectively.

Figure 4 QTLs genotyped by Mass Array in AC's hybrids

Panel A. Schematic localization of QTLs tracked on the genomic map of SBxGN hybrid. alleles conferring a malic acid production were colored in purple and blue for SB and GN, respectively.

Panel B. SNP call detected by mass array genotyping for each locus tracked in this study. The DEMALIC alleles (in green) enhance the consumption of malic acid while the ACIDIC alleles enhance the production of malic acid (in red).

Panel C. Genotypes of the four founders of the study (GN, SB, M2 and F15), the two parental strains GS-8B, FM-1C and the three hybrids AC1, AC2 and AC3. For each locus, the green and red colored areas indicated if the DEMALIC or ACIDIC allele is fixed in the hybrid background.

Figure 5 QTL effect

Panel A. Linear regression of the MAC% according to the number of ACIDIC alleles carried by the progeny clones of the hybrids AC1, AC2 and AC3 that are colored in red, blue and green respectively.

Panel B. The boxplot shows the MAC% values of strains carrying the DEMALIC (C) or ACIDIC (G) allele of the locus VII_480 according to the hybrid background. Colored numbers indicated the MAC% differences between the genotype (same key than panel A). The p value above the box plot indicates a statistical difference of MAC% according to the genotype in indicated (Wilcoxon test).

Panel C. The boxplot shows the MAC% values of strains carrying the DEMALIC (A) or ACIDIC (G) allele of the locus IV_28 according to the hybrid background. Colored numbers indicated the MAC% differences between the genotype (same key than panel A). The p value above the box plot indicates a statistical difference of MAC% according to the genotype in indicated (Wilcoxon test).

Figure 6 Acidity perception estimated by sensory analysis

Panel A. The boxplot figures out the distribution total acidity for each groups of strains. The significative difference between malic acid consumers and producers is confirmed by a Wilcoxon test ($alpha<0.05$).

Panel B. The relative position of the wines tasted are figured out on the first two dimensions of a MDS analysis. Wines are represented by points that are positioned such that the distances between the pairs of points reflect distances between the pairs of wines. The average stress value of the analysis was 0.051 which is significantly lower than explained by chance for 28 tasters and 12 wines (FDR of 5% 0.064). The area of each point is proportional to the individual stress indicating the confidence of the projection. The colors indicate the group of the strain, starter, malic acid consumers or producers.

Figure 1.

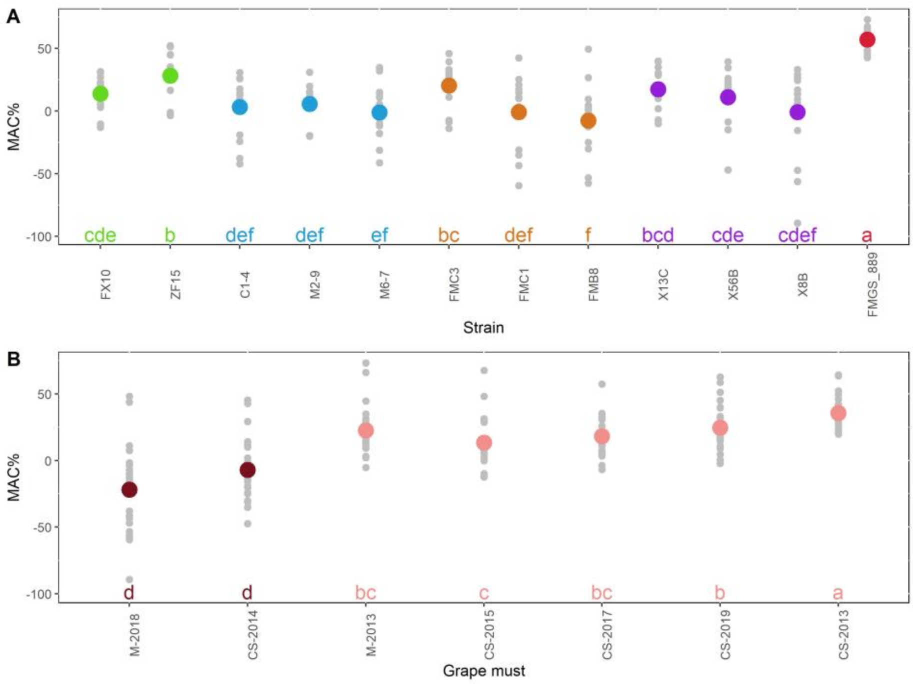

A
MAC%
50
0
-50
-100
cde
b
def
def
ef
bc
def
f
bcd
cde
cdef
a
FX10
ZF15
C1-4
M2-9
M6-7
FMC3
FMC1
FMB8
X13C
X56B
X8B
FMGS_889
Strain
B
MAC%
50
0
-50
-100
d
d
bc
c
bc
b
a
M-2018
CS-2014
M-2013
CS-2015
CS-2017
CS-2019
CS-2013
Grape must

Figure 2

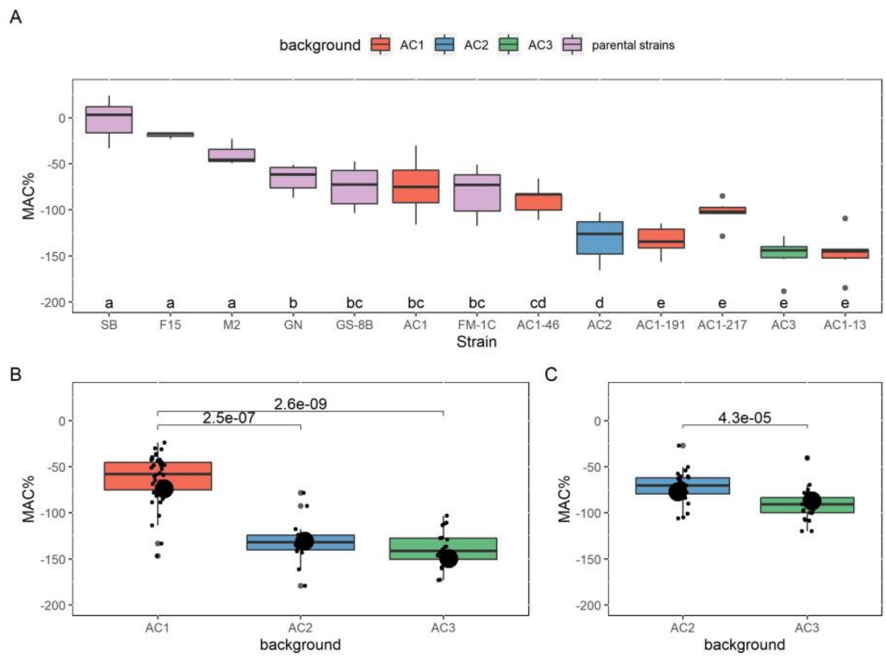

A
background AC1 AC2 AC3 parental strains
MAC%
0
-50
-100
-150
-200
a a a b bc bc bc cd d e e e e
SB F15 M2 GN GS-8B AC1 FM-1C AC1-46 AC2 AC1-191 AC1-217 AC3 AC1-13
Strain
B
2.6e-09
2.5e-07
MAC%
AC1 AC2 AC3
background
C
4.3e-05
MAC%
AC2 AC3
background

Figure 3

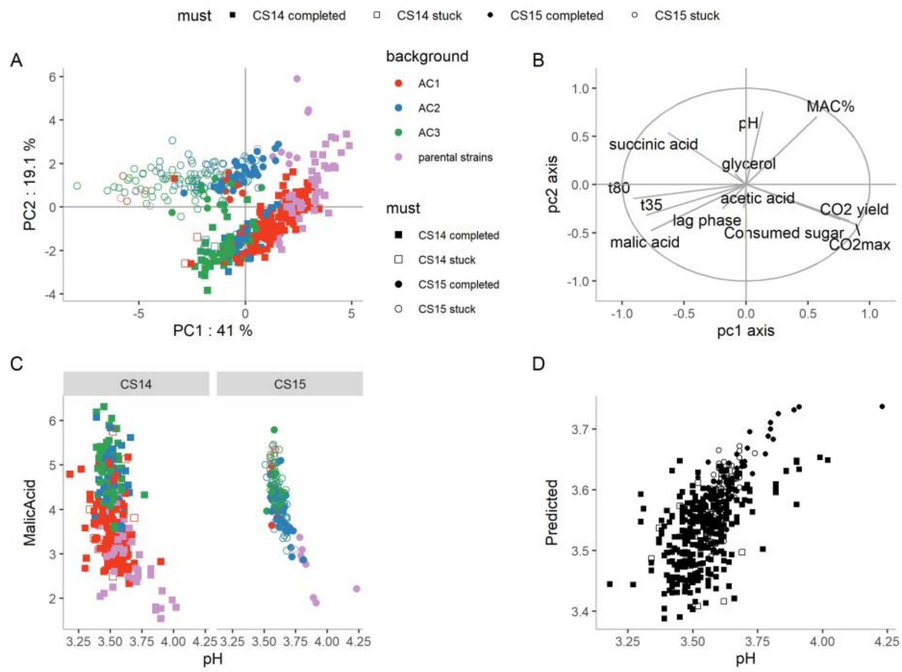
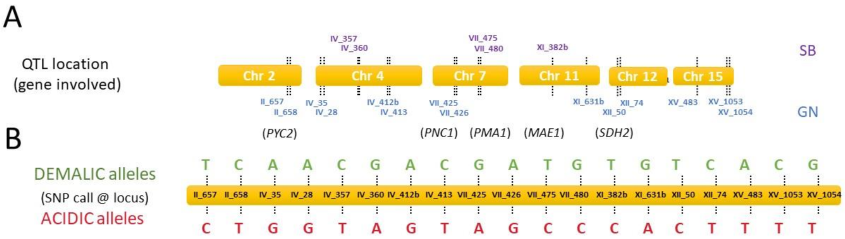

must CS14 completed CS14 stuck CS15 completed CS15 stuck
A
background
AC1
AC2
AC3
parental strains
must
CS14 completed
CS14 stuck
CS15 completed
CS15 stuck
PC2 : 19.1 %
PC1 : 41 %
B
MAC%
pH
succinic acid
glycerol
t80
t35
acetic acid
lag phase
CO2 yield
malic acid
Consumed sugar
CO2max
pc2 axis
pc1 axis
C
CS14
CS15
MalicAcid
pH
D
Predicted
pH

Figure 4



C

	II_657	II_658	IV_28	IV_35	IV_357	IV_360	IV_412b	IV_413	VII_425	VII_426	VII_475	VII_480	XI_382	XI_631	XII_50	XII_74	XV_1053	XV_1054	XV_483
GN	C	T	G	G	C	G	G	T	A	G	T	G	C	A	C	C	T	T	T
SB	T	C	A	A	T	A	A	C	G	A	C	C	T	G	T	C	A	C	G
M2	T	C	A	A	C	G	A	C	G	A	T	G	T	A	T	T	A	C	T
F15	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	C	A	C	G
GS-8B	C	T	G	G	T	A	G	T	G	A	C	C	C	A	T	C	A	C	G
FM-1C	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	T	A	C	T
AC1	T/C	C/T	A/G	G/A	C/T	A/G	A/G	T/C	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/C	A/A	C/C	G/T
AC2	C/C	T/T	A/G	G/G	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T
AC3	C/C	T/T	A/G	G/A	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T

Figure 5

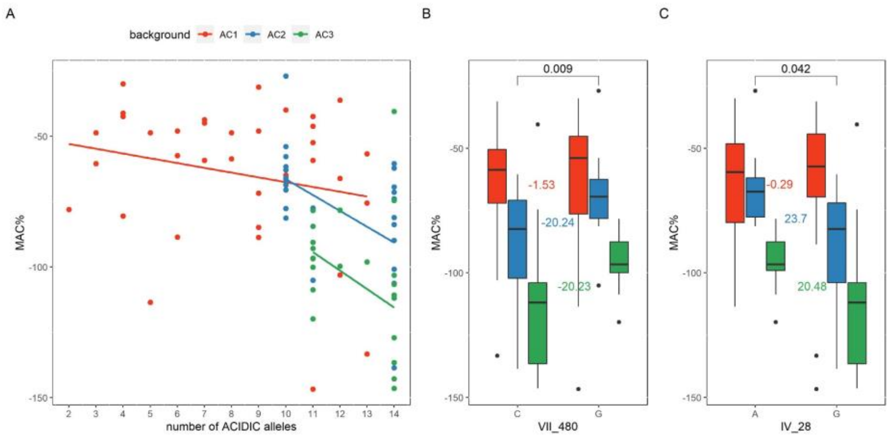

A
B
C
background
AC1
AC2
AC3
MAC%
number of ACIDIC alleles
0.009
-1.53
-20.24
-20.23
VII_480
0.042
-0.29
23.7
20.48
IV_28

Figure 6

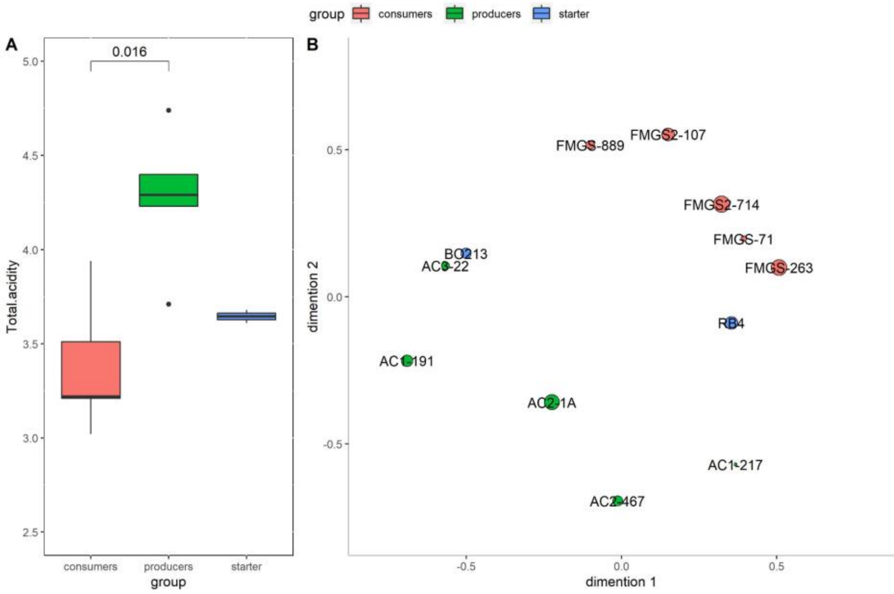

group
consumers
producers
starter
A
0.016
Total.acidity
5.0
4.5
4.0
3.5
3.0
2.5
consumers
producers
starter
group
B
FMGS2-107
FMGS-889
FMGS2-714
FMGS-71
FMGS-263
BO213
AC3-22
RB4
AC1-191
AC2-1A
AC1-217
AC2-467
dimention 2
dimention 1
0.5
0.0
-0.5

Figure S1

Caption: Distribution of MAC% for starters (green), M2xF15 (orange) and SBxGN (purple) progenies and relative position of candidate parental strains (black dots), all the data were obtained in M15_5ml conditions; data obtained from Peltier et al. 2018 (starters) and Vion et al 2021 (M2xF15 and SBxGN progenies).

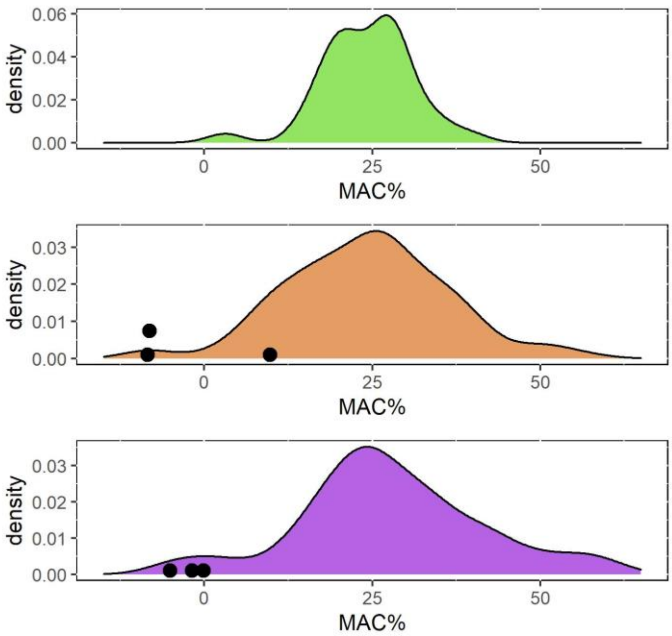


Figure S2

Caption: Correlation matrix between kinetics and metabolic traits measured in seven grape musts and 12 strains, only significative correlations were figured out. The intensity and the size of the color reflects the value of rho parameter (Spearman test with Bonferroni multiple corrections, alpha =0.001). Positive correlations are depicted in blue while negative correlations are depicted in red.

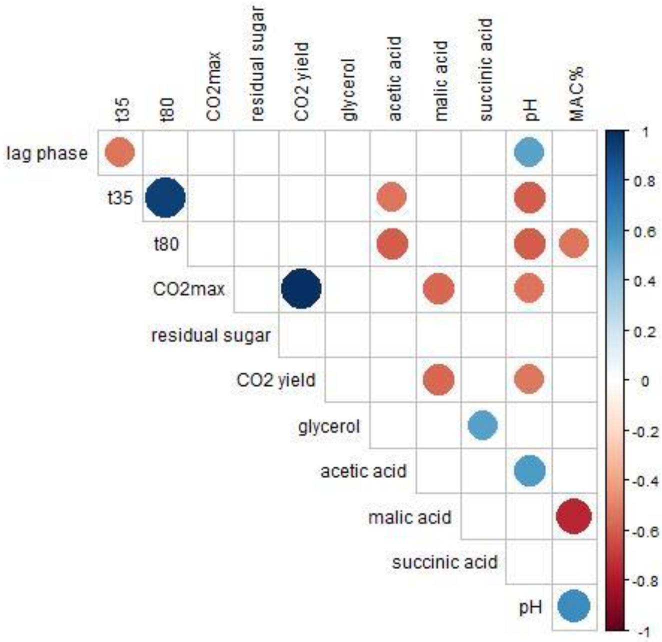


Figure S3

Caption: Panel A the box plot shows the distribution of MAC% values of four strains fermented in two different grape must CS14 and CS15 colored in blue and red, respectively. Panel B part of variance explained by strain and nature origin and their interaction according to a two-way analysis of variance. The number of stars above the bars indicates the significative threshold levels : * <0.05, ***<0.001.

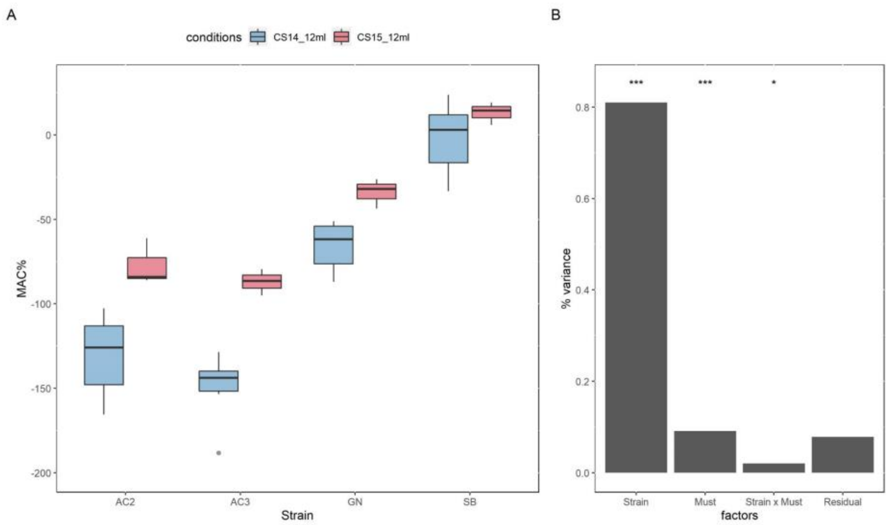


Figure S4

Caption: Correlation matrix between kinetics and metabolic traits measured in two grape musts and 127 strains, only significative correlations were figured out. The intensity and the size of the color reflects the value of rho parameter (Spearman test with Bonferroni multiple corrections, alpha =0.001). Positive correlations are depicted in blue while negative correlations are depicted in red.

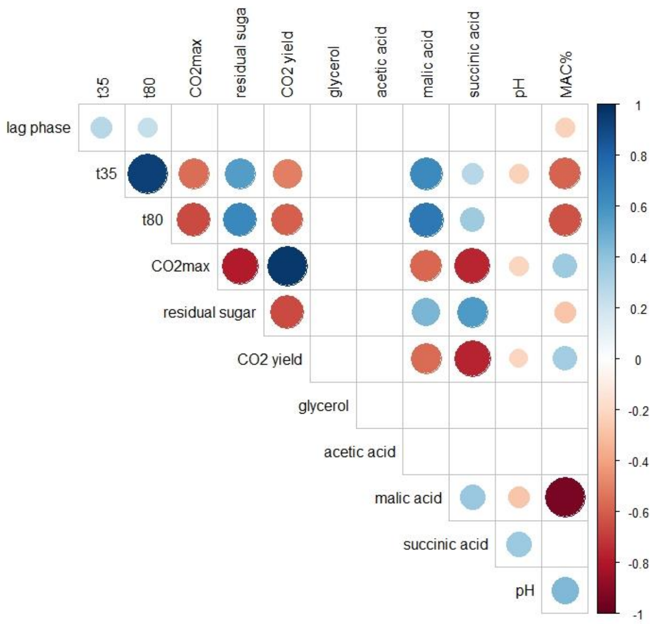

t35
t80
CO2max
residual suga
CO2 yield
glycerol
acetic acid
malic acid
succinic acid
pH
MAC%
lag phase
t35
t80
CO2max
residual sugar
CO2 yield
glycerol
acetic acid
malic acid
succinic acid
pH
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure S5

Caption: Distribution of the average malic acid produced expressed in g/L according to the status of the fermentation (completed or stuck). The vertical line indicates the proportion of strains that have produced more than 3 g/L of malic acid (in addition to the initial quantity present in the grape must).

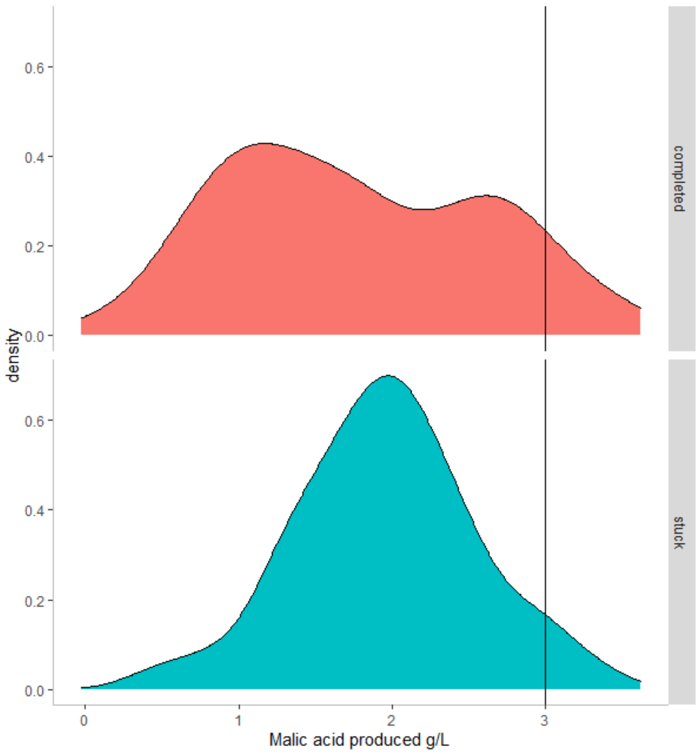


Figure1

Click here to access/download;Figure;Figure1.jpg

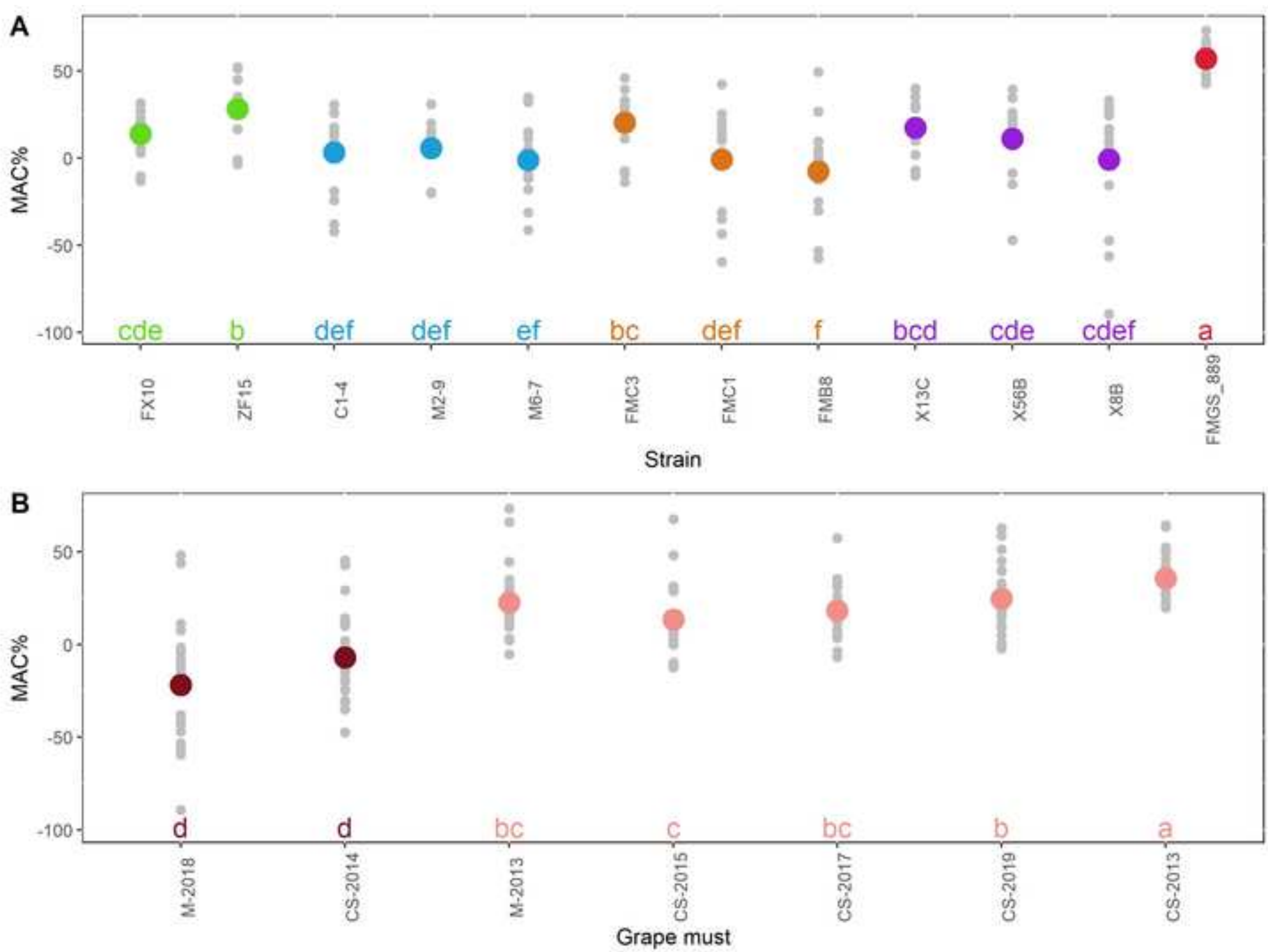


Figure2

Click here to access/download;Figure;Figure2.jpg

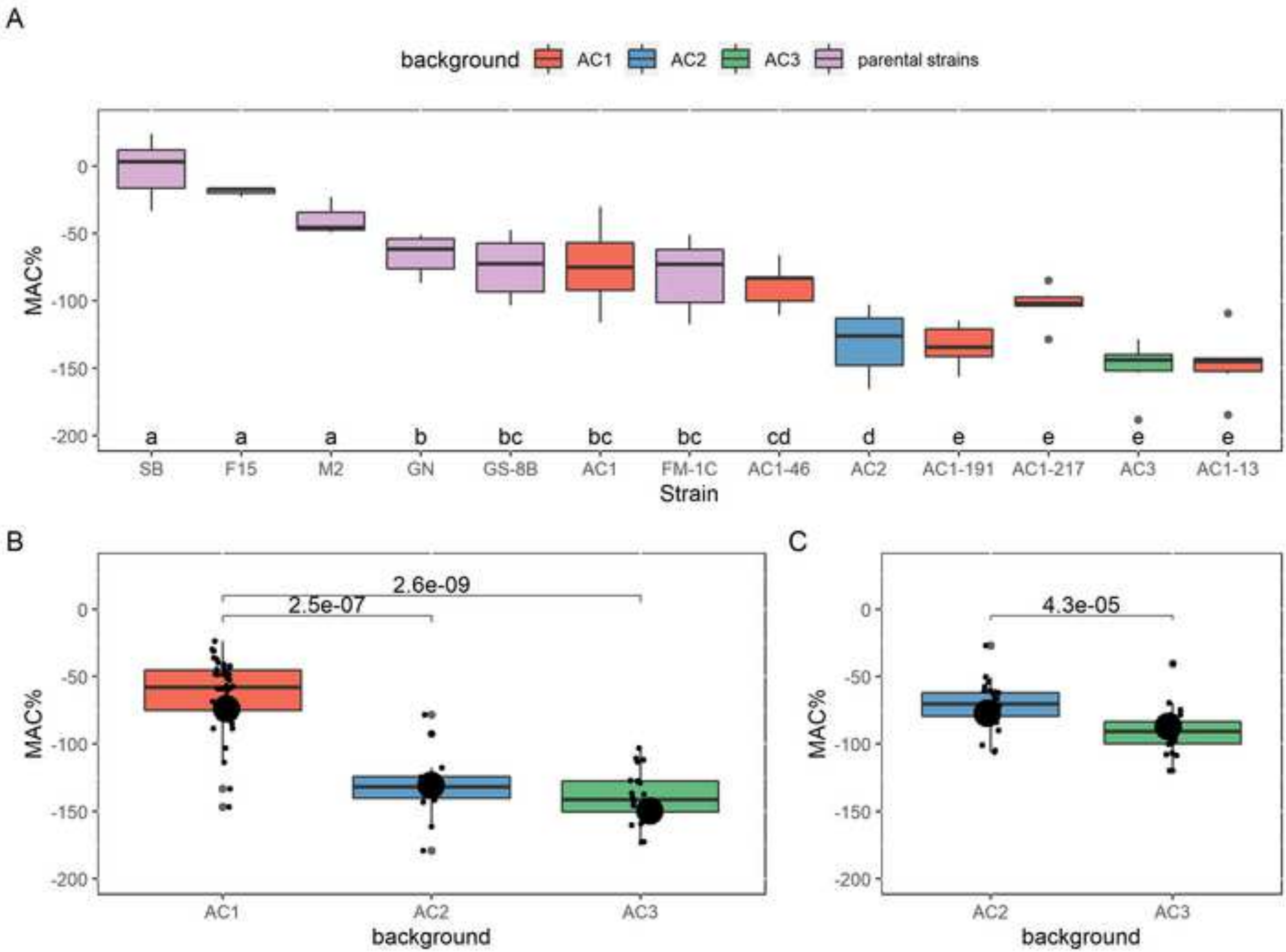
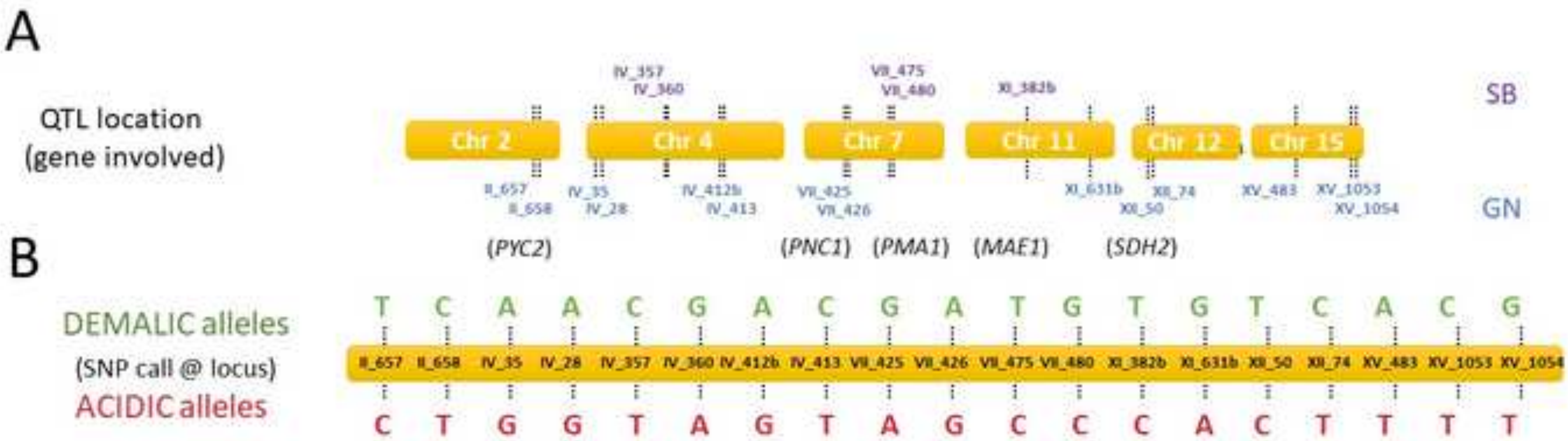


Figure4

Click here to access/download;Figure;Figure4.jpg



C

	II_657	II_658	IV_28	IV_35	IV_357	IV_360	IV_412b	IV_413	VII_425	VII_426	VII_475	VII_480	XI_382	XI_631	XIII_50	XII_74	XV_1053	XV_1054	XV_483
GN	C	T	G	G	C	G	G	T	A	G	T	G	C	A	C	C	T	T	T
SB	T	C	A	A	T	A	A	C	G	A	C	C	T	G	T	C	A	C	G
M2	T	C	A	A	C	G	A	C	G	A	T	G	T	A	T	T	A	C	T
F15	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	C	A	C	G
GS-8B	C	T	G	G	T	A	G	T	G	A	C	C	C	A	T	C	A	C	G
FM-1C	T	C	A	A	C	G	A	C	G	A	T	G	C	A	T	T	A	C	T
AC1	T/C	C/T	A/G	G/A	C/T	A/G	A/G	T/C	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/C	A/A	C/C	G/T
AC2	C/C	T/T	A/G	G/G	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T
AC3	C/C	T/T	A/G	G/A	T/T	A/A	G/G	T/T	G/G	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A	C/C	T/T

Figure5

Click here to access/download;Figure;Figure5.jpg

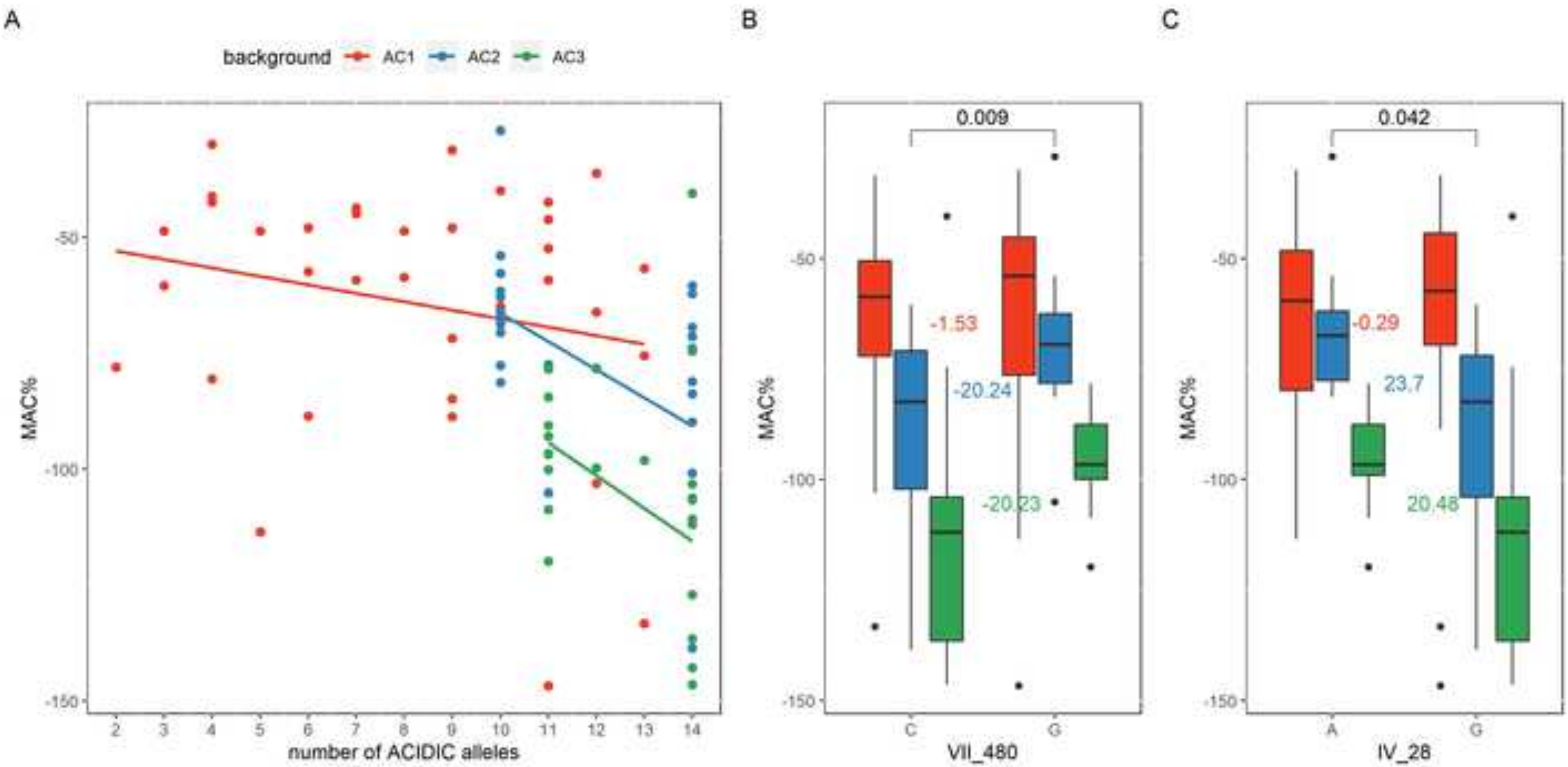


Figure6

Click here to access/download;Figure;Figure6.jpg

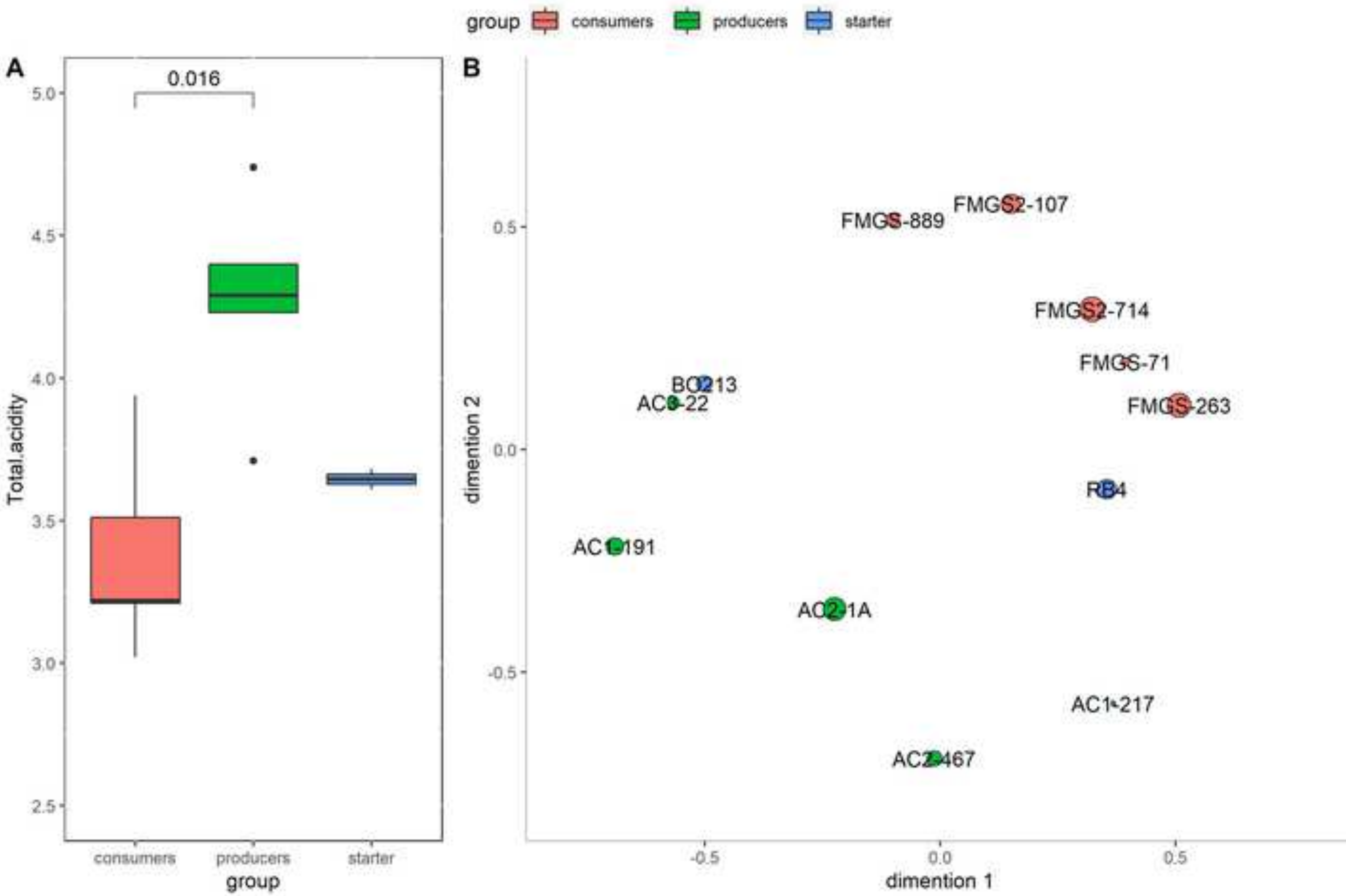


Figure S1

Click here to access/download;Figure;Figure S1.jpg

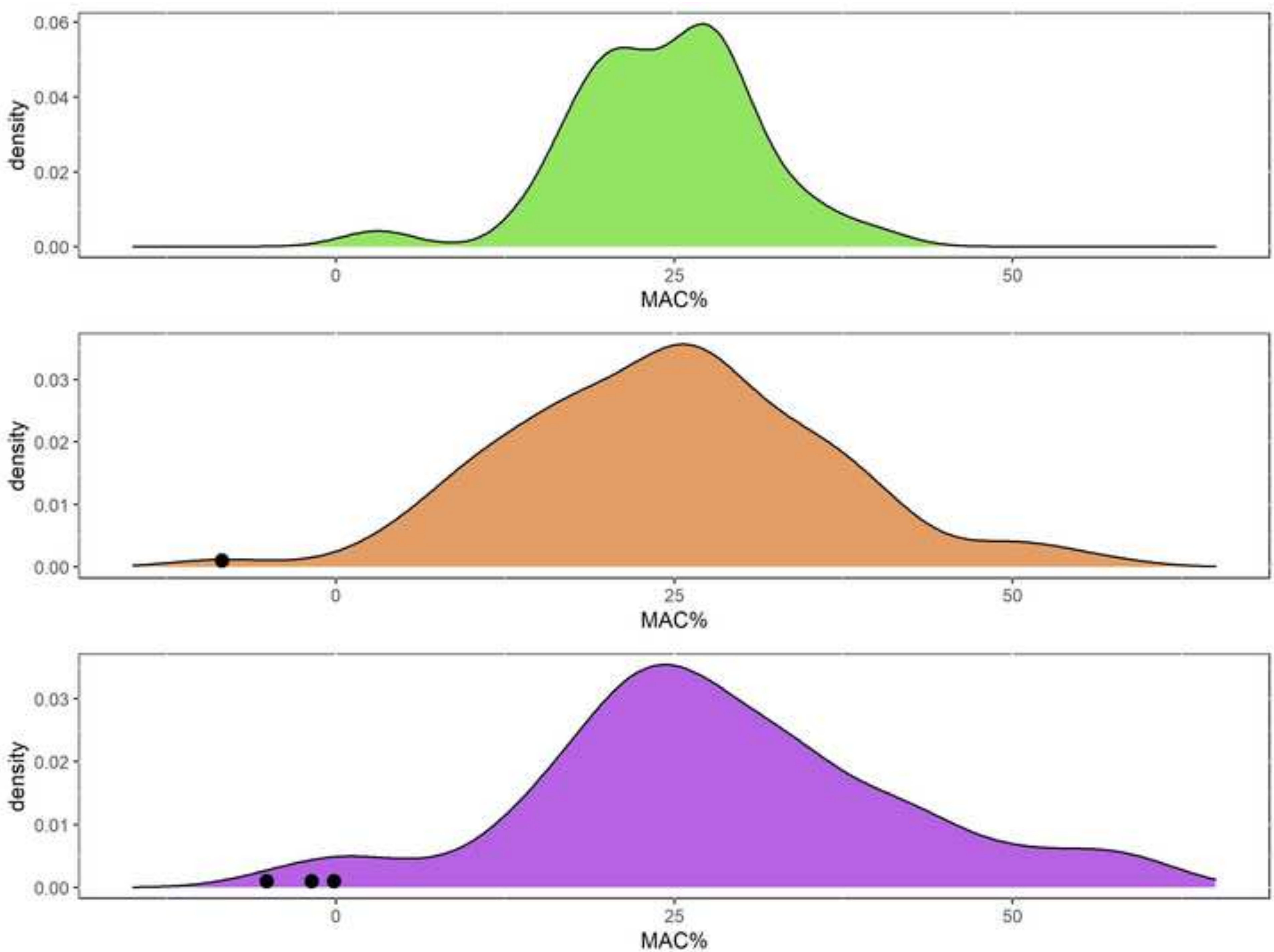


Figure S2

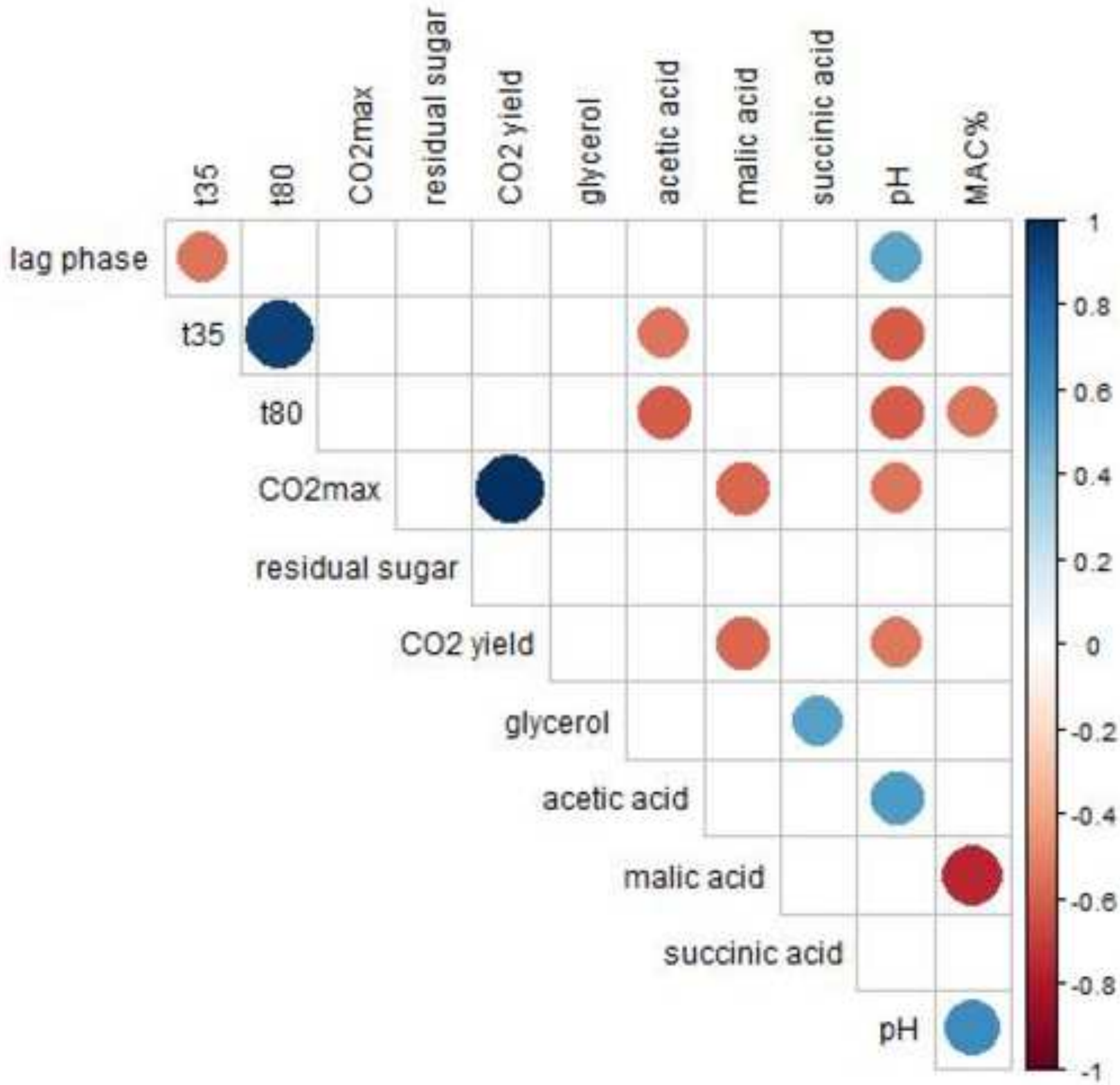

t35
t80
CO2max
residual sugar
CO2 yield
glycerol
acetic acid
malic acid
succinic acid
pH
MAC%
lag phase
t35
t80
CO2max
residual sugar
CO2 yield
glycerol
acetic acid
malic acid
succinic acid
pH
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure S3

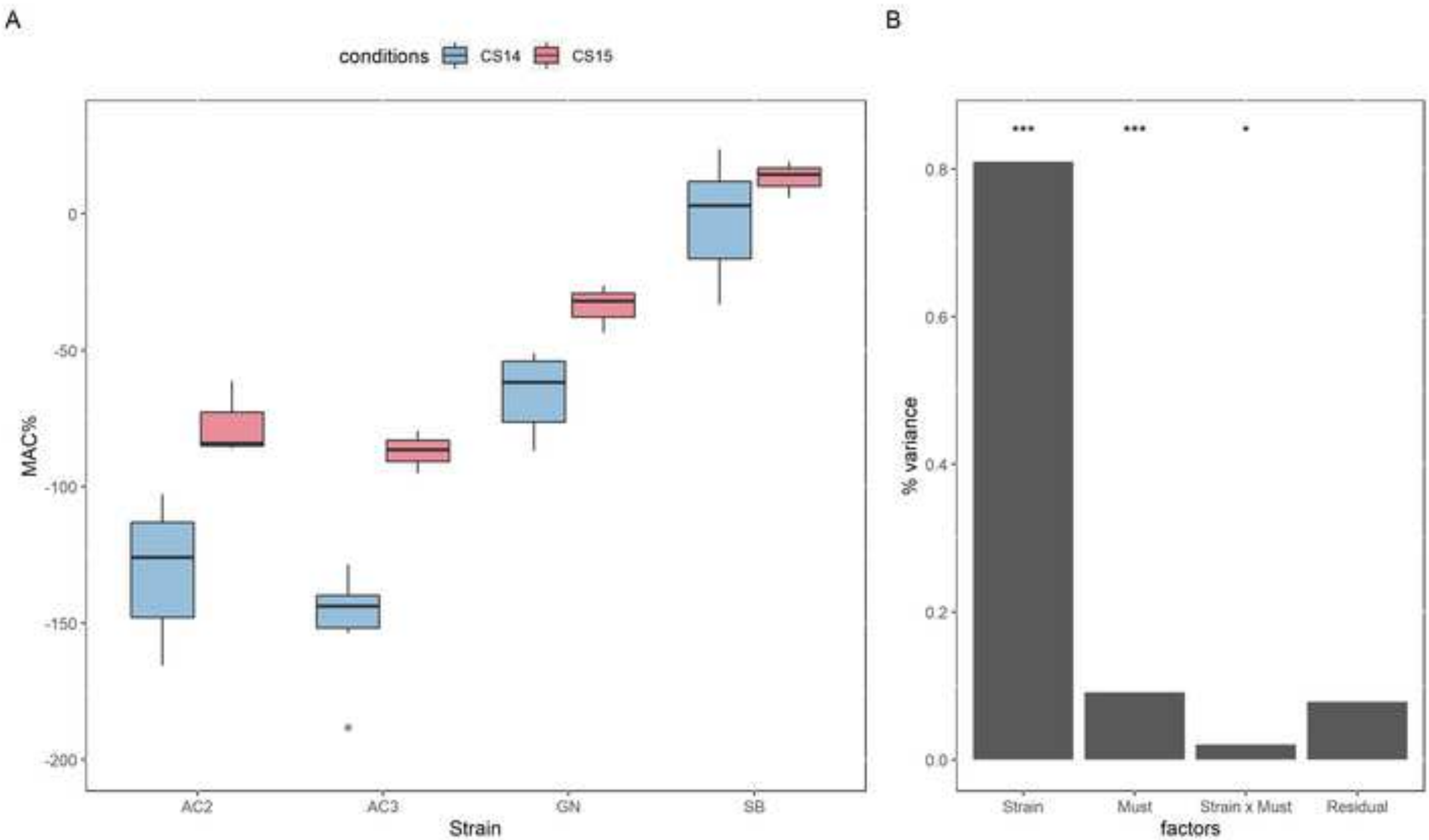

A
conditions
CS14
CS15
MAC%
0
-50
-100
-150
-200
AC2
AC3
GN
SB
Strain
B
***
***
*
% variance
0.8
0.6
0.4
0.2
0.0
Strain
Must
Strain x Must
Residual
factors

Figure S4

Click here to access/download;Figure;Figure S4.jpeg

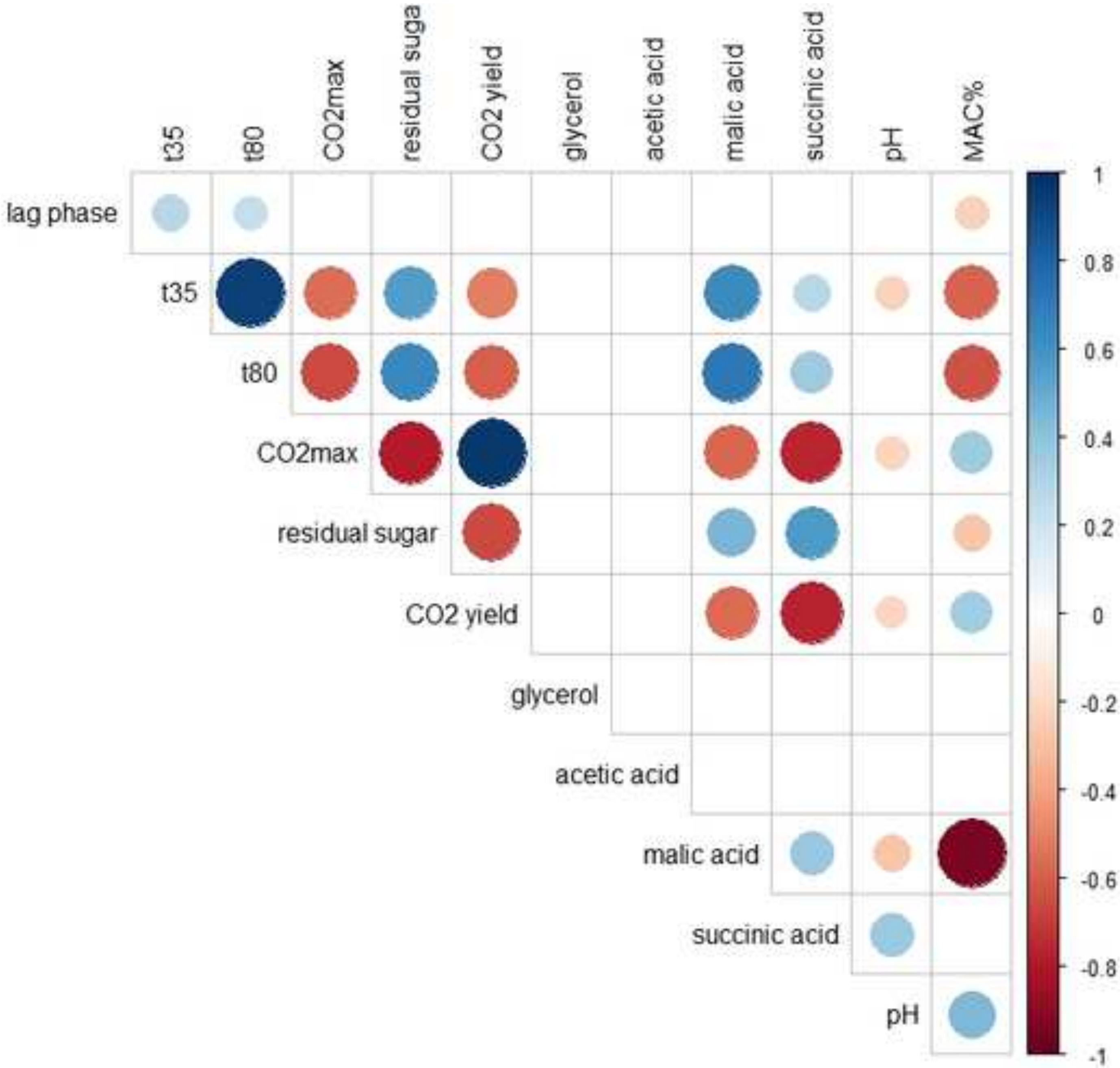
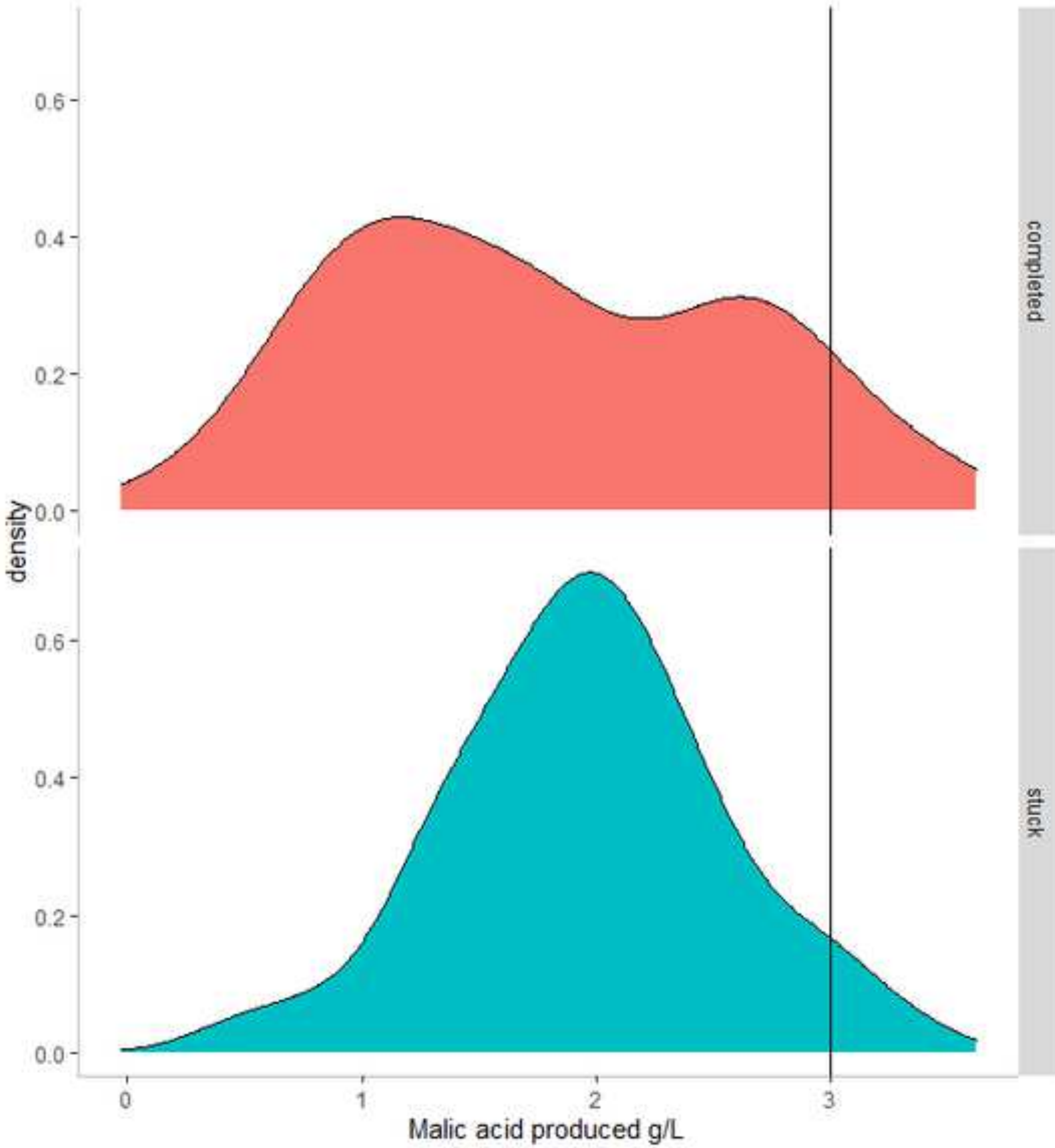


Figure S5

Click here to access/download;Figure;Figure S5.tiff



S tables

Click here to access/download;Table;STables_corrected.xlsx

QTL Vion	locus	Chromosome	Position	GENE	REF	GN
II_661	II_657	chr2	657,626		T	**C**
	II_658	chr2	658,596	YBR218C	C	**T**
IV_31	IV_28	chr4	28,565	YDL239C	A	**G**
	IV_35	chr4	35,209	YDL 234C	A	**G**
IV_360	IV_357	chr4	357,802		C	C
	IV_360	chr4	360,451	MCH1	G	G
IV_414	IV_412b	chr4	412,127	YDL022W	A	**G**
	IV_413	chr4	413,957	YDL021W	C	**T**
VII_427	VII_425	chr7	425,096		G	**A**
	VII_427	chr7	427,894	PNC1	A	**A**
VII_480	VII_475	chr7	470,588	PDR1	T	T
	VII_480	chr7	480,044	PMA1-2	G	G
XI_382	XI_382	chr11	382,913	MAE1	T	**C**
XI_631	XI_631	chr11	631,332	PCK1	A	**A**
XII_53	XII_50	chr12	50,984	YLL043W	T	**C**
	XII_74	chr12	74,779	YLL 032C	C	C
XV_1052	XV_1053	chr15	1,053,754		A	**T**
	XV_1054	chr15	1,054,666		C	**T**
XV_491	XV_483	chr15	483,470	TCB1	G	**T**

SB	M2	F15	AC1	AC2	AC3	SEQUENCE
T	T	T	T/C	C/C	C/C	AAAGTA**W**G
C	C	C	T/C	T/T	T/T	ATTAAGGAA
A	A	A	G/A	G/A	G/A	GCGGTTTCTT
A	A	A	G/A	G/A	G/G	TCTTC**R**TCGT
T	C	C	T/C	T/T	T/T	AAGGTGAGC
A	G	G	A/G	A/A	A/A	GAGGGGCCC
A	A	A	A/G	G/G	G/G	ATCATAAATA
C	C	C	T/C	T/T	T/T	CCCAAGAATA
G	G	G	A/A	A/A	A/A	AATCTAAATA
G	A	A	G/A	A/A	G/A	cggtcagtcaaga
C	T	T	T/C	T/C	T/C	GTTTATTACC
C	G	G	G/C	G/C	G/C	TCTGGATCTC
T	T	C	C/C	C/C	C/C	TACTTGTTCT
G	A	A	A/A	A/A	A/A	TGAAGATGGT
T	T	T	T/T	T/T	T/T	GCCGTGGTG
C	T	C	T/C	T/T	T/T	AATTATGTTC
A	A	A	A/A	A/A	A/A	TAAATTTTGT
C	C	C	C/C	C/C	C/C	TACGTCTGTA
G	T	G	G/T	T/T	T/T	GGTGTATCTT

2nd-PCRP	1st-PCRP	AMP_LEN	UP_CONF	MP_CONF	Tm(NN)	PcGC
ACGTTGGAT(	ACGTTGGAT(	100	92.9	75.9	46.4	33.3
ACGTTGGAT(	ACGTTGGAT(	84	74.2	75.9	46.1	28.6
ACGTTGGAT(	ACGTTGGAT(	98	67.8	75.9	48.1	30.8
ACGTTGGAT(	ACGTTGGAT(	95	86.6	75.9	48.5	64.3
ACGTTGGAT(	ACGTTGGAT(	110	98.2	75.9	45.4	36.8
ACGTTGGAT(	ACGTTGGAT(	100	96.1	72.1	51.6	45.5
ACGTTGGAT(	ACGTTGGAT(	78	92.9	75.9	48.4	60.0
ACGTTGGAT(	ACGTTGGAT(	99	66.9	75.9	56.7	50.0
ACGTTGGAT(	ACGTTGGAT(	91	69.4	75.9	47.1	33.3
ACGTTGGATG	ACGTTGGATG	103	95.6	72.1	47.3	34.8
ACGTTGGAT(	ACGTTGGAT(	99	100.0	75.9	45.9	47.1
ACGTTGGAT(	ACGTTGGAT(	94	99.9	72.1	45.1	47.1
ACGTTGGAT(	ACGTTGGAT(	113	97.9	72.1	46.4	53.3
ACGTTGGATG	ACGTTGGATG	90	98.2	72.1	45.4	53.3
ACGTTGGAT(	ACGTTGGAT(	100	98.6	75.9	45.9	64.3
ACGTTGGAT(	ACGTTGGAT(	89	74.6	75.9	46.5	33.3
ACGTTGGAT(	ACGTTGGAT(	120	65.8	75.9	45.0	30.0
ACGTTGGAT(	ACGTTGGAT(	90	94.1	75.9	48.0	42.1
ACGTTGGAT(	ACGTTGGAT(	101	94.5	72.1	49.1	50.0

PWARN	UEP_DIR	UEP_MASS	UEP_SEQ	EXT1_CALL	EXT1_MASS	EXT1_SEQ
dh	R	6753.5	aTGAAAAAG.	T	7024.7	aTGAAAAAG.
D	F	6450.2	TTCATCGAA/	C	6697.4	TTCATCGAA/
D	F	8346.5	gCGACAGTT1	A	8617.7	gCGACAGTT1
D	R	4297.8	GCCGCGGAA	G	4545.0	GCCGCGGAA
D	F	5839.8	TGGTATAGT	C	6087.0	TGGTATAGT
D	F	6743.4	ACCGCTAGG.	A	7014.6	ACCGCTAGG.
d	F	4503.9	GCCTGGCAT(	A	4775.1	GCCTGGCAT(
DH	R	6821.4	GACATTGGA	T	7092.6	GACATTGGA
d	R	6395.2	ATGAACTCT1	G	6642.4	ATGAACTCT1
dg	F	7286.8	cCAATCTTAAT	A	7558.0	cCAATCTTAAT
	R	5225.4	GTGGACGTT	T	5496.6	GTGGACGTT
	F	5207.4	TGGGTGGTT	C	5454.6	TGGGTGGTT
D	F	4617.0	CGAGTGCTT(	C	4864.2	CGAGTGCTT(
D	R	4595.0	CACCGGAATA	G	4842.2	CACCGGAATA
Ds	F	4239.8	GCCATCTCA(	C	4486.9	GCCATCTCA(
s	R	7386.9	GAGATAGAT	T	7658.1	GAGATAGAT
d	F	6151.1	AAGAAAAAT	A	6422.3	AAGAAAAAT
	R	6020.9	cTGAATGCT/	T	6292.1	cTGAATGCT/
Ds	R	5603.7	GGATTAGAG	G	5850.8	GGATTAGAG

EXT2_CALL	EXT2_MASS	EXT2_SEQ
C	7040.7	aTGAAAAAGAAAATCACCCTAGG
T	6777.3	TTCATCGAAAGTGTTTGATTAT
G	8633.7	gCGACAGTTTTGATATAATATCAGAAGG
A	4624.9	GCCGCGGAATACGAT
T	6166.9	TGGTATAGTTTTCGATTGCT
G	7030.6	ACCGCTAGGATATATGTCACAGG
G	4791.1	GCCTGGCATCACTCTG
C	7108.6	GACATTGGATGGTGTGCTTGCAG
A	6722.3	ATGAACTCTTTGTATCCGTAAT
G	7574.0	cCAATCTTAATATGCTTAGTGACCG
C	5512.6	GTGGACGTTCTCGATAAG
G	5494.6	TGGGTGGTTTCTACTACG
T	4944.1	CGAGTGCTTGCAAGAT
A	4922.1	CACCGGAATAAGCGAT
T	4566.9	GCCATCTCAGGTGCT
C	7674.1	GAGATAGATCCCAATAAAGAATACG
T	6478.2	AAGAAAAATAGCCAGTACATT
C	6308.1	cTGAATGCTAACAACTTCCCG
T	5874.9	GGATTAGAGAACGCTGGTA

Sample Name	background	II_657	II_658	IV_28	IV_35	IV_357	IV_360	IV_412b
AC1	AC1	T/C	T/C	G/A	G/A	T/C	A/G	A/G
AC2	AC2	C/C	T/T	G/A	G/A	T/T	A/A	G/G
AC3	AC3	C/C	T/T	G/A	G/G	T/T	A/A	G/G
AC3-122	AC3	C	T	G	G	T	A	G
AC3-162	AC3	C	T	G	G	T	A	G
AC3-22	AC3	C	T	G	G	T	A	G
AC3-40	AC3	C	T	G	G	T	A	G
AC3-60	AC3	C	T	G	G	T	A	G
AC3-78	AC3	C	T	G	G	T	A	G
AC3-95	AC3	C	T	G	G	T	A	G
AC3-102	AC3	C	T	A	G	T	A	G
AC3-104	AC3	C	T	G	G	T	A	G
AC3-109	AC3	C	T	A	G	T	A	G
AC3-111	AC3	C	T	G	G	T	A	G
AC3-122	AC3	C	T	G	G	T	A	G
AC3-13	AC3	C	T	A	G	T	A	G
AC3-140	AC3	C	T	G	G	T	A	G
AC3-144	AC3	C	T	A	G	T	A	G
AC3-15	AC3	C	T	A	G	T	A	G
AC3-162	AC3	C	T	G	G	T	A	G
AC3-169	AC3	C	T	A	G	T	A	G
AC3-186	AC3	C	T	A	G	T	A	G
AC3-22	AC3	C	T	G	G	T	A	G
AC3-26	AC3	C	T	A	G	T	A	G
AC3-32	AC3	C	T	A	G	T	A	G
AC3-40	AC3	C	T	G	G	T	A	G
AC3-47	AC3	C	T	A	G	T	A	G
AC3-60	AC3	C	T	G	G	T	A	G
AC3-78	AC3	C	T	G	G	T	A	G
AC3-81	AC3	C	T	A	G	T	A	G
AC3-85	AC3	C	T	G	G	T	A	G
AC3-95	AC3	C	T	G	G	T	A	G
AC1-1	AC1	T	C	A	A	T	A	A
AC1-103	AC1	C	T	G	G	C	G	A
AC1-115	AC1	T	C	A	A	C	G	A
AC1-123	AC1	T	C	A	A	C	G	A
AC1-129	AC1	C	T	G	G	T	A	G
AC1-13	AC1	C	T	G	G	T	A	G
AC1-152	AC1	C	T	G	G	T	A	A
AC1-182	AC1	C	T	A	A	T	A	A
AC1-184	AC1	T	C	A	A	C	G	A
AC1-187	AC1	C	T	A	A	C	G	A
AC1-191	AC1	C	T	G	G	T	A	G

AC1-193	AC1	C	T	A	A	C	G	A
AC1-196	AC1	T	C	G	G	C	G	A
AC1-197	AC1	T	C	A	A	T	A	G
AC1-201	AC1	C	T	A	A	C	G	G
AC1-203	AC1	T	C	G	G	T	G	A
AC1-205	AC1	C	T	G	A	T	A	G
AC1-207	AC1	C	T	G	G	C	G	A
AC1-212	AC1	T	C	G	G	T	A	G
AC1-217	AC1	C	T	A	G	T	A	G
AC1-23	AC1	T	C	A	A	C	G	A
AC1-237	AC1	C	T	G	G	T	A	G
AC1-253	AC1	T	C	G	A	T	A	G
AC1-26	AC1	C	T	G	G	T	A	G
AC1-33	AC1	C	T	A	A	T	A	G
AC1-35	AC1	C	T	G	G	T	A	G
AC1-37	AC1	C	T	G	G	T	A	G
AC1-42	AC1	C	T	A	A	C	G	A
AC1-44	AC1	T	C	A	A	T	A	A
AC1-46	AC1	C	T	A	A	T	A	G
AC1-73	AC1	T	C	A	G	T	A	G
AC1-78	AC1	C	T	A	A	T	A	G
AC1-82	AC1	C	T	G	G	T	A	G
AC1-84	AC1	C	T	G	G	C	G	A
AC1-87	AC1	C	T	A	A	T	A	A
AC1-90	AC1	C	T	G	G	T	A	G
AC1-95	AC1	C	T	G	G	C	G	G
AC2-484	AC2	C	T	G	G	T	A	G
AC2-731	AC2	C	T	A	G	T	A	G
AC2-170	AC2	C	T	A	A	T	A	G
AC2-321	AC2	C	T	G	G	T	A	G
AC2-352	AC2	C	T	G	G	T	A	G
AC2-385	AC2	C	T	A	A	T	A	G
AC2-400	AC2	C	T	A	A	T	A	G
AC2-437	AC2	C	T	A	A	T	A	G
AC2-45	AC2	C	T	G	G	T	A	G
AC2-467	AC2	C	T	A	A	T	A	G
AC2-484	AC2	C	T	G	G	T	A	G
AC2-575	AC2	C	T	A	G	T	A	G
AC2-603	AC2	C	T	G	G	T	A	G
AC2-616	AC2	C	T	G	G	T	A	G
AC2-626	AC2	C	T	G	G	T	A	G
AC2-652	AC2	C	T	G	G	T	A	G
AC2-66	AC2	C	T	G	G	T	A	G
AC2-681	AC2	C	T	A	A	T	A	G

AC2-701	AC2	C	T	G	G	T	A	G
AC2-711	AC2	C	T	A	A	T	A	G
AC2-718	AC2	C	T	A	A	T	A	G
AC2-730	AC2	C	T	G	A	T	A	G
AC2-731	AC2	C	T	A	G	T	A	G
AC2-749	AC2	C	T	A	A	T	A	G
AC2-755	AC2	C	T	G	G	T	A	G
AC2-772	AC2	C	T	A	A	T	A	G
AC2-79	AC2	C	T	A	A	T	A	G
AC2-818	AC2	C	T	G	G	T	A	G

IV_413	VII_425	VII_427	VII_475	VII_480	XI_382	XI_631	XII_50	XII_74	XV_1053
T/C	A/A	G/A	T/C	G/C	C/C	A/A	T/T	T/C	A/A
T/T	A/A	A/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A
T/T	A/A	G/A	T/C	G/C	C/C	A/A	T/T	T/T	A/A
T	A	G	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	G	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	G	C	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	G	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T/C	A	NA	T	G	C	A	T	C	A
C	A	A/G	T	C	C	A	T	C	A
C	A	A	T	G	C	A	T	C	A
C	A	G	C	C	C	A	T	C	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
C	A	G	C	G	C	A	T	T	A
C	A	A	T	G	C	A	T	C	A
C	A	NA	T	G	C	A	T	T	A
C	A	A	T	G	C	A	T	C	A
T	A	A	C	C	C	A	T	T	A

C	A	A	T	G	C	A	T	T	A
C	A	G	C	C	C	A	T	C	A
T	A	G	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	C	A
C	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	C	A
C	A	G	C	G	C	A	T	C	A
T	A	A	C	C	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
C	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	C	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	G	C	A	T	T	A
T	A	A/G	C	C	C	A	T	T	A
C	A	A	T	G	C	A	T	T	A
C	A	A	T	G	C	A	T	C	A
T	A	A	T	G	C	A	T	T	A
T	A	G	C	C	C	A	T	T	A
T	A	G	T	G	C	A	T	T	A
T	A	A	C	G	C	A	T	T	A
C	A	G	C	G	C	A	T	C	A
C	A	G	C	C	C	A	T	C	A
T	A	A	T	G	C	A	T	C	A
T	A	A	T	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A

T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	T	G	C	A	T	T	A
T	A	A	C	C	C	A	T	T	A

XV_1054	XV_483
C/C	G/T
C/C	T/T
C/C	T/T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	G
C	T
C	G
C	T
C	T
C	T
C	T
C	G
C	T
C	G
C	T

C	T
C	G
C	T
C	G
C	G
C	T
C	G
C	T
C	T
C	G
C	G
C	T
C	T
C	T
C	T
C	T
C	G
C	G
C	T
C	T
C	G
C	G
C	G
C	T
C	G
C	G
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T

C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T
C	T

NAME	grape variety	Year	Reducing sugars (g/L)	YAN (mg/L N)
M-2018	Merlot	2018	244	69
CS-2014	Cabernet Sauvignon	2014	237	134
M-2013	Merlot	2013	235	112
CS-2015	Cabernet Sauvignon	2015	218	134
CS-2017	Cabernet Sauvignon	2017	210	104
CS-2019	Cabernet Sauvignon	2019	203	102
CS-2013	Cabernet Sauvignon	2013	184	121
SB-2021	Sauvignon Blanc	2021	194	143

L-malic acid (g/L)	pH	total SO_2 **(mg/mL)**	TA (g/L H2SO4)
1.45	4.08	17	2.39
2.02	3.85	24	3.06
2.21	3.69	15	3.17
2.35	3.99	20	2.89
3.95	3.57	31	3.39
3.8	3.66	27	3.40
7.02	3.31	22	4.55
4.49	3.3	50	4.39

Vial	Strain	Must	pH must	pH wine	Malic Acid mu	Acetic acid (g/L)
83	C1-4	CS-2013	3.58	3.39	4.40	0.09
105	C1-4	CS-2013	3.58	3.32	4.40	0.08
135	FMB8	CS-2013	3.58	3.43	4.40	0.19
9	FMB8	CS-2013	3.58	3.44	4.40	0.23
90	FMC1	CS-2013	3.58	3.44	4.40	0.16
84	FMC1	CS-2013	3.58	3.39	4.40	0.21
107	FMC3	CS-2013	3.58	3.38	4.40	0.29
60	FMC3	CS-2013	3.58	3.54	4.40	0.30
100	FMGS_889	CS-2013	3.58	3.7	4.40	0.22
75	FMGS_889	CS-2013	3.58	3.6	4.40	0.25
32	FX10	CS-2013	3.58	3.48	4.40	0.30
97	FX10	CS-2013	3.58	3.52	4.40	0.30
151	M2-9	CS-2013	3.58	3.45	4.40	0.22
70	M2-9	CS-2013	3.58	3.36	4.40	0.17
23	M6-7	CS-2013	3.58	3.44	4.40	0.25
163	M6-7	CS-2013	3.58	3.5	4.40	0.23
46	X13C	CS-2013	3.58	3.49	4.40	0.10
113	X13C	CS-2013	3.58	3.4	4.40	0.13
98	X56B	CS-2013	3.58	3.51	4.40	0.23
25	X56B	CS-2013	3.58	3.48	4.40	0.12
47	X8B	CS-2013	3.58	3.48	4.40	0.16
141	X8B	CS-2013	3.58	3.44	4.40	0.12
27	ZF15	CS-2013	3.58	3.52	4.40	0.19
130	ZF15	CS-2013	4.11	3.49	4.40	0.21
48	C1-4	CS-2014	4.11	3.83	2.03	0.17
166	C1-4	CS-2014	4.11	3.88	2.03	0.10
134	FMB8	CS-2014	4.11	3.78	2.03	0.29
73	FMB8	CS-2014	4.11	3.7	2.03	0.23
164	FMC1	CS-2014	4.11	3.78	2.03	0.31
31	FMC1	CS-2014	4.11	3.76	2.03	0.30
161	FMC3	CS-2014	4.11	3.87	2.03	0.38
74	FMC3	CS-2014	4.11	3.77	2.03	0.29
138	FMGS_889	CS-2014	4.11	4.11	2.03	0.23
37	FMGS_889	CS-2014	4.11	4.13	2.03	0.29
155	FX10	CS-2014	4.11	3.87	2.03	0.33
4	FX10	CS-2014	4.11	3.91	2.03	0.36
82	M2-9	CS-2014	4.11	3.8	2.03	0.26
109	M2-9	CS-2014	4.11	3.76	2.03	0.30
40	M6-7	CS-2014	4.11	3.82	2.03	0.29
126	M6-7	CS-2014	4.11	3.75	2.03	0.14
125	X13C	CS-2014	4.11	3.81	2.03	0.15
7	X13C	CS-2014	4.11	3.94	2.03	0.15
11	X56B	CS-2014	4.11	3.89	2.03	0.15
104	X56B	CS-2014	4.11	3.78	2.03	0.13
69	X8B	CS-2014	4.11	3.62	2.03	0.11
145	X8B	CS-2014	4.11	3.68	2.03	0.11
99	ZF15	CS-2014	4.11	3.9	2.03	0.31
44	ZF15	CS-2014	4.11	3.89	2.03	0.26
88	C1-4	CS-2015	3.99	3.7	2.35	0.13

38	C1-4	CS-2015	3.99	3.73	2.35	0.12
85	FMB8	CS-2015	3.99	3.6	2.35	0.13
54	FMB8	CS-2015	3.99	3.71	2.35	0.15
79	FMC1	CS-2015	3.99	3.6	2.35	0.18
154	FMC1	CS-2015	3.99	3.71	2.35	0.22
21	FMC3	CS-2015	3.99	3.72	2.35	0.28
94	FMC3	CS-2015	3.99	3.76	2.35	0.29
110	FMGS_889	CS-2015	3.99	3.9	2.35	0.16
18	FMGS_889	CS-2015	3.99	3.96	2.35	0.23
59	FX10	CS-2015	3.99	3.8	2.35	0.39
153	FX10	CS-2015	3.99	3.82	2.35	0.26
20	M2-9	CS-2015	3.99	3.7	2.35	0.19
101	M2-9	CS-2015	3.99	3.64	2.35	0.23
12	M6-7	CS-2015	3.99	3.72	2.35	0.12
127	M6-7	CS-2015	3.99	3.66	2.35	0.13
96	X13C	CS-2015	3.99	3.77	2.35	0.09
22	X13C	CS-2015	3.99	3.74	2.35	0.08
58	X56B	CS-2015	3.99	3.82	2.35	0.09
167	X56B	CS-2015	3.99	3.77	2.35	0.13
35	X8B	CS-2015	3.99	3.63	2.35	0.07
92	X8B	CS-2015	3.99	3.71	2.35	0.12
160	ZF15	CS-2015	3.99	3.86	2.35	0.29
39	ZF15	CS-2015	3.99	3.78	2.35	0.20
114	C1-4	CS-2017	3.89	3.56	2.86	0.09
17	C1-4	CS-2017	3.89	3.64	2.86	0.10
140	FMB8	CS-2017	3.89	3.6	2.86	0.19
61	FMB8	CS-2017	3.89	3.65	2.86	0.19
78	FMC1	CS-2017	3.89	3.58	2.86	0.26
148	FMC1	CS-2017	3.89	3.64	2.86	0.31
124	FMC3	CS-2017	3.89	3.63	2.86	0.34
71	FMC3	CS-2017	3.89	3.59	2.86	0.28
13	FMGS_889	CS-2017	3.89	3.96	2.86	0.20
103	FMGS_889	CS-2017	3.89	3.85	2.86	0.23
111	FX10	CS-2017	3.89	3.58	2.86	0.31
1	FX10	CS-2017	3.89	3.66	2.86	0.26
102	M2-9	CS-2017	3.89	3.49	2.86	0.21
15	M2-9	CS-2017	3.89	3.61	2.86	0.19
65	M6-7	CS-2017	3.89	3.69	2.86	0.18
91	M6-7	CS-2017	3.89	3.64	2.86	0.18
68	X13C	CS-2017	3.89	3.65	2.86	0.25
149	X13C	CS-2017	3.89	3.73	2.86	0.14
142	X56B	CS-2017	3.89	3.75	2.86	0.13
49	X56B	CS-2017	3.89	3.69	2.86	0.09
156	X8B	CS-2017	3.89	3.65	2.86	0.09
30	X8B	CS-2017	3.89	3.64	2.86	0.13
34	ZF15	CS-2017	3.89	3.73	2.86	0.21
89	ZF15	CS-2017	3.89	3.72	2.86	0.19
147	C1-4	CS-2019	3.95	3.7	2.95	0.11
5	C1-4	CS-2019	3.95	3.7	2.95	0.07
43	FMB8	CS-2019	3.95	3.61	2.95	0.13

122	FMB8	CS-2019	3.95	3.53	2.95	0.20
132	FMC1	CS-2019	3.95	3.61	2.95	0.13
6	FMC1	CS-2019	3.95	3.69	2.95	0.15
41	FMC3	CS-2019	3.95	3.68	2.95	0.27
121	FMC3	CS-2019	3.95	3.61	2.95	0.26
168	FMGS_889	CS-2019	3.95	3.95	2.95	0.13
80	FMGS_889	CS-2019	3.95	3.89	2.95	0.13
117	FX10	CS-2019	3.95	3.64	2.95	0.27
10	FX10	CS-2019	3.95	3.69	2.95	0.28
86	M2-9	CS-2019	3.95	3.57	2.95	0.12
56	M2-9	CS-2019	3.95	3.66	2.95	0.32
29	M6-7	CS-2019	3.95	3.61	2.95	0.19
143	M6-7	CS-2019	3.95	3.64	2.95	0.11
2	X13C	CS-2019	3.95	3.76	2.95	0.32
112	X13C	CS-2019	3.95	3.62	2.95	0.46
24	X56B	CS-2019	3.95	3.67	2.95	0.08
137	X56B	CS-2019	3.95	3.72	2.95	0.09
93	X8B	CS-2019	3.95	3.62	2.95	0.06
57	X8B	CS-2019	3.95	3.65	2.95	0.05
116	ZF15	CS-2019	3.95	3.66	2.95	0.16
36	ZF15	CS-2019	3.95	3.74	2.95	0.16
87	C1-4	M-2013	3.69	3.48	2.21	0.25
62	C1-4	M-2013	3.69	3.53	2.21	0.19
42	FMB8	M-2013	3.69	3.44	2.21	0.38
106	FMB8	M-2013	3.69	3.37	2.21	0.39
45	FMC1	M-2013	3.69	3.48	2.21	0.45
115	FMC1	M-2013	3.69	3.41	2.21	0.47
162	FMC3	M-2013	3.69	3.54	2.21	0.50
26	FMC3	M-2013	3.69	3.5	2.21	0.51
139	FMGS_889	M-2013	3.69	3.64	2.21	0.31
14	FMGS_889	M-2013	3.69	3.65	2.21	0.38
95	FX10	M-2013	3.69	3.53	2.21	0.49
67	FX10	M-2013	3.69	3.51	2.21	0.51
72	M2-9	M-2013	3.69	3.41	2.21	0.44
119	M2-9	M-2013	3.69	3.43	2.21	0.41
77	M6-7	M-2013	3.69	3.41	2.21	0.29
146	M6-7	M-2013	3.69	3.45	2.21	0.31
120	X13C	M-2013	3.69	3.46	2.21	0.44
3	X13C	M-2013	3.69	3.59	2.21	0.26
118	X56B	M-2013	3.69	3.42	2.21	0.24
8	X56B	M-2013	3.69	3.59	2.21	0.24
16	X8B	M-2013	3.69	3.44	2.21	0.25
131	X8B	M-2013	3.69	3.46	2.21	0.26
157	ZF15	M-2013	3.69	3.53	2.21	0.59
50	ZF15	M-2013	3.69	3.5	2.21	0.38
28	C1-4	M-2018	4.08	3.55	1.46	0.12
165	C1-4	M-2018	4.08	3.56	1.46	0.12
123	FMB8	M-2018	4.08	3.47	1.46	0.12
55	FMB8	M-2018	4.08	3.53	1.46	0.15
81	FMC1	M-2018	4.08	3.46	1.46	0.09

150	FMC1	M-2018	4.08	3.52	1.46	0.09
52	FMC3	M-2018	4.08	3.64	1.46	0.22
128	FMC3	M-2018	4.08	3.57	1.46	0.21
133	FMGS_889	M-2018	4.08	3.8	1.46	0.13
33	FMGS_889	M-2018	4.08	3.84	1.46	0.12
64	FX10	M-2018	4.08	3.7	1.46	0.30
136	FX10	M-2018	4.08	3.59	1.46	0.25
152	M2-9	M-2018	4.08	3.55	1.46	0.15
19	M2-9	M-2018	4.08	3.56	1.46	0.20
76	M6-7	M-2018	4.08	3.52	1.46	0.20
144	M6-7	M-2018	4.08	3.52	1.46	0.10
108	X13C	M-2018	4.08	3.49	1.46	0.09
66	X13C	M-2018	4.08	3.61	1.46	0.10
159	X56B	M-2018	4.08	3.63	1.46	0.16
53	X56B	M-2018	4.08	3.66	1.46	0.11
63	X8B	M-2018	4.08	3.56	1.46	0.09
158	X8B	M-2018	4.08	3.47	1.46	0.10
51	ZF15	M-2018	4.08	3.64	1.46	0.16
129	ZF15	M-2018	4.08	3.58	1.46	0.19

Malic acid (g/l	Glycerol (g/L)	Glucose (g/L)	Fructose (g/L)	Residual guga	Succinic acid (	CO2max
3.05	6.18	0.69	1.76	2.45	1.64	82.66
3.26	5.42	0.77	2.08	2.84	1.83	82.73
2.23	5.88	0.69	1.43	2.12	2.28	84.39
3.23	7.31	0.65	0.50	1.15	2.47	82.33
2.54	7.78	0.72	0.68	1.40	2.64	82.23
3.48	7.33	0.69	0.44	1.13	3.28	74.86
2.38	6.17	0.69	1.81	2.50	3.73	83.09
2.67	7.74	0.74	0.60	1.34	2.87	83.47
1.58	7.19	0.54	0.58	1.12	2.09	85.46
1.61	7.23	0.65	0.15	0.80	2.23	85.26
3.39	6.74	0.73	0.52	1.24	2.20	83.56
3.50	6.51	0.09	0.88	0.98	-0.41	83.76
3.04	6.34	0.68	0.72	1.40	2.76	80.87
3.53	6.45	0.64	0.72	1.36	2.34	79.78
2.88	6.64	0.68	0.72	1.40	3.24	81.82
2.99	5.41	0.68	0.45	1.13	3.04	83.68
3.07	4.69	0.61	0.93	1.55	1.88	86.91
3.15	5.57	0.77	0.68	1.45	1.71	83.67
2.68	6.00	0.96	0.93	1.89	2.24	83.03
2.88	5.96	0.70	0.59	1.29	2.04	83.13
2.94	8.33	0.70	0.25	0.95	2.18	82.00
3.12	8.62	0.68	0.64	1.32	2.28	82.45
2.10	6.05	0.66	0.29	0.95	2.19	84.37
2.85	7.00	0.64	0.63	1.27	2.60	83.40
2.41	7.63	0.80	0.79	1.60	2.89	106.08
2.52	5.17	0.59	2.08	2.67	1.86	105.11
2.54	7.40	0.74	0.36	1.10	3.16	107.57
2.64	7.70	0.77	1.01	1.78	0.01	105.42
2.66	8.25	0.32	0.35	0.67	3.56	101.61
2.74	9.04	0.70	0.71	1.41	3.73	108.63
2.21	7.94	0.80	-0.09	0.71	3.08	104.31
2.31	8.62	0.65	0.68	1.33	3.46	105.95
1.11	6.87	0.97	0.85	1.81	2.48	108.64
1.16	7.77	0.73	0.85	1.57	2.59	107.97
1.74	4.83	-0.05	0.79	0.75	-0.22	104.73
1.79	8.11	0.78	-0.82	-0.04	2.23	106.53
2.43	6.03	0.68	0.52	1.19	2.99	106.99
2.44	6.44	0.74	0.46	1.20	2.31	107.30
2.15	4.90	0.70	0.46	1.17	3.30	106.95
2.66	6.94	0.70	0.91	1.61	3.10	105.76
1.82	5.43	0.70	0.15	0.85	1.34	108.55
1.99	5.54	0.88	0.86	1.74	2.49	105.71
1.81	6.97	1.38	1.73	3.11	2.00	102.20
2.20	7.20	0.68	-0.34	0.34	1.36	108.23
2.34	9.91	0.88	0.65	1.54	2.52	102.37
2.99	7.78	0.80	0.59	1.40	2.66	100.56
1.44	6.12	0.70	0.65	1.36	2.59	106.53
2.05	8.03	0.85	0.73	1.59	3.42	106.26
2.13	7.40	0.18	0.40	0.58	2.88	100.95

2.29	5.54	0.78	0.31	1.09	2.81	101.45
2.63	6.80	0.66	0.90	1.56	2.66	99.30
2.64	6.74	0.72	0.68	1.40	2.96	100.41
2.10	7.68	0.68	0.67	1.34	4.10	99.56
2.35	6.58	0.66	0.39	1.05	3.42	99.42
1.64	7.30	0.73	0.99	1.71	2.18	100.89
1.67	6.83	0.70	0.38	1.08	2.62	103.12
0.77	7.70	0.82	0.35	1.17	3.40	103.98
1.22	7.63	0.70	0.96	1.66	2.46	102.80
1.61	8.12	0.74	0.60	1.34	2.56	102.73
2.22	6.72	0.63	0.52	1.14	1.99	100.81
2.01	5.68	0.73	0.55	1.28	1.92	101.51
2.15	5.70	0.68	0.50	1.18	2.34	102.68
2.58	6.75	0.74	0.46	1.20	-0.37	100.82
2.62	5.41	0.54	0.49	1.03	2.47	101.55
2.03	6.31	0.70	0.13	0.84	1.45	102.54
2.06	6.04	0.75	0.83	1.59	2.05	102.22
1.96	5.21	0.82	0.46	1.28	1.77	102.16
2.16	7.41	0.17	0.53	0.70	1.75	99.25
2.21	9.00	0.74	0.54	1.28	3.26	99.88
2.23	5.51	0.46	0.54	1.00	2.18	93.04
1.62	6.50	0.79	0.41	1.20	3.02	93.43
1.96	7.91	0.75	0.62	1.37	2.34	91.28
2.12	6.66	0.68	0.39	1.07	1.92	88.73
2.45	7.00	0.73	0.77	1.50	2.20	93.24
2.73	7.55	0.58	1.05	1.62	2.82	88.24
3.06	7.88	0.84	0.26	1.10	3.14	88.17
2.54	8.88	0.78	0.69	1.47	3.64	92.11
2.77	8.34	0.72	0.44	1.15	2.91	86.27
2.28	8.03	0.77	0.58	1.34	3.22	88.90
2.32	8.76	0.73	0.26	0.99	3.28	88.91
1.22	7.36	0.61	0.58	1.19	2.78	91.78
1.22	8.37	0.70	0.63	1.33	2.72	101.44
1.98	7.10	0.47	0.86	1.33	2.20	90.03
2.52	8.23	0.72	0.43	1.14	2.50	89.95
2.64	7.61	0.72	1.19	1.90	3.68	91.18
2.76	7.26	0.74	-0.51	0.23	3.02	88.04
2.71	7.85	0.83	0.46	1.29	3.18	89.06
2.97	7.49	0.79	0.67	1.46	3.11	88.82
2.41	7.21	0.83	0.13	0.96	2.32	90.86
2.44	6.64	0.72	0.50	1.22	2.15	97.84
2.23	6.04	0.65	0.71	1.36	2.06	99.73
2.66	6.57	0.61	0.46	1.08	2.05	96.92
1.92	7.57	0.69	0.40	1.09	1.92	95.17
2.38	6.83	0.70	0.68	1.38	2.27	97.69
1.85	8.83	0.74	0.52	1.26	2.41	98.22
1.97	9.49	0.82	0.46	1.28	2.98	97.93
2.43	5.66	0.64	0.85	1.48	2.03	91.71
2.82	6.47	0.70	1.07	1.78	2.16	92.29
2.93	7.20	0.68	-0.65	0.02	3.00	90.50

3.02	6.26	0.84	0.64	1.48	2.43	92.24
2.52	7.03	0.74	1.10	1.84	2.71	90.96
2.66	8.45	0.78	0.59	1.37	3.32	90.99
1.97	6.68	0.73	0.85	1.57	2.98	91.86
2.01	5.67	0.79	0.74	1.54	3.12	91.99
1.10	5.48	0.73	1.02	1.75	2.28	91.96
1.22	7.16	0.78	-1.01	-0.23	3.17	94.26
2.33	6.22	0.59	0.83	1.42	1.94	93.08
2.45	7.25	0.70	0.82	1.52	2.29	92.67
2.49	7.10	0.70	1.05	1.75	2.81	91.13
2.69	8.01	0.16	0.36	0.52	3.28	92.33
2.51	6.78	0.77	0.57	1.33	2.98	92.36
2.63	5.58	0.78	0.82	1.60	1.94	93.09
1.77	6.59	0.73	-0.59	0.14	3.40	93.17
1.79	6.43	0.69	0.71	1.40	1.44	94.12
2.24	6.42	0.70	0.59	1.29	2.26	87.91
2.37	6.56	0.72	0.49	1.20	1.74	92.70
2.16	6.56	0.85	0.81	1.66	2.12	90.26
2.23	6.57	0.69	0.60	1.29	1.81	86.24
1.44	6.32	0.21	0.69	0.90	2.17	94.99
1.62	6.24	0.75	0.67	1.42	2.04	92.63
1.64	8.35	0.68	0.86	1.54	2.64	106.95
1.93	7.41	0.70	1.42	2.12	2.36	105.68
2.00	7.45	0.66	0.71	1.37	0.16	104.54
2.16	8.30	0.83	0.68	1.51	2.87	104.55
1.65	9.73	0.52	2.05	2.58	3.00	105.46
1.83	7.34	0.85	0.64	1.50	2.10	105.98
1.57	8.69	0.69	0.15	0.84	2.64	104.68
1.62	9.01	0.74	0.82	1.56	3.26	105.58
0.60	7.08	0.69	0.57	1.26	3.74	109.29
0.75	8.42	0.69	-0.55	0.14	2.78	105.04
1.61	7.62	0.69	0.59	1.28	2.30	106.00
2.14	8.86	0.72	0.71	1.42	2.50	106.18
1.96	7.45	0.65	1.19	1.84	2.54	104.73
1.97	7.87	0.56	0.69	1.26	2.18	105.75
1.90	7.61	0.49	1.00	1.48	2.22	105.44
2.32	7.82	0.73	0.77	1.50	0.19	104.02
1.44	7.32	0.69	0.25	0.94	3.22	108.33
1.85	7.92	0.21	0.46	0.67	2.10	108.15
1.63	7.29	0.69	0.69	1.38	3.08	106.21
1.82	8.51	0.68	0.88	1.56	1.99	106.28
1.91	7.28	0.41	0.74	1.15	2.58	104.29
2.00	8.87	0.66	0.58	1.24	2.83	106.29
1.22	7.79	0.64	0.50	1.14	5.42	120.95
1.52	6.61	0.68	0.73	1.41	3.14	106.85
2.01	5.95	0.65	0.73	1.38	3.10	116.05
2.07	7.28	0.68	0.87	1.55	2.92	112.79
2.23	8.55	0.66	0.87	1.54	3.94	115.28
2.30	9.04	0.80	0.65	1.46	4.36	115.09
2.09	8.64	0.75	0.85	1.60	3.88	117.92

2.32	9.72	0.70	1.02	1.73	4.38	112.26
1.30	8.45	0.58	0.52	1.09	3.78	116.28
1.56	9.61	0.77	0.59	1.36	3.78	118.52
0.75	8.43	0.63	0.59	1.22	3.31	119.39
0.82	8.80	0.65	0.78	1.43	3.20	118.78
1.61	7.96	0.46	0.48	0.94	2.89	118.05
1.65	8.46	0.52	0.71	1.23	2.99	118.00
1.35	8.01	0.64	0.08	0.72	0.55	112.11
1.74	8.66	0.69	1.28	1.97	4.30	114.24
1.72	7.78	0.74	0.59	1.33	4.01	115.91
2.06	7.40	0.69	1.00	1.69	3.62	114.99
1.56	7.35	0.68	0.87	1.55	2.41	117.15
1.61	5.94	0.14	0.44	0.58	2.63	116.64
1.68	7.25	0.72	0.65	1.37	2.75	115.29
2.14	7.81	0.75	0.93	1.69	2.38	116.05
2.28	9.13	0.69	0.77	1.46	2.22	114.39
2.76	9.98	0.11	0.76	0.86	3.23	112.28
1.48	9.25	0.58	0.62	1.19	4.33	118.59
1.51	1.00	0.40	0.50	0.90	2.80	116.67

lp (h)	V50-80 (g.h-1,	t35g (h)	t50g (h)	t80g (h)	MAC%	delta pH
7	0.94	38	57	171	30.66	-0.19
11	1.08	31	48	151	26.02	-0.26
6	1.13	38	55	150	49.29	-0.15
11	1.35	32	49	135	26.62	-0.14
10	1.28	29	46	142	42.25	-0.14
10	0.78	33	53	NA	20.87	-0.19
10	1.62	27	42	106	45.95	-0.2
9	1.84	28	42	99	39.42	-0.04
10	1.78	31	45	98	64.15	0.12
10	1.49	32	47	118	63.38	0.02
10	1.62	27	41	112	23.02	-0.1
10	1.62	27	41	112	20.61	-0.06
6	0.59	46	73	265	30.92	-0.13
6	0.78	41	64	NA	19.84	-0.22
11	0.95	41	67	164	34.69	-0.14
6	0.72	46	71	204	32.03	-0.08
10	1.35	32	49	115	30.40	-0.09
7	1.06	36	53	158	28.51	-0.18
10	1.08	37	59	143	39.25	-0.07
11	1.08	35	55	145	34.52	-0.1
11	1.31	30	46	145	33.15	-0.1
9	1.20	35	51	152	29.20	-0.14
10	1.90	27	41	92	52.21	-0.06
10	1.78	26	40	105	35.21	-0.62
7	1.21	31	44	82	-19.01	-0.28
8	1.15	31	44	85	-24.05	-0.23
7	1.21	37	51	91	-24.98	-0.33
8	1.23	35	50	89	-30.20	-0.41
7	0.91	37	55	107	-30.95	-0.33
7	1.06	39	54	100	-35.05	-0.35
6	1.25	32	44	80	-8.95	-0.24
10	1.45	28	40	73	-13.79	-0.34
11	1.73	28	40	70	45.30	0
11	1.55	27	40	72	42.69	0.02
10	1.45	26	37	69	14.17	-0.24
10	1.58	26	37	69	11.56	-0.2
7	0.85	39	55	110	-19.76	-0.31
6	0.91	37	52	103	-20.13	-0.35
6	1.17	43	62	107	-5.97	-0.29
6	0.79	41	59	119	-31.32	-0.36
7	0.96	37	52	99	10.07	-0.3
8	1.09	36	54	100	1.86	-0.17
7	0.79	41	57	112	10.81	-0.22
7	0.89	39	54	108	-8.58	-0.33
9	1.02	37	54	105	-15.47	-0.49
9	0.89	36	53	107	-47.35	-0.43
10	1.65	28	40	71	29.27	-0.21
10	1.51	27	39	71	-1.12	-0.22
10	1.31	27	41	86	9.17	-0.29

10	1.36	26	40	82	2.41	-0.26
10	1.28	30	45	88	-12.07	-0.39
7	1.19	34	48	95	-12.39	-0.28
10	1.42	29	44	85	10.62	-0.39
6	1.11	36	52	102	0.00	-0.28
10	1.45	26	38	77	30.09	-0.27
7	1.42	30	41	79	28.80	-0.23
10	1.82	27	39	70	67.42	-0.09
10	1.72	28	40	73	48.11	-0.03
10	1.92	25	36	66	31.38	-0.19
8	1.52	30	41	77	5.63	-0.17
6	0.93	36	52	111	14.32	-0.29
6	1.02	36	52	106	8.53	-0.35
6	0.74	41	60	135	-9.82	-0.27
6	0.84	39	58	125	-11.59	-0.33
6	0.92	38	55	114	13.68	-0.22
8	1.07	34	50	102	12.39	-0.25
6	0.85	40	59	125	16.74	-0.17
7	0.98	36	54	112	7.88	-0.22
8	1.26	34	49	95	5.95	-0.36
7	0.77	41	57	127	5.31	-0.28
6	1.02	34	49	96	31.06	-0.13
6	0.88	36	52	112	16.57	-0.21
10	0.98	30	46	106	26.01	-0.33
10	1.17	29	45	97	14.39	-0.25
10	1.02	32	48	106	4.62	-0.29
11	1.07	33	50	106	-6.73	-0.24
7	1.11	34	50	105	11.22	-0.31
10	0.85	32	49	117	3.43	-0.25
9	1.31	28	42	86	20.46	-0.26
10	1.19	28	43	91	19.14	-0.3
10	1.37	31	46	90	57.43	0.07
11	1.91	29	41	73	57.43	-0.04
10	1.47	28	40	81	30.89	-0.31
10	1.40	28	41	84	12.01	-0.23
7	0.86	38	61	131	7.79	-0.4
7	0.77	38	60	137	3.83	-0.28
6	0.65	47	70	161	5.41	-0.2
6	0.62	47	71	167	-3.56	-0.25
6	0.95	42	60	123	15.97	-0.24
6	1.26	37	53	101	14.92	-0.16
7	1.24	40	55	104	22.18	-0.14
7	1.11	37	55	110	7.00	-0.2
10	1.47	31	45	87	33.14	-0.24
6	1.37	37	52	95	16.77	-0.25
10	1.75	26	38	72	35.25	-0.16
10	1.75	26	38	73	31.29	-0.17
6	1.22	35	51	106	17.68	-0.25
10	1.22	29	44	97	4.61	-0.25
11	1.24	31	47	100	0.77	-0.34

11	1.24	31	47	98	-2.18	-0.42
11	1.24	32	48	100	14.74	-0.34
10	1.22	32	48	101	9.99	-0.26
11	1.60	27	40	81	33.19	-0.27
10	1.56	28	40	82	32.03	-0.34
11	1.52	30	45	89	62.66	0
11	1.69	30	44	82	58.69	-0.06
10	1.69	26	37	75	21.14	-0.31
10	1.69	27	38	76	17.04	-0.26
7	0.92	38	59	130	15.50	-0.38
6	0.91	41	60	132	8.97	-0.29
6	0.82	45	67	146	14.99	-0.34
7	0.80	44	64	145	10.89	-0.31
11	1.74	30	44	82	39.98	-0.19
10	1.85	31	45	80	39.47	-0.33
8	0.86	39	59	137	24.22	-0.28
9	1.00	35	53	118	19.73	-0.23
10	1.00	36	53	119	26.91	-0.33
11	0.86	33	52	131	24.35	-0.3
10	1.56	27	39	80	51.25	-0.29
10	1.56	28	40	83	44.97	-0.21
6	1.14	37	52	95	25.67	-0.21
8	1.14	34	50	94	12.66	-0.16
7	1.19	36	51	92	9.58	-0.25
7	1.22	34	49	89	2.22	-0.32
6	1.10	38	53	98	25.32	-0.21
6	1.07	37	53	97	17.28	-0.28
6	1.24	36	50	87	29.09	-0.15
11	1.53	28	41	75	26.69	-0.19
8	1.68	32	43	74	73.06	-0.05
10	1.53	30	43	78	66.05	-0.04
11	1.60	28	40	72	27.03	-0.16
9	1.60	29	41	73	3.08	-0.18
7	0.79	40	57	119	11.29	-0.28
6	0.92	37	53	105	10.78	-0.26
6	0.71	43	63	132	14.20	-0.28
6	0.71	43	63	132	-5.13	-0.24
7	1.36	37	52	89	34.91	-0.23
7	1.04	39	54	99	16.43	-0.1
9	1.31	34	49	89	26.18	-0.27
7	0.95	41	57	107	17.45	-0.1
9	1.22	34	48	89	13.52	-0.25
7	1.12	40	54	97	9.41	-0.23
6	2.82	27	37	58	44.66	-0.16
10	1.53	28	40	72	31.31	-0.19
6	0.98	38	56	107	-37.88	-0.53
6	0.87	39	57	113	-42.03	-0.52
7	0.99	40	55	103	-53.19	-0.61
7	1.09	40	56	101	-57.60	-0.55
6	0.94	40	56	107	-43.33	-0.62

6	0.80	39	56	113	-59.41	-0.56
6	1.22	36	51	89	11.16	-0.44
6	1.36	34	47	81	-7.26	-0.51
7	1.49	37	51	85	48.26	-0.28
7	1.44	36	50	84	43.85	-0.24
7	1.38	35	48	82	-10.38	-0.38
8	1.53	30	42	75	-12.97	-0.49
6	0.71	44	66	137	7.52	-0.53
6	0.94	43	62	116	-19.46	-0.52
6	0.72	50	77	147	-17.90	-0.56
7	0.56	47	74	158	-41.25	-0.56
7	0.82	39	56	113	-7.01	-0.59
7	0.92	45	66	122	-10.12	-0.47
7	0.92	43	62	119	-15.05	-0.45
7	0.80	44	63	127	-46.96	-0.42
7	0.89	41	57	111	-56.30	-0.52
7	0.86	40	57	116	-89.25	-0.61
6	1.38	36	50	84	-1.56	-0.44
6	1.31	34	48	82	-3.63	-0.5

background	Strain	Must	lag phase (h)	CO2max	t35g (h)	t80g (h)
AC1	AC1	CS14	10	108.13	54	154
AC1	AC1	CS14	10	108.38	54	150
AC1	AC1	CS14	11	107.78	58	167
AC1	AC1	CS14	9	103.77	57	169
AC1	AC1	CS14	8	108.59	55	159
AC1	AC1	CS14	9	108.16	50	156
AC1	AC1-1	CS14	9	106.45	61	204
AC1	AC1-1	CS14	9	110.17	59	184
AC1	AC1-1	CS14	9	104.69	62	212
AC1	AC1-103	CS14	10	109.99	85	228
AC1	AC1-103	CS14	11	109.68	82	216
AC1	AC1-103	CS14	10	106.98	96	261
AC1	AC1-115	CS14	10	108.81	69	200
AC1	AC1-115	CS14	10	109.51	69	199
AC1	AC1-115	CS14	10	108.54	69	198
AC1	AC1-123	CS14	9	108.54	54	142
AC1	AC1-123	CS14	9	109.12	56	146
AC1	AC1-123	CS14	9	105.12	60	161
AC1	AC1-129	CS14	10	108.61	58	176
AC1	AC1-129	CS14	9	109.81	54	164
AC1	AC1-129	CS14	10	109.78	59	180
AC1	AC1-13	CS14	10	101.01	79	296
AC1	AC1-13	CS14	9	107.54	82	263
AC1	AC1-13	CS14	9	105.33	82	284
AC1	AC1-13	CS14	10	100.25	81	275
AC1	AC1-13	CS14	9	102.81	78	255
AC1	AC1-13	CS14	9	97.93	80	289
AC1	AC1-13	CS15	9	83.39	79	375
AC1	AC1-13	CS15	9	87.59	79	316
AC1	AC1-13	CS15	10	87.68	86	370
AC1	AC1-152	CS14	9	86.96	85	308
AC1	AC1-152	CS14	9	107.39	70	212
AC1	AC1-152	CS14	9	106.48	69	216
AC1	AC1-178	CS14	9	107.83	70	210
AC1	AC1-178	CS14	9	108.05	72	211
AC1	AC1-178	CS14	10	108.79	71	208
AC1	AC1-182	CS14	10	107.9	46	141
AC1	AC1-182	CS14	9	109.11	47	142
AC1	AC1-182	CS14	9	110.92	46	128
AC1	AC1-184	CS14	9	109.5	59	183
AC1	AC1-184	CS14	9	109.3	59	185
AC1	AC1-184	CS14	9	104.73	62	198
AC1	AC1-187	CS14	9	107.89	58	186
AC1	AC1-187	CS14	9	109.09	58	179
AC1	AC1-187	CS14	10	108.59	57	173
AC1	AC1-191	CS14	10	108.44	54	175

AC1	AC1-191	CS14	10	108.21	56	182
AC1	AC1-191	CS14	10	108.02	56	184
AC1	AC1-191	CS14	9	102.06	56	190
AC1	AC1-191	CS14	9	106.28	54	178
AC1	AC1-191	CS14	9	107.41	52	170
AC1	AC1-191	CS15	9	100.19	58	172
AC1	AC1-191	CS15	9	102.72	57	170
AC1	AC1-191	CS15	10	102.81	57	174
AC1	AC1-193	CS14	10	108.09	55	165
AC1	AC1-193	CS14	10	107.34	54	166
AC1	AC1-193	CS14	9	108.43	55	165
AC1	AC1-196	CS14	9	109.02	60	190
AC1	AC1-196	CS14	9	108.02	59	173
AC1	AC1-196	CS14	9	109.58	63	190
AC1	AC1-197	CS14	10	107.99	66	207
AC1	AC1-197	CS14	10	108.05	60	191
AC1	AC1-197	CS14	10	109.05	67	197
AC1	AC1-201	CS14	10	109.26	62	190
AC1	AC1-201	CS14	9	108.8	60	172
AC1	AC1-201	CS14	9	109.01	62	186
AC1	AC1-203	CS14	10	109.78	65	191
AC1	AC1-203	CS14	9	108.8	65	192
AC1	AC1-203	CS14	10	109.63	65	185
AC1	AC1-205	CS14	9	108.76	56	155
AC1	AC1-205	CS14	10	109.19	56	153
AC1	AC1-205	CS14	9	107.76	57	151
AC1	AC1-207	CS14	9	108.01	50	161
AC1	AC1-207	CS14	9	108.68	48	139
AC1	AC1-207	CS14	9	108.52	47	133
AC1	AC1-212	CS14	9	106.64	58	170
AC1	AC1-212	CS14	9	108.52	56	163
AC1	AC1-212	CS14	10	109.43	56	157
AC1	AC1-217	CS14	9	108.28	62	181
AC1	AC1-217	CS14	9	107.06	60	194
AC1	AC1-217	CS14	10	107.2	62	194
AC1	AC1-217	CS14	9	107.6	59	187
AC1	AC1-217	CS14	9	107.49	61	190
AC1	AC1-217	CS14	9	107.63	58	182
AC1	AC1-217	CS15	8	98.18	62	181
AC1	AC1-217	CS15	8	101.25	62	176
AC1	AC1-217	CS15	10	101.44	60	201
AC1	AC1-23	CS14	9	109.23	47	140
AC1	AC1-23	CS14	9	108.21	46	146
AC1	AC1-23	CS14	9	107.73	47	149
AC1	AC1-237	CS14	10	108.77	57	181
AC1	AC1-237	CS14	9	108.89	61	181
AC1	AC1-237	CS14	9	108.4	55	180

AC1	AC1-253	CS14	10	109.08		57	165
AC1	AC1-253	CS14	10	109.14		53	160
AC1	AC1-253	CS14	10	109.04		56	158
AC1	AC1-26	CS14	10	108.04		50	153
AC1	AC1-26	CS14	9	107.96		53	152
AC1	AC1-26	CS14	10	107.91		50	149
AC1	AC1-262	CS14	9	109.87		67	199
AC1	AC1-262	CS14	9	108.95		69	211
AC1	AC1-262	CS14	10	109.83		64	194
AC1	AC1-264	CS14	10	106.57		60	196
AC1	AC1-264	CS14	9	107.6		58	193
AC1	AC1-264	CS14	9	107.19		60	195
AC1	AC1-33	CS14	9	109.13		59	177
AC1	AC1-33	CS14	9		110	61	177
AC1	AC1-33	CS14	10	108.75		61	179
AC1	AC1-35	CS14	9	109.38		56	165
AC1	AC1-35	CS14	9	110.14		56	167
AC1	AC1-35	CS14	9	109.84		55	162
AC1	AC1-37	CS14	9	107.02		75	206
AC1	AC1-37	CS14	10	107.92		75	202
AC1	AC1-37	CS14	10	107.49		71	198
AC1	AC1-42	CS14	10	107.79		69	216
AC1	AC1-42	CS14	10	108.07		69	211
AC1	AC1-42	CS14	10	107.46		71	205
AC1	AC1-42	CS14	10		109	70	210
AC1	AC1-42	CS14	10	110.15		67	206
AC1	AC1-42	CS14	9	108.24		72	223
AC1	AC1-44	CS14	9	109.35		49	135
AC1	AC1-44	CS14	9	109.53		50	136
AC1	AC1-44	CS14	9	109.07		47	127
AC1	AC1-46	CS14	9	109.29		62	173
AC1	AC1-46	CS14	9	105.88		70	215
AC1	AC1-46	CS14	9	104.29		70	220
AC1	AC1-46	CS14	9	106.92		63	209
AC1	AC1-46	CS14	8	103.54		68	232
AC1	AC1-46	CS14	9	109.38		66	199
AC1	AC1-46	CS15	8	97.26		66	197
AC1	AC1-46	CS15	8	97.88		65	191
AC1	AC1-46	CS15	9	99.23		65	218
AC1	AC1-60	CS14	10	109.1		50	149
AC1	AC1-60	CS14	10	108.69		51	147
AC1	AC1-60	CS14	10	109.78		51	150
AC1	AC1-73	CS14	10	109.18		49	138
AC1	AC1-73	CS14	10	108.69		50	139
AC1	AC1-73	CS14	10	109.36		50	142
AC1	AC1-78	CS14	8	108.09		56	159
AC1	AC1-78	CS14	9	109.42		58	170

AC1	AC1-78	CS14	9	108.02	57	149
AC1	AC1-82	CS14	9	108.45	61	200
AC1	AC1-82	CS14	9	107.57	65	214
AC1	AC1-82	CS14	9	107.74	66	213
AC1	AC1-84	CS14	10	109.94	61	173
AC1	AC1-84	CS14	9	109.34	59	166
AC1	AC1-84	CS14	9	109.13	61	168
AC1	AC1-87	CS14	8	107.79	52	151
AC1	AC1-87	CS14	8	107.85	53	156
AC1	AC1-87	CS14	8	105.98	53	160
AC1	AC1-90	CS14	10	108.47	53	155
AC1	AC1-90	CS14	9	108.81	51	150
AC1	AC1-90	CS14	9	108.32	49	152
AC1	AC1-95	CS14	9	110.2	62	185
AC1	AC1-95	CS14	10	109.37	56	179
AC1	AC1-95	CS14	9	110.15	59	182
AC2	AC2	CS14	10	107.3	62	193
AC2	AC2	CS14	9	107.71	58	184
AC2	AC2	CS14	9	107.99	58	194
AC2	AC2	CS14	11	105.15	60	199
AC2	AC2	CS14	11	106.23	59	195
AC2	AC2	CS14	11	106.15	58	191
AC2	AC2	CS15	8	100.36	58	169
AC2	AC2	CS15	8	101.58	57	168
AC2	AC2	CS15	9	96.34	58	184
AC2	AC2-170	CS15	8	98.66	63	206
AC2	AC2-170	CS15	10	98.38	64	225
AC2	AC2-170	CS15	9	98.03	65	230
AC2	AC2-1A	CS14	8	106.79	59	197
AC2	AC2-1A	CS14	8	106.36	59	198
AC2	AC2-1A	CS14	9	105.14	61	209
AC2	AC2-1B	CS14	10	106.91	62	208
AC2	AC2-1B	CS14	9	107.41	59	192
AC2	AC2-1B	CS14	9	108.02	60	202
AC2	AC2-1C	CS14	9	106.71	55	185
AC2	AC2-1C	CS14	9	108.45	53	171
AC2	AC2-1C	CS14	9	106.85	55	188
AC2	AC2-1D	CS14	9	106.48	66	210
AC2	AC2-1D	CS14	9	106.39	69	219
AC2	AC2-1D	CS14	10	106.76	69	218
AC2	AC2-2A	CS14	9	106.09	60	210
AC2	AC2-2A	CS14	9	106.93	59	201
AC2	AC2-2A	CS14	9	106.72	57	194
AC2	AC2-2B	CS14	8	105.91	62	197
AC2	AC2-2B	CS14	8	105.56	59	176
AC2	AC2-2B	CS14	8	104.74	62	208
AC2	AC2-2C	CS14	9	105.91	53	167

AC2	AC2-2C	CS14	9	106.21	52	166
AC2	AC2-2C	CS14	9	106.68	52	166
AC2	AC2-2D	CS14	10	108.31	60	187
AC2	AC2-2D	CS14	9	108.5	63	202
AC2	AC2-2D	CS14	9	106.25	63	204
AC2	AC2-321	CS15	7	101.23	51	139
AC2	AC2-321	CS15	8	101.3	51	141
AC2	AC2-321	CS15	9	98.83	52	155
AC2	AC2-352	CS15	8	101.2	65	214
AC2	AC2-352	CS15	8	94.84	67	236
AC2	AC2-352	CS15	8	87.68	68	248
AC2	AC2-385	CS15	7	99.41	56	160
AC2	AC2-385	CS15	8	98.89	58	183
AC2	AC2-385	CS15	9	92.67	59	199
AC2	AC2-3A	CS14	9	104.67	61	216
AC2	AC2-3A	CS14	9	104.93	64	222
AC2	AC2-3A	CS14	9	104.21	61	222
AC2	AC2-3B	CS14	9	107.9	56	179
AC2	AC2-3B	CS14	9	107.54	57	181
AC2	AC2-3B	CS14	9	107.02	54	173
AC2	AC2-3C	CS14	8	105.08	65	206
AC2	AC2-3C	CS14	8	103.56	64	206
AC2	AC2-3C	CS14	8	102.7	65	210
AC2	AC2-3D	CS14	10	105.35	56	183
AC2	AC2-3D	CS14	9	107.95	61	175
AC2	AC2-3D	CS14	9	105.85	60	206
AC2	AC2-400	CS15	8	102.34	48	128
AC2	AC2-400	CS15	10	102.55	49	139
AC2	AC2-400	CS15	10	102.43	50	139
AC2	AC2-437	CS15	9	102.07	54	155
AC2	AC2-437	CS15	9	98.41	57	170
AC2	AC2-437	CS15	10	103	56	165
AC2	AC2-45	CS15	8	103.54	60	181
AC2	AC2-45	CS15	8	98.67	60	175
AC2	AC2-45	CS15	9	93.24	64	205
AC2	AC2-467	CS15	8	101.61	53	158
AC2	AC2-467	CS15	9	102.3	54	169
AC2	AC2-467	CS15	10	103.54	54	173
AC2	AC2-484	CS14	11	101.26	76	251
AC2	AC2-484	CS14	12	103.47	73	240
AC2	AC2-484	CS14	11	102.44	73	240
AC2	AC2-484	CS15	9	96.37	67	231
AC2	AC2-484	CS15	9	84.11	71	273
AC2	AC2-484	CS15	10	85.25	71	281
AC2	AC2-575	CS15	8	95.79	60	187
AC2	AC2-575	CS15	10	98.78	61	219
AC2	AC2-575	CS15	10	100.21	60	214

AC2	AC2-603	CS15	9	94.79	62	221
AC2	AC2-603	CS15	9	88.85	66	234
AC2	AC2-603	CS15	10	94.79	65	238
AC2	AC2-616	CS15	8	96.71	70	253
AC2	AC2-616	CS15	10	100.06	71	248
AC2	AC2-616	CS15	10	91.18	70	280
AC2	AC2-626	CS15	10	105.01	60	194
AC2	AC2-626	CS15	10	102.58	62	201
AC2	AC2-626	CS15	10	102.3	66	220
AC2	AC2-652	CS15	9	101.83	60	192
AC2	AC2-652	CS15	9	95.42	62	194
AC2	AC2-652	CS15	11	89.29	63	226
AC2	AC2-66	CS15	9	94.46	72	266
AC2	AC2-66	CS15	9	93.5	72	259
AC2	AC2-66	CS15	10	92.68	75	291
AC2	AC2-681	CS15	22	90.89	57	196
AC2	AC2-681	CS15	8	96.72	54	156
AC2	AC2-681	CS15	8	98.64	53	153
AC2	AC2-701	CS15	9	85.64	68	262
AC2	AC2-701	CS15	9	88.64	68	279
AC2	AC2-701	CS15	10	95.27	70	252
AC2	AC2-711	CS15	8	101.25	56	162
AC2	AC2-711	CS15	10	99.82	57	185
AC2	AC2-711	CS15	10	100.67	57	180
AC2	AC2-718	CS15	9	97.76	62	203
AC2	AC2-718	CS15	9	91.92	64	209
AC2	AC2-718	CS15	10	89.99	64	225
AC2	AC2-730	CS15	8	93.92	61	209
AC2	AC2-730	CS15	9	97.78	60	223
AC2	AC2-730	CS15	9	94.09	60	234
AC2	AC2-731	CS14	12	107.43	54	172
AC2	AC2-731	CS14	11	108.49	50	150
AC2	AC2-731	CS14	11	107.66	53	167
AC2	AC2-731	CS15	9	101.03	56	156
AC2	AC2-731	CS15	10	104.27	54	154
AC2	AC2-731	CS15	11	103.94	56	162
AC2	AC2-749	CS15	8	93.74	71	225
AC2	AC2-749	CS15	10	99.63	69	232
AC2	AC2-749	CS15	9	92.48	73	259
AC2	AC2-755	CS15	8	102.68	56	166
AC2	AC2-755	CS15	8	99.51	57	177
AC2	AC2-755	CS15	9	99.8	58	184
AC2	AC2-772	CS15	8	98.71	60	185
AC2	AC2-772	CS15	9	101.48	60	186
AC2	AC2-772	CS15	10	103.38	58	188
AC2	AC2-79	CS15	7	100.04	55	160
AC2	AC2-79	CS15	7	98.94	53	159

AC2	AC2-79	CS15	7	98.18	55	173
AC2	AC2-818	CS15	10	100.69	56	170
AC2	AC2-818	CS15	11	99.91	57	175
AC2	AC2-818	CS15	11	103.83	54	174
AC3	AC3	CS14	10	106.17	66	215
AC3	AC3	CS14	9	103.82	68	223
AC3	AC3	CS14	9	107.54	66	215
AC3	AC3	CS14	12	106.3	66	201
AC3	AC3	CS14	12	103.74	66	215
AC3	AC3	CS14	11	105.17	66	213
AC3	AC3	CS15	9	95.11	67	252
AC3	AC3	CS15	9	96.35	65	229
AC3	AC3	CS15	9	94.47	66	241
AC3	AC3-102	CS15	9	94.7	68	208
AC3	AC3-102	CS15	10	99.77	66	226
AC3	AC3-102	CS15	10	90.82	66	230
AC3	AC3-104	CS15	12	95.87	72	232
AC3	AC3-104	CS15	9	97	69	224
AC3	AC3-104	CS15	8	98.45	67	220
AC3	AC3-109	CS15	9	87.33	75	293
AC3	AC3-109	CS15	10	88.53	78	342
AC3	AC3-109	CS15	10	82.79	77	389
AC3	AC3-111	CS15	11	97.92	68	214
AC3	AC3-111	CS15	11	95.46	70	220
AC3	AC3-111	CS15	11	100.82	68	203
AC3	AC3-122	CS14	12	107.94	67	190
AC3	AC3-122	CS14	12	105.44	70	216
AC3	AC3-122	CS14	12	105.17	69	219
AC3	AC3-122	CS15	9	100.91	68	219
AC3	AC3-122	CS15	9	98.9	67	229
AC3	AC3-122	CS15	10	97.27	74	255
AC3	AC3-13	CS15	9	89.84	76	278
AC3	AC3-13	CS15	9	97.13	74	240
AC3	AC3-13	CS15	10	92.32	77	272
AC3	AC3-140	CS15	8	99.72	61	206
AC3	AC3-140	CS15	8	106.36	55	140
AC3	AC3-140	CS15	10	99.46	63	225
AC3	AC3-144	CS15	9	84.13	87	410
AC3	AC3-144	CS15	9	83.87	86	409
AC3	AC3-144	CS15	10	80.67	95	524
AC3	AC3-15	CS15	9	94.16	69	247
AC3	AC3-15	CS15	9	84.47	73	293
AC3	AC3-15	CS15	10	84.72	77	363
AC3	AC3-162	CS14	13	105.55	74	228
AC3	AC3-162	CS14	13	106.32	71	210
AC3	AC3-162	CS14	13	104.77	73	225
AC3	AC3-162	CS15	8	81.35	83	342

AC3	AC3-162	CS15	10	87.63		81	325
AC3	AC3-162	CS15	9	86.38		84	347
AC3	AC3-169	CS15	8	95.81		61	199
AC3	AC3-169	CS15	9	101.6		60	195
AC3	AC3-169	CS15	10	100.62		61	191
AC3	AC3-171	CS15	10	100.17		65	206
AC3	AC3-171	CS15	10	95.2		66	234
AC3	AC3-171	CS15	10	90.42		70	282
AC3	AC3-186	CS15	9	92.85		71	214
AC3	AC3-186	CS15	10	89.21		76	273
AC3	AC3-186	CS15	11	91.6		74	257
AC3	AC3-1A	CS14	9	106.17		68	226
AC3	AC3-1A	CS14	9	106.34		69	226
AC3	AC3-1A	CS14	9	106.15		69	230
AC3	AC3-1B	CS14	9	105.92		63	220
AC3	AC3-1B	CS14	9	99.13		68	235
AC3	AC3-1B	CS14	9	105.01		67	197
AC3	AC3-1C	CS14	10	104.48		69	229
AC3	AC3-1C	CS14	9	104.72		61	176
AC3	AC3-1C	CS14	10	105.82		72	235
AC3	AC3-1D	CS14	9	102.73		77	267
AC3	AC3-1D	CS14	9	102.5		78	263
AC3	AC3-1D	CS14	8	101.83		76	268
AC3	AC3-22	CS14	11	106.74		62	194
AC3	AC3-22	CS14	12	108.65		58	158
AC3	AC3-22	CS14	13	106.95		62	191
AC3	AC3-22	CS15	9	100.83		65	207
AC3	AC3-22	CS15	10	101.35		67	233
AC3	AC3-22	CS15	10	100.73		68	223
AC3	AC3-26	CS15	9	100.61		63	218
AC3	AC3-26	CS15	9	84.75		69	341
AC3	AC3-26	CS15	10	95.91		68	260
AC3	AC3-2A	CS14	10	104.32		80	273
AC3	AC3-2A	CS14	10	103.62		80	279
AC3	AC3-2A	CS14	9	101.37		80	278
AC3	AC3-2B	CS14	10	105.77		59	158
AC3	AC3-2B	CS14	9	105.51		65	206
AC3	AC3-2B	CS14	9	107.22		64	202
AC3	AC3-2C	CS14	9	101.5		73	270
AC3	AC3-2C	CS14	9	102.1		77	254
AC3	AC3-2C	CS14	10	105.6		62	171
AC3	AC3-2D	CS14	9	104.99		72	225
AC3	AC3-2D	CS14	9	104.91		64	181
AC3	AC3-2D	CS14	9	105.35		61	171
AC3	AC3-32	CS15	8	82.89		70	297
AC3	AC3-32	CS15	9		87	71	300
AC3	AC3-32	CS15	10	86.25		74	344

AC3	AC3-3A	CS14	9	104.87	67	230
AC3	AC3-3A	CS14	9	103.69	67	236
AC3	AC3-3A	CS14	9	106.13	62	181
AC3	AC3-3B	CS14	9	105.12	74	254
AC3	AC3-3B	CS14	9	103.05	74	251
AC3	AC3-3B	CS14	9	107.64	77	227
AC3	AC3-3C	CS14	10	100.47	67	238
AC3	AC3-3C	CS14	9	105.64	65	215
AC3	AC3-3C	CS14	10	105.56	66	216
AC3	AC3-3D	CS14	10	104.78	80	244
AC3	AC3-3D	CS14	10	105.25	79	238
AC3	AC3-3D	CS14	8	107.94	81	236
AC3	AC3-40	CS14	12	105.78	75	237
AC3	AC3-40	CS14	12	104.79	75	235
AC3	AC3-40	CS14	12	102.84	74	241
AC3	AC3-40	CS15	9	96.37	77	248
AC3	AC3-40	CS15	11	93.2	79	285
AC3	AC3-40	CS15	11	84.39	79	354
AC3	AC3-47	CS15	8	102.42	61	199
AC3	AC3-47	CS15	10	93.04	66	250
AC3	AC3-47	CS15	10	95.17	65	227
AC3	AC3-60	CS14	12	105.43	72	236
AC3	AC3-60	CS14	12	105.34	70	220
AC3	AC3-60	CS14	12	104.69	71	235
AC3	AC3-60	CS15	9	91.63	68	253
AC3	AC3-60	CS15	11	97.81	72	268
AC3	AC3-60	CS15	11	97.36	71	267
AC3	AC3-78	CS14	12	104.04	68	228
AC3	AC3-78	CS14	12	103.46	67	224
AC3	AC3-78	CS14	12	105.22	70	224
AC3	AC3-78	CS15	9	96.47	68	234
AC3	AC3-78	CS15	11	94.87	70	261
AC3	AC3-78	CS15	10	100.04	70	247
AC3	AC3-81	CS15	9	92.47	68	288
AC3	AC3-81	CS15	9	93.05	66	270
AC3	AC3-81	CS15	10	88.67	69	336
AC3	AC3-85	CS15	9	86.78	75	323
AC3	AC3-85	CS15	11	84.63	78	368
AC3	AC3-85	CS15	11	87.28	78	355
AC3	AC3-95	CS14	11	104	70	235
AC3	AC3-95	CS14	12	104.59	69	223
AC3	AC3-95	CS14	11	105.96	69	227
AC3	AC3-95	CS15	9	90.62	71	275
AC3	AC3-95	CS15	9	86.94	69	261
AC3	AC3-95	CS15	10	83.52	75	371
Temoins	F15	CS14	7	109.83	38	102
Temoins	F15	CS14	7	109.85	38	108

Temoins	F15	CS14	8	110.04	39	111
Founder	F15	CS15	10	85.67	84	381
Founder	F15	CS15	10	90.58	79	314
Founder	F15	CS15	10	85.94	79	346
M2xF15	FM-1C	CS14	11	109.55	57	167
M2xF15	FM-1C	CS14	11	109.46	57	172
M2xF15	FM-1C	CS14	10	106.92	56	156
M2xF15	FM-1C	CS14	9	109.34	56	173
M2xF15	FM-1C	CS14	9	108.95	57	184
M2xF15	FM-1C	CS14	9	108.66	55	174
M2xF15	FM-3C	CS14	7	108.51	40	114
M2xF15	FM-3C	CS14	7	110.31	41	122
M2xF15	FM-3C	CS14	7	107.94	42	126
FMGS	FMGS-889	CS14	8	109.8	45	118
FMGS	FMGS-889	CS14	8	109.83	45	124
FMGS	FMGS-889	CS14	8	109.54	46	122
Founder	GN	CS14	8	110.78	53	163
Founder	GN	CS14	8	109.22	52	176
Founder	GN	CS14	8	110.19	54	178
Founder	GN	CS14	7	110.02	53	177
Founder	GN	CS14	7	108.74	52	176
Founder	GN	CS14	6	110.31	55	178
Founder	GN	CS14	8	110.9	52	166
Founder	GN	CS14	8	110.46	53	175
Founder	GN	CS14	10	109.31	52	162
Founder	GN	CS14	10	109.81	51	158
Temoins	GN	CS14	10	109.91	50	159
Founder	GN	CS15	7	103.47	54	159
Founder	GN	CS15	7	102.81	54	157
Temoins	GN	CS15	7	96.77	55	169
SBxGN	GS-13C	CS14	8	106.16	51	165
SBxGN	GS-13C	CS14	8	110.84	48	153
SBxGN	GS-13C	CS14	9	110.78	47	150
SBxGN	GS-56B	CS14	9	110.87	52	158
SBxGN	GS-56B	CS14	8	110.71	52	152
SBxGN	GS-56B	CS14	8	110.49	50	144
SBxGN	GS-8B	CS14	11	107.76	56	160
SBxGN	GS-8B	CS14	11	108.15	54	151
SBxGN	GS-8B	CS14	11	108.93	53	154
SBxGN	GS-8B	CS14	10	107.85	51	157
SBxGN	GS-8B	CS14	9	107.99	52	158
SBxGN	GS-8B	CS14	10	108.95	54	156
Founder	M2	CS14	9	109.46	43	125
Founder	M2	CS14	8	110.35	46	130
Founder	M2	CS14	9	111.57	46	124
Temoins	M2	CS15	9	105.23	52	117
Founder	M2	CS15	9	104.84	51	117

Founder	M2	CS15	9	105.83	51	114
Founder	SB	CS14	8	105.93	42	117
Founder	SB	CS14	8	106.13	40	113
Founder	SB	CS14	8	105.53	42	112
Founder	SB	CS14	8	111.67	40	100
Founder	SB	CS14	9	111.26	40	100
Founder	SB	CS14	10	110.95	41	104
Founder	SB	CS14	11	111.91	41	99
Founder	SB	CS14	12	112.03	43	108
Founder	SB	CS14	12	110.27	42	112
Founder	SB	CS14	12	110.66	43	105
Founder	SB	CS14	12	110.07	42	110
Founder	SB	CS15	8	102.83	44	98
Founder	SB	CS15	8	104.29	44	101
Founder	SB	CS15	8	101.11	45	99

Glycerol (g/L)	Acetic acid (g/L)	Malic acid (g/L)	Succinic acid (g/L)	pH	Glucose (g/L)
7.672531007	0.38435013157634	3.796074437	0.421900161	3.5	0.2215792
6.697566503	0.32021573244164	3.906720346	0.727214171	3.51	0.521026774
6.91339834	0.25057952949090	4.360987094	0.541706924	3.62	0.937073405
7.168962599	0.217966034	3.279907141	0.222049228	3.52	-0.738231073
6.753370565	0.220676025	2.63695289	0.15543446	3.56	0.316183098
8.72743273	0.18239741	3.128623788	0.621737839	3.42	0.341590668
10.70149489	0.177316178	3.809398877	-0.244254151	3.69	0.621073942
8.831330738	0.19086613	3.128623788	0.421893533	3.54	0.430517165
8.207942686	0.109735792	3.998503068	0.222049228	3.34	0.722704224
7.272860608	0.187478642	3.620294685	0.288663997	3.57	0.239960387
8.207942686	0.24167845	2.674773729	0.288663997	3.3	-1.030418132
10.38980087	0.21627229	3.355548818	0.288663997	3.49	0.354294454
9.870310825	0.268778354	4.074144745	0.222049228	3.61	-0.674712147
7.688452643	0.232362858	3.128623788	0.355278765	3.46	0.417813379
9.974208834	0.292490771	3.582473847	0.621737839	3.3	0.252664172
10.59759689	0.211191058	3.166444626	0.488508302	3.49	0.544851231
8.415738704	0.211191058	2.674773729	0.421893533	3.56	-0.776342428
10.18200485	0.160378738	2.712594567	0.355278765	3.62	-0.407932658
5.402696452	0.087547746	3.506832171	0.488508302	3.62	0.214552816
6.026084504	0.15699125	3.166444626	0.222049228	3.5	-0.509562939
6.961166582	0.150216274	2.977340435	0.488508302	3.64	0.316183098
6.556159438	0.23983181971073	5.750589761	0.665378422	3.52	1.461769155
6.786876229	0.23250674369983	4.904595259	0.518518519	3.51	0.743624972
8.878212301	0.19687296782150	4.986377018	0.523027375	3.41	1.347820078
9.974208834	0.113292655	5.133128216	0.355278765	3.62	1.84063732
8.935228747	0.10617893	4.225428098	0.15543446	3.34	-0.166560739
7.792350652	0.126503858	4.906203187	0.222049228	3.27	0.81163072
11.35938608	0.126575488	4.897659847	1.607280104	3.55	9.166159721
8.457337563	0.074410228	5.155340069	1.472414589	3.55	5.103130538
6.66331013	0.175095144	5.355905604	1.623062239	3.59	3.992206688
9.143024764	0.310105708	3.431190494	0.288663997	3.67	0.570258802
8.000146669	0.28402205	3.317727979	0.754967376	3.56	0.176441461
11.948271	0.325349404	3.582473847	0.222049228	NA	-0.026819102
9.350820782	0.260309634	2.977340435	0.222049228	3.48	-1.170159769
10.8053929	0.281989558	3.128623788	0.222049228	3.63	0.278071742
13.50674113	0.328059395	3.052982111	0.688352607	3.51	0.430517165
7.792350652	0.116341394	3.09080295	0.355278765	3.52	0.176441461
10.38980087	0.121422626	3.166444626	0.421893533	3.58	0.506739876
6.961166582	0.157668748	2.712594567	0.15543446	3.48	0.379702024
9.974208834	0.211191058	3.242086303	0.355278765	3.57	0.189145246
9.454718791	0.221353522	3.052982111	0.288663997	3.5	0.278071742
11.11708693	0.21627229	3.431190494	0.55512307	3.55	0.252664172
6.129982513	0.25014717	2.939519597	0.15543446	3.63	0.316183098
10.07810684	0.201028594	2.788236244	-0.044409846	3.54	-0.420636443
8.415738704	0.201028594	2.901698758	0.288663997	3.59	0.201849031
7.672531007	0.18209486687375	5.17605572	0.801288245	3.64	-1.323358287

6.221247967	0.19456988715432	4.899784567	0.573268921	3.5	-1.053060476
7.583221281	0.17748870553939	4.388476761	0.723993559	3.49	1.443219305
10.70149489	0.041816658	4.792740672	0.355278765	3.18	-0.674712147
11.84437299	0.080095272	4.679278157	0.222049228	3.53	0.303479313
11.53267896	0.023185474	4.338890613	0.55512307	3.34	0.303479313
10.66780245	0.029138472	4.809995441	1.7995352	3.55	0.449680287
7.553817012	0.133734125	4.452962225	1.670121695	3.59	0.219208906
11.64940503	0.024366047	4.977354761	1.842577385	3.56	0.387530701
11.53267896	0.187986765	3.015161273	0.15543446	3.46	0.366998239
12.77945507	0.162072482	2.750415405	0.421893533	3.5	0.417813379
11.948271	0.163766226	3.242086303	0.222049228	3.46	0.646481513
7.480656625	0.204416082	2.826057082	0.421893533	3.9	0.417813379
8.104044678	0.08348276	3.393369656	0.288663997	NA	-0.573081865
10.59759689	0.209497314	3.317727979	0.288663997	3.6	-1.602088465
10.07810684	0.134972578	3.998503068	0.55512307	3.44	0.341590668
7.89624866	0.16545997	3.620294685	0.621737839	3.47	0.532147446
12.57165905	0.172234946	3.582473847	0.55512307	3.49	0.036699824
9.454718791	0.232362858	3.393369656	0.288663997	3.52	0.671889083
9.350820782	0.22474101	3.317727979	0.55512307	3.51	0.354294454
9.350820782	0.177316178	2.901698758	0.55512307	3.31	0.290775528
8.311840695	0.27555333	4.187607259	0.288663997	3.41	0.265367957
9.143024764	0.228805996	3.847219715	0.088819691	3.43	0.265367957
9.350820782	0.255736526	3.393369656	0.355278765	3.63	0.328886883
6.129982513	0.272673966	3.204265464	0.488508302	3.6	0.201849031
7.376758617	0.236597218	2.901698758	0.421893533	3.6	0.455924735
9.974208834	0.187478642	3.128623788	0.688352607	3.63	-0.115745598
10.8053929	0.146828786	3.204265464	0.421893533	3.51	0.265367957
11.74047498	0.15699125	2.826057082	0.355278765	3.54	-0.458747798
11.63657697	0.21627229	2.750415405	0.421893533	3.54	-0.166560739
10.49369888	0.150216274	3.09080295	0.222049228	3.5	0.366998239
9.454718791	0.297572003	2.750415405	0.288663997	3.5	0.074811179
8.623534721	0.155297506	2.788236244	0.288663997	3.69	-1.818052813
9.123814046	0.41067562309148	4.128699407	0.457326892	3.59	1.665817502
9.436398085	0.37360242179616	4.08883939	0.348470209	3.5	0.87347392
8.05209734	0.36538170330358	4.619389963	0.565539452	3.5	1.586318146
11.84437299	0.206109826	3.96068223	0.754967376	3.37	0.278071742
10.59759689	0.177316178	4.074144745	0.488508302	3.43	0.265367957
10.70149489	0.431377779	3.7337572	0.621737839	3.42	0.316183098
10.38893808	0.275316075	3.945305621	1.803839418	3.61	0.449680287
8.267709793	0.283999237	4.015702795	1.674712861	3.61	0.535135968
8.269568889	0.271339054	3.821778503	1.805274158	3.6	0.573979459
6.33777853	0.075691538	2.674773729	0.355278765	3.62	0.214552816
7.065064591	0.145473791	2.750415405	0.222049228	NA	-0.623897006
9.143024764	0.128197602	3.582473847	0.488508302	3.48	0.265367957
6.026084504	0.084160258	3.166444626	0.55512307	3.54	-0.522266724
7.688452643	0.424602803	2.826057082	0.421893533	3.5	0.227256602
7.272860608	0.112953906	2.86387792	0.888196912	3.48	0.341590668

7.584554634	0.226434754	2.712594567	0.15543446	3.57	-0.24278345
9.974208834	0.270641473	2.599132052	0.288663997	3.58	0.239960387
7.89624866	0.219659778	2.63695289	0.55512307	3.61	0.239960387
9.662514808	0.18239741	2.86387792	0.55512307	3.53	0.252664172
10.38980087	0.286223918	3.885040553	0.288663997	3.6	0.366998239
10.59759689	0.229822242	2.750415405	0.621737839	3.58	0.316183098
8.831330738	0.26708461	2.334386184	0.288663997	3.65	0.430517165
9.454718791	0.268778354	2.674773729	0.15543446	3.61	-0.547674295
10.18200485	0.362103649	2.485669537	0.15543446	3.52	0.900557217
11.42878096	0.190696756	3.998503068	0.421893533	3.55	0.341590668
9.870310825	0.160378738	3.355548818	0.355278765	3.39	0.239960387
10.90929091	0.184091154	3.658115524	0.288663997	3.38	-0.509562939
6.857268573	0.106856427	3.128623788	0.15543446	3.46	0.290775528
8.623534721	0.102791442	3.506832171	0.15543446	NA	0.519443661
6.857268573	0.07653841	3.771578039	0.222049228	3.52	-0.573081865
8.104044678	0.262511502	NA	0.222049228	NA	-0.001411532
10.90929091	0.502515027	2.712594567	0.222049228	3.67	0.316183098
8.415738704	0.239984706	2.788236244	0.688352607	3.58	0.227256602
12.36386303	0.509290003	3.582473847	-0.044409846	3.58	0.341590668
10.49369888	0.180703666	3.469011332	0.621737839	3.49	0.011292253
11.11708693	0.206109826	3.582473847	0.088819691	3.61	-0.204672095
8.788902575	0.31276270750479	4.765085198	0.379388084	3.54	0.809874435
6.623141732	0.22658910587443	5.070220502	0.479871176	3.49	-0.03281874
8.364681379	0.24082342388687	4.050353856	0.627375201	3.47	1.538618533
10.38980087	0.1427638	4.111965583	0.421893533	3.46	0.049403609
7.272860608	0.133278834	3.771578039	0.222049228	3.48	-1.182863554
9.039126756	0.104485186	4.111965583	0.15543446	3.39	0.176441461
11.84437299	0.177316178	2.712594567	0.15543446	3.67	-1.61479225
11.53267896	0.189172386	2.599132052	0.088819691	3.45	0.328886883
11.22098494	0.223047266	2.561311214	0.821582144	3.58	0.328886883
7.538566418	0.30339044867863	3.708107502	1.122061192	3.57	1.172921494
6.139380719	0.33959999472375	4.154127349	0.704025765	3.53	0.711825229
7.985115046	0.30127929140038	4.256526359	0.349114332	3.53	1.713517116
9.454718791	0.225757257	3.355548818	0.222049228	3.37	0.760815579
11.53267896	0.134972578	3.695936362	0.288663997	3.37	-0.293598591
8.831330738	0.150216274	3.695936362	0.088819691	3.47	0.405109594
6.66331013	0.253442459	4.184390364	1.422198706	3.63	0.449680287
10.59901591	0.250989963	4.055550252	1.580020053	3.63	0.845883898
12.74255335	0.245488417	3.646449692	1.58862849	3.56	0.620591648
7.065064591	0.170541202	2.826057082	0.288663997	3.58	0.544851231
7.480656625	0.24167845	2.674773729	-0.044409846	3.61	-1.500458184
7.792350652	-0.049645519	2.826057082	0.488508302	3.6	0.214552816
8.104044678	0.194253618	3.317727979	0.355278765	3.38	0.062107394
9.246922773	0.226434754	3.279907141	0.15543446	3.54	-0.53497051
9.350820782	0.211191058	3.393369656	0.421893533	3.54	0.12562632
7.376758617	0.226096005	3.204265464	0.222049228	3.46	0.328886883
11.22098494	0.163766226	2.826057082	0.688352607	3.58	0.278071742

8.311840695	0.091443357	2.939519597	0.222049228	3.57	0.328886883
8.72743273	0.351771811	3.317727979	0.488508302	3.47	0.265367957
12.05216901	0.57534602	3.317727979	0.088819691	3.45	-0.458747798
8.104044678	0.238290962	3.431190494	0.488508302	3.5	0.379702024
7.065064591	0.285715794	2.561311214	0.421893533	3.64	-0.611193221
8.935228747	0.314509443	3.052982111	0.022204923	3.49	0.328886883
11.11708693	0.14005381	3.09080295	0.355278765	3.55	-1.652903606
11.74047498	0.311121955	3.204265464	0.222049228	3.52	0.214552816
9.143024764	0.141747554	2.788236244	0.488508302	3.55	0.328886883
10.59759689	0.228128498	3.015161273	1.088041218	3.46	0.214552816
7.480656625	0.729476724	3.015161273	0.355278765	3.63	0.392405809
7.376758617	0.270472098	2.63695289	0.288663997	3.46	0.265367957
9.662514808	0.290797027	2.826057082	0.421893533	3.51	0.417813379
7.065064591	0.180703666	3.582473847	0.355278765	3.55	-1.030418132
8.935228747	0.15699125	3.355548818	0.088819691	3.62	0.189145246
8.207942686	0.172234946	3.279907141	0.421893533	3.55	-1.055825702
6.987823111	0.20890016686122	4.096399049	0.51078905	3.56	0.886723813
7.962787614	0.25173106982449	5.144442603	0.545571659	3.45	-0.128217967
5.328150712	0.23276264155174	5.361610972	0.476650564	3.52	1.220621108
8.92312967334397	0.217865142	4.59753557	1.12156378124838	3.45	0.31335865243
8.43571720581835	0.188837675	4.229922989	1.17001859760088	3.47	-0.13682179480
8.92312967334397	0.212899917	4.529607158	1.12303210901664	3.45	0.45306982572
7.531507862	0.103840184	3.788572289	1.637409634	3.62	0.379762003
6.864092475	0.113451318	4.327841209	1.59006323	3.63	0.403068097
10.34246069	0.137247161	4.370345164	1.572846355	3.59	1.731515498
11.69216423	0.071228611	4.270726521	1.614453801	3.55	0.535135968
5.836012506	0.028674486	3.959916355	1.565672658	3.56	0.457448985
6.918006253	0.11258963	4.290650249	1.714885568	3.57	0.488523778
5.663062183	0.18286256042948	5.839931178	0.503703704	3.5	-0.941761378
10.49322984	0.18798051746766	6.075655073	0.634460548	3.39	0.664125615
8.476318536	0.22374224227195	5.006994268	0.503703704	3.42	-0.84636215
6.228690444	0.21465786852918	4.500497154	0.51078905	3.46	0.870823941
8.096752203	0.23081142043093	4.546542346	0.669243156	3.64	0.889373791
8.074424771	0.32658119150788	4.567159596	0.603542673	3.53	0.571376367
4.993239242	0.19722482736788	4.727974148	0.754267311	3.53	0.507776882
10.04668121	0.23960790909030	4.376793652	0.59194847	3.65	0.632325873
7.627876144	0.26977186838408	4.938270101	0.437359098	3.52	0.841674177
9.868061758	0.23596136470060	5.102520861	0.858615137	3.48	0.441527418
7.925575229	0.29507376849158	4.18711495	0.696296296	3.57	0.600526131
7.568336327	0.29622530882517	5.440643765	0.692431562	3.59	1.236520979
7.025035497	0.27172308950489	4.483316112	0.186795491	3.49	1.538618533
7.032477974	0.30236685727099	5.102520861	0.217069243	3.49	0.775424714
6.340327601	0.27300257876443	4.871607658	0.302093398	3.62	0.680025487
5.305823281	0.26251076683616	4.837932816	0.465056361	3.56	1.204721237
8.007442477	0.27169110227340	4.377480894	0.704025765	3.42	0.523676753
5.797026771	0.24888420622201	5.015241168	0.519162641	3.5	1.697617245
4.680655202	0.13776056403052	4.969883218	0.279549114	3.44	1.236520979

9.957371484	0.19335437235775	4.451015753	0.642190016	3.49	0.902623684
9.689442307	0.14997968645917	4.286077751	0.796779388	3.56	0.759524843
7.345062013	0.212354787862	4.500497154	0.387117552	3.49	0.409727676
9.280106065	0.22662109310592	4.574032013	0.658293076	3.47	1.586318146
8.081867248	0.22655711864295	4.500497154	0.789049919	3.62	0.902623684
12.11789716	0.035899408	3.706220878	1.882750092	3.65	0.403068097
11.32592236	0.027547663	3.985153078	2.0391367	3.65	0.403068097
10.47631559	0.021118146	4.250802792	1.796665721	3.64	0.535135968
6.942174498	0.028343068	4.802025949	1.554194742	3.55	0.309843718
7.419962115	0.082761972	4.724987532	1.432241882	3.52	0.581748157
11.71633247	0.120212254	5.009232727	1.581454792	3.51	2.267555677
7.486889563	0.175426563	4.334482452	1.492500942	3.64	0.566210761
11.7497962	0.246681523	3.742083589	1.67901708	3.64	-0.14074078
4.990123924	0.226796418	3.937336129	1.512587295	3.63	1.133325733
10.1136635	0.21350632819559	5.283952663	0.657648953	3.47	1.37961982
4.3234163	0.22371025504046	4.92589975	0.715619968	3.49	0.046680616
9.280106065	0.24232682376684	5.618639359	0.665378422	3.66	0.046680616
6.645469163	0.40027977285768	3.763086836	0.684702093	3.5	0.682675465
6.831531091	0.31618534127408	3.848992046	0.669243156	3.38	0.521026774
10.22530066	0.31541764771835	4.055164548	0.87020934	3.61	0.568726388
8.253044222	0.21721684704827	4.825562466	0.615780998	3.56	1.411419563
8.677265418	0.28742882016580	4.686739648	0.61900161	3.5	0.682675465
9.079159183	0.26977186838408	5.140319153	0.522383253	3.52	0.921173533
7.583221281	0.26251076683616	4.693612064	0.445732689	3.49	1.459119176
9.399185699	0.23461790097808	3.832498245	0.823832528	3.45	1.157021623
7.40460183	0.28742882016580	4.670245847	0.472141707	3.41	0.841674177
10.66780245	0.1667434	3.144371732	1.574281095	3.71	0.387530701
6.293350069	0.156336862	2.868096029	1.49967464	3.81	0.364224606
7.553817012	0.312700073	2.938758853	1.357348479	3.72	0.281358491
11.59177306	0.023902061	3.521594326	1.176858248	3.73	0.34868721
10.72171623	0.08037576	3.414006192	1.323201679	3.66	0.65943514
1.731129013	0.075603334	3.916084152	1.267246837	3.65	0.379762003
5.902939954	0.258148601	3.517609581	1.581454792	3.67	0.34868721
11.34823151	0.247079225	3.829747995	1.766536191	3.68	0.977951768
10.34246069	0.220764603	4.083443472	1.67758234	3.65	1.529529343
11.66985508	0.046571081	3.625197715	1.654626508	3.63	0.737122122
11.68100965	0.017936529	5.103538375	1.722059265	3.63	0.550673364
11.90596024	0.056977619	4.052893755	2.135264248	3.66	0.356455908
9.24437879966768	0.32480844	4.601531359	1.04667906506725	3.49	0.43754636202
8.08123541125427	0.276302016	4.677451348	1.04374240953074	3.49	-0.18339218589
9.00067256590486	0.337794412	5.176924963	1.10688050356581	3.51	-0.10577486740
8.368100965	0.262523324	3.680984155	1.613019062	3.63	1.607216326
9.673186207	0.260137111	3.354235006	1.46811037	3.65	8.070773268
10.05430084	0.293610372	4.276039515	1.522630472	3.6	5.639170717
11.66985508	0.130486225	3.995779066	1.512587295	3.62	1.032332655
10.48932926	0.074874214	4.311902226	1.561368439	3.59	0.465217683
8.658119908	0.052934314	4.202985844	1.541282086	3.57	0.768196915

8.580037885	0.15037133	4.015702795	1.667539164	3.6	1.747052894
10.18815574	0.184639995	4.427459852	1.661800206	3.61	3.774683137
9.650877057	0.229116347	4.509811264	1.681886559	3.61	1.669365912
5.590611862	0.163097797	4.709048549	1.898532227	3.59	0.61282295
11.36868156	0.125780083	4.114258839	1.820769345	3.61	0.234746302
5.345211219	0.214401369	4.561612958	1.776579367	3.58	1.949039049
10.81281192	0.143676678	4.315886972	2.18834961	3.67	0.356455908
12.84294452	0.17231123	4.076802229	2.261521325	3.63	0.418605494
6.830628751	0.08362366	4.371673412	2.324649864	3.58	0.48075508
10.63247963	0.216058461	3.608196133	1.793509294	3.69	0.203671509
10.50048383	0.209297525	3.965229349	1.934400715	3.65	1.381924077
12.36329781	0.160380166	3.738098843	1.91718384	3.59	3.091037691
8.178473195	0.313429193	4.294634995	1.59006323	3.66	1.420767568
6.282195495	0.292748684	3.967885846	1.466675631	3.61	1.405230171
7.299120889	0.273725266	4.299947989	1.61875802	3.64	2.127719108
11.03590342	0.09674783	4.299947989	1.627366457	3.65	0.465217683
8.078082023	0.132872438	3.557457038	1.541282086	3.58	0.939108277
6.43835954	0.043787166	3.621212969	1.526934691	3.62	0.643897743
6.451373211	0.14964221	4.479261546	2.093656801	3.57	4.893375685
8.738061027	0.113053616	4.897659847	2.064962011	3.57	3.712533551
10.9559623	0.07281942	4.780773972	2.00757243	3.55	0.876958691
5.244820046	0.088727504	3.601289241	1.713450828	3.62	0.426374192
11.32406326	0.161175571	4.050502907	1.592645761	3.63	0.172596716
11.15674464	0.053332017	4.083443472	1.690494996	3.64	0.690509933
13.85801082	0.28963335	3.746068335	1.951617589	3.65	0.620591648
7.834540475	0.28764484	4.021015789	1.417894487	3.6	2.547228814
12.46182988	0.30905447	3.928038389	1.687625517	3.63	2.042263428
7.946086222	0.203331994	4.802025949	1.390634436	3.57	1.071176147
8.392269211	0.124586977	4.586849681	1.694799215	3.6	0.682741234
10.80165735	0.12697319	5.069003912	1.538412607	3.59	1.257624905
8.45787231797861	0.351926205	3.63455044	1.24637164155027	3.58	-0.16786872220
9.8536443840747	0.241545444	3.602584129	1.27720652468367	3.59	-0.27653296808
6.98455735932163	0.249566191	3.570617818	1.28161150798845	3.58	-0.29205643178
5.971726498	0.092373107	3.518937829	1.882750092	3.67	0.371993304
10.1193692	0.126509204	3.501670598	1.763666712	3.69	0.690509933
11.5267047	0.196173356	3.582693761	1.778014107	3.7	0.325381115
11.37054066	0.297587393	3.614571726	1.531238909	3.62	1.646059817
9.316239816	0.224741624	3.704892629	1.466675631	3.6	0.333149813
7.509198713	0.31230237	3.803448673	1.467823422	3.62	1.368976246
10.33130611	0.094295333	4.019687541	1.531238909	3.64	2.415160944
7.933072552	0.036230826	4.286665504	1.584324271	3.57	0.379762003
11.31476778	0.012037281	3.773961555	1.813882595	3.6	0.371993304
2.179171097	0.062943151	4.128603924	1.763666712	3.66	0.589516855
11.50253646	0.106226397	3.669029918	1.610149583	3.67	0.550673364
7.286107219	0.160777869	3.596241896	1.681599611	3.7	0.125984527
4.296681196	0.152359841	4.043596015	1.522630472	3.63	0.410836796
11.28130406	0.069306384	4.240176804	2.113743155	3.6	0.43414289

10.6454933	0.11941685	3.594647998	1.538412607	3.63	0.511829873
5.501375265	0.22394622	4.083443472	1.646018071	3.66	0.426374192
11.72748705	0.216456163	4.056878501	1.762231972	3.65	0.573979459
11.42631353	0.147255997	4.116649686	1.76940567	3.68	0.449680287
8.922867163	0.28784465417515	5.823437378	0.542351047	3.55	1.713517116
8.587955693	0.25694498855714	5.118327419	0.519162641	3.56	1.554518404
9.823406896	0.29776069593663	4.838620058	0.45668277	3.44	0.680025487
9.38838702870933	0.176615584	4.861257638	1.42257097374116	3.5	-0.10577486740
9.48808503343048	0.235052458	4.985127095	0.935086154679686	3.44	-0.18339218589
7.50520249508763	0.272482612	4.617514514	0.964452710044835	3.42	0.01841284217(
5.233665472	0.169858733	4.382299401	1.459501933	3.57	0.488523778
12.78717165	0.202138887	4.220253075	1.713450828	3.62	1.381924077
10.09706005	0.124918395	4.581536686	1.638844373	3.59	2.290861772
9.896277701	0.048957293	4.187046861	1.440850319	3.61	1.871352066
8.646965333	-0.002346278	5.063690918	1.667539164	3.61	0.410836796
4.787482483	0.112191928	4.613414652	1.403547092	3.62	4.792382608
8.972307096	0.078851235	5.798212378	2.066396751	3.58	0.527367269
14.49382158	0.07407881	4.547002224	1.752188795	3.55	0.403068097
7.631899035	0.031856103	4.218924826	1.971703942	3.56	0.426374192
5.670552981	0.560468484	4.706392052	1.568542137	3.64	9.492445048
12.57523473	0.524277592	4.451368326	1.486761984	3.59	7.4881209
13.41182783	0.504392487	4.949461541	1.435111361	3.58	10.09840351
7.299120889	0.242704502	3.355563255	1.475284068	3.68	0.620591648
13.26681836	0.14208587	3.47112088	1.43941558	3.68	0.900264785
7.589139831	0.228387226	3.076631055	1.327505897	3.68	0.496292476
8.77912144430231	0.349634563	3.622563074	1.36971117408389	3.53	-0.21443911329
8.01477007477351	0.438626665	4.429712435	1.1245004367849	3.59	-0.01263408521
10.0862730617574	0.327482023	4.257893512	1.21260010288035	3.5	-0.15234525850
5.066346851	0.479668673	4.286665504	1.613019062	3.61	0.581748157
11.34637241	0.42239957	3.641136698	1.755058274	3.6	0.566210761
6.929160828	0.44831649	4.022609687	1.629948988	3.62	0.592106421
10.42054271	0.068842399	4.298619741	1.49967464	3.61	4.077662369
12.32983408	0.136120338	4.459337818	1.719189786	3.58	0.884727389
4.865564506	0.060888357	5.207141764	1.595802188	3.58	2.228712186
9.773577379	0.14281499	4.055550252	1.684756038	3.58	2.275324375
7.910763402	0.156734564	3.967885846	2.227087577	3.52	0.333149813
6.897556199	0.131745615	4.284009006	1.85979426	3.58	0.294306322
12.12905174	0.109474297	5.435600518	1.503978858	3.57	7.394896521
10.06545542	0.138904253	5.025171709	1.485327245	3.55	8.202841139
11.17905379	0.093897631	5.035797698	1.470979849	3.58	12.66984263
12.17367004	0.071692597	4.495200529	1.668973903	3.58	2.718140175
9.128471142	0.049818981	4.598803918	1.49967464	3.56	8.06300457
7.074170299	0.030265295	4.784758718	1.386330218	3.56	9.096241437
8.86774189294333	0.493626076	4.217935622	0.914529565924082	3.48	0.35992904353
8.95636234158435	0.380189791	4.405737702	1.07751394820066	3.52	0.25126479764(
9.24437879966767	0.304470117	4.217935622	0.945364449057488	3.52	0.31335865243
4.687091311	0.636761004	3.983824829	1.664669685	3.6	12.07942156

6.717223908	0.579094199	4.816636684	1.43798084	3.58	6.703482377
3.683179587	0.663009343	4.394253638	1.496805161	3.61	8.055235872
10.1193692	0.246217537	4.055550252	1.547021044	3.58	1.047870052
12.65331675	0.198161866	4.399566632	1.808143637	3.64	0.403068097
8.068786544	0.200150377	4.112664941	1.763666712	3.63	0.511829873
11.03590342	0.518709763	4.303932735	1.307419544	3.64	0.651666441
8.635810759	0.454215738	4.426131604	1.328940637	3.62	2.927895028
13.83570167	0.55211674	3.957259858	1.478153547	3.6	6.726788471
8.334637241	0.073681108	4.189969008	1.312871554	3.61	3.419912584
8.715751877	0.060556938	4.423475106	1.67758234	3.55	7.239522556
13.13296346	0.042262641	4.391597141	1.557064221	3.61	5.747932492
7.099460268	0.21024363058375	4.582966155	0.526247987	3.51	0.886723813
8.766575144	0.34519776023426	5.490812407	0.333011272	3.56	0.905273662
8.342353948	0.36403823958106	4.821439016	0.360708535	3.5	1.316020335
6.310557693	0.23273065432025	4.497060945	0.939774557	3.57	0.714475208
9.324760928	0.25556953760313	5.358862006	0.503059581	3.47	1.429969413
7.493911556	0.20183098870224	4.690863098	0.526247987	3.59	0.921173533
11.20770764	0.38316660401126	6.191111674	0.360064412	3.39	1.347820078
8.565628261	0.35940009101521	4.010493839	0.854750403	3.52	-0.112318096
7.724628346	0.31560957110728	6.314815176	0.479871176	3.45	0.489227032
9.637344967	0.25576146099206	5.890787062	0.64605475	3.49	0.650875723
10.3741502	0.31029969068017	5.398722023	0.577133655	3.48	1.284220593
12.43571637	0.35629732956081	5.250965063	0.769726248	3.46	1.236520979
9.22222368750742	0.265225746	4.361784023	0.835239866438179	3.48	0.68592178119(
8.14770074773504	0.208698574	4.321826134	1.50039234545881	3.53	-0.30757989548
8.76804388822218	0.338176353	4.086074588	0.946832776825746	3.5	-0.09025140371
4.70940046	0.244626729	4.768819735	1.34902699	3.58	0.535135968
7.84569505	0.231171141	4.132588669	1.515456774	3.63	0.807040406
9.215848643	0.206049625	4.398238384	1.425068185	3.56	0.535135968
7.721135632	0.186562222	4.382299401	1.968834463	3.61	0.395299399
6.259886345	0.156336862	4.804682447	1.747884577	3.56	8.599044749
8.479646713	0.228320943	4.250802792	1.928661757	3.65	0.97018307
6.533832006	0.35095546190221	5.511429658	0.649919485	3.47	0.078480359
8.565628261	0.37283472824043	4.971257701	0.665378422	3.47	0.984773018
10.1806458	0.21657710241849	4.582966155	0.607407407	3.45	1.220621108
9.525707811	0.34481391345640	3.956888989	0.781320451	3.61	1.366369928
7.032477974	0.32002380905271	4.451015753	0.56489533	3.61	0.552826517
6.980380634	0.33521774400981	4.517678195	0.653784219	3.59	0.650875723
8.945194595	0.33003581250865	5.107331553	0.45668277	3.49	0.778074693
7.382274399	0.30451000178073	5.255775755	0.831561997	3.42	0.809874435
9.778752033	0.38316660401126	4.013930048	0.939774557	3.55	0.632325873
11.65425627	0.27760874009879	4.714916556	0.615136876	3.53	0.680025487
10.00202635	0.29085145393508	4.327312251	1.024798712	3.77	0.711825229
9.592690105	0.33582550140809	4.743093465	0.944283414	3.48	1.586318146
9.206553165	0.216058461	5.045095438	1.384895478	3.57	8.715575223
3.538170116	0.23349107	4.481918044	1.493935682	3.58	6.874393738
9.840504827	0.209231242	5.187218035	1.644583331	3.55	6.594720601

9.101486614	0.30163115094675	5.276393005	0.348470209	3.5	1.445869284
10.15831837	0.20544554586045	4.929335959	0.734943639	3.44	0.791324585
10.26995552	0.28087143771063	5.556100367	0.866344605	3.49	0.584626259
10.29228295	0.39429816056930	6.050914373	0.580354267	3.51	0.791324585
9.793636987	0.37708903002842	5.336870272	0.488244767	3.46	1.872515828
9.309875974	0.24344637686894	4.327312251	0.445088567	3.4	0.632325873
8.260486699	0.21120324752840	4.929335959	0.835426731	3.59	0.664125615
10.49322984	0.26244679237318	4.846866958	0.615136876	3.44	0.680025487
9.466167994	0.23423405420022	4.096399049	0.584219002	3.54	0.711825229
8.721920281	0.23986380694221	5.20079642	0.666022544	3.56	1.37961982
8.654937987	0.33963198195524	5.202170904	0.746537842	3.51	1.00067289
7.449256693	0.23909611338649	4.920401817	0.511433172	3.57	1.284220593
8.51326009837925	0.199532005	5.344748097	1.22287839725815	3.44	1.12057876473
7.84860673357159	0.228177532	5.412676509	1.16267695875959	3.42	-0.12129833110
9.00067256590486	0.244600967	3.574613606	1.11715879794361	3.54	-0.16786872220
8.280723464	0.294803478	4.299947989	1.789492023	3.57	1.7237468
12.41721159	0.284396939	4.378314655	1.651757029	3.6	3.005582011
11.09167629	0.236738971	4.758193747	1.73927614	3.58	9.772118185
10.54324303	0.472178617	3.779540199	1.453476027	3.68	0.592106421
13.22220006	0.535413251	4.443398835	1.67901708	3.67	3.650383965
8.615360705	0.487291297	4.355734429	1.4982399	3.66	3.432860414
8.18093341597542	0.266371567	4.329817712	1.14212037000399	3.47	-0.12129833110
9.12252568278627	0.198386184	4.549586103	1.17001859760088	3.46	-0.10577486740
8.69050099566129	0.348488742	4.881236583	0.971794348886122	3.43	0.00288937847
7.734149303	0.265771224	4.188375109	1.552760002	3.62	6.074217819
10.55439761	0.28638545	4.382299401	1.785187804	3.66	1.218781413
8.381114636	0.300769009	4.535047986	1.654626508	3.61	0.822577803
10.2413588468792	0.211372156	5.396693353	0.965921037813093	3.39	0.65487485379
8.32494164501708	0.151789461	5.152950229	1.06723565382286	3.41	-0.02815754891
8.31386408893695	0.186927974	4.161994578	1.1245004367849	3.45	-0.05920447631
4.363608644	0.068444697	5.235034984	1.347592251	3.55	0.752659519
5.947558253	0.130883927	5.46216549	1.278724754	3.57	1.715978101
6.350982039	0.141688168	4.813980187	1.538412607	3.59	0.371993304
12.26104754	0.264909536	3.816465509	1.463806152	3.67	2.943432425
8.992757149	0.276442897	4.430116349	1.422198706	3.57	3.254180354
7.053720245	0.243102204	5.351920858	1.338983813	3.62	8.078541967
5.936403678	0.178210477	4.274711266	1.668973903	3.58	7.029767703
8.682288153	0.311109264	5.045095438	1.654626508	3.57	7.255059952
8.916534222	0.264578118	4.762178492	1.572846355	3.6	6.571414506
9.90903216447533	0.312204409	5.404684931	1.10688050356581	3.42	-0.13682179480
7.78214139709082	0.277065896	4.601531359	0.888099666095447	3.47	0.43754636202
9.12252568278627	0.238871861	4.925190261	1.10981715910232	3.4	-0.10577486740
8.100391172	0.31859932	5.262928204	1.671843382	3.58	3.891213611
6.774855878	0.296791988	4.543017478	1.674712861	3.57	9.399220669
7.62074446	0.189743839	4.840545158	1.555629481	3.59	10.33923316
8.104044678	0.234903474	2.372207023	0.222049228	3.71	-0.496859154
9.662514808	0.18239741	2.372207023	0.222049228	3.7	0.252664172

7.168962599	0.192559874	2.485669537	0.55512307	3.72	0.023996039
8.236105165	0.169129613	5.379814078	1.463806152	3.58	5.141974029
11.12328092	0.179801286	5.422318032	1.429372403	3.56	3.471703905
6.873387954	0.126111502	5.079629901	1.456632454	3.56	6.532571015
6.295672738	0.31983188566378	3.626325743	0.518518519	3.41	0.536926646
6.273345307	0.31023571621719	4.393287452	0.406441224	3.58	0.870823941
7.159000085	0.34753282813293	4.211168408	0.936553945	3.47	-1.10076009
10.38980087	0.096016466	3.052982111	1.154655986	3.5	0.252664172
6.649472556	0.206109826	3.242086303	0.488508302	3.52	0.582962587
7.168962599	0.197641106	3.355548818	0.821582144	3.5	0.303479313
5.922186495	0.201028594	3.242086303	0.15543446	3.63	-1.576680895
7.168962599	0.226434754	2.485669537	0.754967376	3.72	0.12562632
9.662514808	0.16545997	2.977340435	0.355278765	3.73	0.227256602
8.081867248	0.27089142148618	2.042920923	0.421900161	3.42	0.966223169
7.315292105	0.35287469579153	2.249780668	0.25958132	3.52	1.2709707
6.868743477	0.36746087335034	2.381731069	0.309822866	NA	0.825774306
5.238840987	0.22697295265230	3.090964478	0.23252818	3.4	0.682675465
6.198920536	0.35018776834649	3.775457186	0.112721417	3.61	0.74627495
5.908663928	0.39644130507904	3.581655034	0.190016103	3.63	0.571376367
6.233880521	0.180703666	3.052982111	0.288663997	3.53	0.392405809
8.000146669	0.129891346	3.128623788	0.288663997	3.55	-0.115745598
6.33777853	0.163766226	3.128623788	0.15543446	3.57	-0.700119717
6.233880521	0.163766226	3.544653009	0.15543446	3.57	0.163737676
6.33777853	0.153942511	3.09080295	0.355278765	3.61	0.062107394
7.70459850452993	0.230087233	3.390807316	0.838176521974694	3.6	-0.18339218589
7.73783117277031	0.151789461	3.26693786	0.855796455193783	3.67	0.09603016066
7.54951271940814	0.271718732	3.578609395	0.882226355022418	3.65	-0.26100950438
10.66594336	0.239456602	2.967714672	0.876997688	3.79	0.472986382
8.435028414	0.194118562	3.374158735	1.070687523	3.78	0.426374192
5.568302713	0.216787582	3.103196026	0.858346074	3.74	1.218781413
6.233880521	0.109566418	3.052982111	0.421893533	3.53	0.201849031
6.026084504	0.104485186	2.599132052	0.355278765	3.53	0.379702024
7.89624866	0.179518045	2.674773729	0.355278765	3.7	0.252664172
6.33777853	0.234903474	2.523490376	0.022204923	3.82	-0.204672095
7.272860608	0.146828786	2.63695289	0.488508302	3.72	0.176441461
6.129982513	0.397502899	2.447848699	0.088819691	3.68	-0.369821302
9.376858268	0.36838850306351	3.651066443	0.155233494	3.47	0.7277251
8.878212301	0.32562157456322	4.11358009	0.503059581	3.55	0.714475208
5.372805575	0.36077554196922	3.988502106	0.152012882	3.46	-1.021260734
9.143024764	0.194253618	2.977340435	0.488508302	3.51	0.354294454
10.49369888	0.195947362	3.317727979	0.288663997	3.56	-0.064930458
10.8053929	0.245065938	3.128623788	0.288663997	3.54	0.151033891
7.272860608	0.112953906	2.485669537	0.688352607	3.64	0.189145246
5.091002426	0.208819817	3.015161273	0.222049228	3.6	0.239960387
7.792350652	0.14005381	2.939519597	0.488508302	3.47	0.163737676
11.25899491	0.37076458	2.869424277	1.237117307	3.8	0.418605494
7.868004199	0.390251983	3.096554783	1.043427472	3.8	0.403068097

7.63375813	0.441157852	2.769805635	1.106556011	3.83	0.418605494
5.61840732	0.32542965117429	2.690989823	0.421900161	3.54	0.269278813
9.354530837	0.37126735389749	2.661438431	0.224798712	3.82	0.521026774
7.359946967	0.21392216220494	2.542545621	0.336876006	3.82	0.603176109
8.104044678	0.263697122	1.76707361	0.222049228	3.9	0.201849031
10.59759689	0.150216274	1.540148581	0.488508302	3.9	0.239960387
11.22098494	0.10617893	1.729252772	0.821582144	3.86	0.481332305
6.961166582	0.14852253	1.804894449	0.55512307	3.86	-1.741830102
10.70149489	0.417827827	2.107461155	0.288663997	3.45	0.328886883
9.07821545846576	0.338176353	2.164100118	0.89250464940022	3.91	-0.12129833110
7.52735760724789	0.283940823	1.792491749	0.910124582619309	4.02	0.34440557983
7.48304738292738	0.397759048	1.960314883	1.00997087086082	3.99	0.32888211613
6.873387954	0.13525865	1.89980282	1.307419544	3.91	0.410836796
12.06026519	0.144008096	2.0113757	1.369113343	3.89	0.364224606
8.738061027	0.2800885	2.211941234	1.660365466	4.23	0.403068097

Fructose (g/L)	MAC%	Residual sugar (g/L)	CO2 yield
0.969528009	-87.92447707	1.191107209	0.45854928845214
0.702021043	-93.40199732	1.223047817	0.459671732103315
0.986423185	-115.8904501	1.92349659	0.458489038404742
0.393111575	-62.37164064	0	0.437848101265823
2.387605849	-30.54222227	2.703788947	0.463473137324598
1.472933316	-54.88236574	1.814523984	0.45989234468136
16.20932413	-88.58410282	16.830398072	0.483490904592775
2.400309634	-54.88236574	2.830826799	0.470471832368111
9.22224228	-97.94569643	9.944946504	0.461077603814902
1.079115975	-79.22250915	1.319076362	0.466690295939869
1.612674953	-32.41454103	0.582256821	0.463924570656939
2.41301342	-66.11627811	2.767307874	0.456725314596361
1.485637101	-101.6903339	0.810924954	0.460690232936508
1.104523545	-54.88236574	1.522336924	0.465054725656318
-0.343707966	-77.35019044	0	0.457974683544304
0.253369938	-56.75468445	0.798221169	0.459522364891499
1.320487894	-32.41454103	0.544145466	0.461481489705765
0.583668353	-34.28685975	0.175735695	0.443873436315709
1.472933316	-73.60555301	1.687486132	0.461556413701506
0.291481293	-56.75468445	0	0.463333333333333
0.609075923	-47.39309084	0.925259021	0.465022219423998
3.824812885	-184.6826614	5.28658204	0.435926416731883
0.659783101	-142.8017454	1.403408073	0.456458215801871
1.220139797	-146.8503474	2.567959875	0.449298653647503
12.33466965	-154.1152582	14.17530697	0.449905253483297
5.004585598	-109.1796088	4.838024859	0.442837376523696
3.759614649	-142.8813458	4.571245369	0.421333410986399
21.38755406	-108.4110573	30.553713781	0.403930734368063
22.99055883	-119.3761731	28.093689368	0.419278861107717
21.27092478	-127.9108767	25.263131468	0.414098879462501
2.146233931	-69.86091554	2.716492733	0.371174228243461
1.638082523	-64.24395935	1.814523984	0.456618332981983
3.327685953	-77.35019044	3.300866851	0.455628562097025
1.384006819	-47.39309084	0.21384705	0.455389804921445
2.298679353	-54.88236574	2.576751095	0.460918447742302
0.367704005	-51.13772826	0.79822117	0.460580781985976
0.113628301	-53.01004702	0.290069762	0.455832165095533
0.672594849	-56.75468445	1.179334725	0.46268209731646
1.320487894	-34.28685975	1.700189918	0.471398595525195
0.367704005	-60.49932193	0.556849251	0.463113436160566
-0.242077684	-51.13772826	0.035994058	0.461251486551728
1.43482196	-69.86091554	1.687486132	0.445067702853869
1.701601449	-45.52077212	2.017784547	0.459141130285154
3.264167027	-38.03149722	2.843530584	0.465885056569553
2.044603649	-43.64845336	2.24645268	0.462570219873941
-0.680567591	-156.2403821	0	0.457552742616034

-0.562301353	-142.5635924	0	0.456582278481013
0.687941729	-117.2513248	2.131161034	0.459916268482245
1.142634901	-137.2643897	0.467922754	0.431484816724688
0.634483493	-131.6474335	0.937962806	0.450220633793214
1.256968968	-114.796565	1.560448281	0.456210518648095
1.696642656	-104.6806570	2.146322943	0.426606052140642
1.843077206	-89.48775425	2.062286112	0.437222267553727
1.618889798	-111.8023302	2.006420499	0.437501314794698
0.545556997	-49.26540955	0.912555236	0.457838832166827
-0.127743618	-36.15917846	0.290069761	0.453466400381351
0.08822073	-60.49932193	0.734702243	0.458933245928993
2.41301342	-39.90381594	2.830826799	0.465560852907066
1.384006819	-67.98859683	0.810924954	0.457345455029883
1.358599249	-64.24395935	0	0.462362869198312
1.218857612	-97.94569643	1.56044828	0.458673995983601
1.917565797	-79.22250915	2.449713243	0.460668803666578
0.507445642	-77.35019044	0.544145466	0.461185451360096
1.460229531	-67.98859683	2.132118614	0.465197707558973
1.333191679	-64.24395935	1.687486133	0.462363850574876
2.031899864	-43.64845336	2.322675392	0.464510153173461
1.168042471	-107.3072900	1.433410428	0.46602534000878
0.863151627	-90.45642153	1.128519584	0.46126814402535
0.507445642	-67.98859683	0.836332525	0.464211964406444
1.485637101	-58.62700316	1.687486132	0.462193863863142
-0.165854973	-43.64845336	0.290069762	0.461281873093431
1.168042471	-54.88236574	1.052296873	0.456711375325394
0.304185079	-58.62700316	0.569553036	0.456836255173371
0.367704005	-39.90381594	0	0.458565400843882
0.875855412	-36.15917846	0.709294673	0.459264785086745
0.177147227	-53.01004702	0.544145466	0.450993274030634
0.532853212	-36.15917846	0.607664391	0.459067337020162
1.447525745	-38.03149722	0	0.461729957805907
0.932921792	-104.3910597	2.598739294	0.461942907959916
1.315879133	-102.4177915	2.189353053	0.455941846726247
0.89068385	-128.6826714	2.477001996	0.457098028391107
0.774225131	-96.07337772	1.052296873	0.456033258955201
-0.089632262	-101.6903339	0.175735695	0.453880856826251
0.355000219	-84.83946534	0.671183317	0.45542478276938
2.532485885	-67.88534557	2.982166172	0.419540675144276
3.435714924	-70.88097	3.970850892	0.434494999392005
4.106981269	-62.62887246	4.680960728	0.436640924126901
1.472933316	-32.41454103	1.687486132	0.464191207702933
2.565458842	-36.15917846	1.941561836	0.460353607576096
1.676193879	-77.35019044	1.941561836	0.458311562186238
0.863151627	-56.75468445	0.340884903	0.459606214429637
0.825040271	-39.90381594	1.052296873	0.461500572189887
1.345895464	-41.77613465	1.687486132	0.460663983475217

1.447525745	-34.28685975	1.204742295	0.4626047235287
1.155338686	-28.66990356	1.395299073	0.463233541481059
0.812336486	-30.54222227	1.052296873	0.462136306286943
0.291481293	-41.77613465	0.544145465	0.456914040942082
1.371303034	-92.32874024	1.738301273	0.458893226497008
0.100924516	-36.15917846	0.417107614	0.456119201653592
1.536452242	-15.56367247	1.966969407	0.467466209846303
1.549156027	-32.41454103	1.001481732	0.461655440888304
5.258661301	-23.05294737	6.159218518	0.475782482171869
4.000986568	-97.94569643	4.342577236	0.458055447936862
2.590866412	-66.11627811	2.830826799	0.459496860876906
1.638082523	-81.09482792	1.128519584	0.454442392997034
1.40941439	-54.88236574	1.700189918	0.463791279567838
0.710706205	-73.60555301	1.230149866	0.46655668626621
0.672594849	-86.71178410	0.099512984	0.459053509639493
1.981084723	NA	1.979673191	0.465406552212365
1.396710605	-34.28685975	1.712893703	0.46810894882175
1.333191679	-38.03149722	1.560448281	0.466531639217082
0.456630501	-77.35019044	0.798221169	0.453087188968935
1.803231731	-71.73323425	1.814523984	0.458871873502333
0.698002419	-77.35019044	0.493330324	0.454490353896805
1.104689423	-135.8953068	1.914563858	0.458514154551416
0.251482995	-151.0010149	0.218664255	0.456412663016587
0.755522436	-100.5125671	2.294140969	0.457849669555146
1.968380938	-103.5626526	2.017784547	0.463864892029676
0.850447842	-86.71178410	0	0.464767932489451
0.469334286	-103.5626526	0.645775747	0.457956697588518
0.685298634	-34.28685975	0	0.461392405063291
1.06641219	-28.66990356	1.395299073	0.464888856500095
0.850447842	-26.79758485	1.179334725	0.462512476897684
0.671046552	-83.56967831	1.843968046	0.464755248214848
1.817102711	-105.6498687	2.52892794	0.451569564935097
2.808286415	-110.7191266	4.521803531	0.448601208990825
10.34017538	-66.11627811	11.100990959	0.473308849179566
1.955677153	-82.96714663	1.662078562	0.439963093781627
3.823133575	-82.96714663	4.228243169	0.469902369123817
5.623162009	-78.05916442	6.072842296	0.421171771077124
8.820100373	-72.57660646	9.665984271	0.430555892333687
4.359678059	-55.168072	4.980269707	0.427679145539433
1.77782416	-39.90381594	2.322675391	0.464893658480952
1.231561397	-32.41454103	0	0.458607594936709
-0.686710166	-39.90381594	0	0.463206751054852
0.316888864	-64.24395935	0.378996258	0.461412969573253
1.218857612	-62.37164064	0.683887102	0.459934782555066
0.151739656	-67.98859683	0.277365976	0.461975258305518
1.40941439	-58.62700316	1.738301273	0.459445802631174
-0.62319124	-39.90381594	0	0.46168776371308

0.990189479	-45.52077212	1.319076362	0.458331537116326
1.231561397	-64.24395935	1.496929354	0.460503549709627
19.8426067	-64.24395935	19.383858902	0.494310759566118
3.213351886	-69.86091554	3.59305391	0.461597230951543
1.701601449	-26.79758485	1.090408228	0.46602598552353
1.739712805	-51.13772826	2.068599688	0.465412455954339
2.082715005	-53.01004702	0.429811399	0.461300727050013
0.329592649	-58.62700316	0.544145465	0.455856761136126
1.485637101	-38.03149722	1.814523984	0.458574236075117
-0.178558758	-49.26540955	0.035994058	0.447240919897092
2.43842099	-49.26540955	2.830826799	0.46321212359961
1.930269582	-30.54222227	2.195637539	0.463407063052642
0.761521345	-39.90381594	1.179334724	0.459332094043685
0.583668353	-77.35019044	0	0.464978902953587
0.443926716	-66.11627811	0.633071962	0.462712786885384
0.367704005	-62.37164064	0	0.464767932489451
1.014581813	-102.7920321	1.901305626	0.456404065899681
0.378196821	-154.6753763	0.249978854	0.454952440885219
0.899131439	-165.4262857	2.119752547	0.459766204996054
2.208550472171:	-127.6007707	2.52190912460946	0.448442750482305
2.360516980637!	-109.4021281	2.2236951858334	0.452473259957323
0.878843523090:	-124.2379781	1.33191334881069	0.450421614179403
2.677624554	-61.21584208	3.057386557	0.428994096128847
1.949339446	-84.16345570	2.352407543	0.432904505587948
16.20662193	-85.97213463	17.938137428	0.43978444658908
1.414140604	-81.73304344	1.949276572	0.419739188891377
1.472455248	-68.50707893	1.929904233	0.418513463735147
2.036163472	-82.58086165	2.52468725	0.418082393622908
-0.393349585	-189.1055038	0	0.450590717299578
1.048372167	-200.7750036	1.712497782	0.452042709440022
-0.173712287	-147.8710033	0	0.443628691983122
1.360932937	-122.7968888	2.231756878	0.455385270930545
1.214508072	-125.0763537	2.103881863	0.457265964426686
0.944185243	-126.0970097	1.51556161	0.458713963175357
0.64851965	-134.0581261	1.156296532	0.452460669633602
0.642887924	-116.6729530	1.275213797	0.460070414091311
1.130032188	-144.4688168	1.971706365	0.454626114785731
0.811839692	-152.6000426	1.25336711	0.451671350274105
1.03147699	-107.2829183	1.632003121	0.452015573105692
1.377828114	-169.3388002	2.614349093	0.455488634167116
1.482015038	-121.9463421	3.020633571	0.453416049539533
0.862525222	-152.6000426	1.637949936	0.454321331628967
0.870972811	-141.1686959	1.550998298	0.453261637248613
0.797760378	-139.5016245	2.002481615	0.450685610332642
0.572491354	-116.7069749	1.096168107	0.447470476223037
1.558043333	-148.2792657	3.255660578	0.448096412768753
1.118768737	-146.0338226	2.355289716	0.451363254137768

1.056819755	-120.3473145	1.959443439	0.451879460949265
0.870972811	-112.1820668	1.630497654	0.453244787182229
0.744258985	-122.7968888	1.153986661	0.459240325781202
1.025845264	-126.4372283	2.61216341	0.462907980117553
1.234219111	-122.7968888	2.136842795	0.452391091324979
1.405069437	-57.71152672	1.808137534	0.43041455150105
1.346754793	-69.58098204	1.74982289	0.430605414390967
1.083690955	-80.88522519	1.618826923	0.419872153358968
5.195521287	-104.3415297	5.505365005	0.437159159227106
11.36650649	-101.0632992	11.948254647	0.421414194549972
23.91452196	-113.1588394	26.182077637	0.415903918496203
1.219758458	-84.44606178	1.785969219	0.422636352389018
2.124283378	-59.23759953	1.983542598	0.420779042851654
13.90384144	-67.54621825	15.037167173	0.41750233054661
3.151821677	-161.581815	4.531441497	0.450254437305547
1.391907428	-143.8564232	1.438588044	0.445446472445154
1.755153729	-178.1504633	1.801834345	0.443073183456967
0.969528009	-86.29142752	1.652203474	0.458470406745787
0.668230689	-90.54416069	1.189257463	0.45604368504597
0.913210753	-100.7507201	1.481937141	0.454402514613373
1.186349444	-138.8892309	2.597769007	0.448289248591401
4.373906131	-132.0168142	5.056581596	0.446488202651298
1.932553085	-154.4712451	2.853726618	0.438614710866866
0.831550731	-132.3570328	2.290669907	0.448853055642299
0.947001106	-89.72763589	2.104022729	0.459565128590763
1.22295566	-131.2002894	2.064629837	0.450549442285172
1.24956372	-33.80305242	1.637094421	0.434817881552917
1.278073102	-22.04663953	1.642297708	0.435719753385295
4.097910102	-25.05356821	4.379268593	0.440330487228954
2.638748125	-49.85507770	2.987435335	0.436173160813472
9.169988236	-45.27685923	9.829423376	0.433198706727248
5.14627781	-66.64187880	5.526039813	0.444974458106604
1.725152038	-49.68551408	2.073839248	0.440734227591205
15.1867636	-62.96799978	16.164715368	0.446803599182186
18.71415161	-73.763552	20.243680953	0.43016046964602
5.535042103	-54.26373255	6.272164225	0.440388996233153
2.541557052	-117.1718457	3.092230416	0.437351868139901
2.240912666	-72.46356404	2.597368574	0.441718590657918
7.926290353219(	-127.7985821	8.36383671524151	0.442887068017688
5.608801099106(	-131.5569974	5.42540891320844	0.4468106777795
6.3876294549968	-156.2834140	6.28185458758877	0.444004955990294
17.54785874	-56.63762361	19.155075066	0.442378907974088
28.6665175	-42.73340451	36.737290768	0.419998312828977
27.33564774	-81.95912829	32.974818457	0.417840579066133
14.35999154	-70.03315174	15.392324195	0.432250370624747
3.757093406	-83.48520110	4.222311089	0.424353383960984
7.031784628	-78.85046144	7.799981543	0.437216369678436

19.70550056	-70.88097	21.452553454	0.439763966212288
26.13047843	-88.40254689	29.905161567	0.429030489954703
18.73358983	-91.90686229	20.402955742	0.437632934118393
7.303919633	-100.3850446	7.916742583	0.422160925640929
5.380832267	-75.07484421	5.615578569	0.432440522059254
15.70122835	-94.11118970	17.650267399	0.4156832056224
1.599451583	-83.65476476	1.955907491	0.446767237921451
1.405069437	-73.48094591	1.823674931	0.436183361441265
1.385631222	-86.02865582	1.866386302	0.435071780640397
3.514763663	-53.54026097	3.718435172	0.436511132266623
16.83901184	-68.73316378	18.220935917	0.436147765783474
23.75901625	-59.06803587	26.850053941	0.424887094545966
16.38156586	-82.75042531	17.802333428	0.430935244326484
14.06841832	-68.84620621	15.473648491	0.422071682953716
18.6467658	-82.97651017	20.774484908	0.428626565928477
4.106981269	-82.97651017	4.572198952	0.391046163970849
12.68830508	-51.38115055	13.627413357	0.432998522574216
7.653807496	-54.09416889	8.297705239	0.431303061926342
16.27530362	-90.60687429	21.168679305	0.396791344853147
21.50418335	-108.4110573	25.216716901	0.418541060951277
10.51122504	-103.4371902	11.388183731	0.422273981813117
1.433578818	-53.24635068	1.85995301	0.430594453374018
1.920830065	-72.36182582	2.093426781	0.424934894890912
2.36661312	-73.763552	3.057123053	0.430318722731647
8.674961704	-59.40716319	9.295553352	0.429328462571149
21.21261013	-71.10705485	23.759838944	0.431063264747115
19.88044449	-67.15056974	21.922707918	0.41840772277201
13.39844786	-104.3415297	14.469624007	0.422054740081661
7.936309548	-95.18509280	8.619050782	0.428144292835321
14.73968466	-115.7022941	15.997309565	0.425741423395355
2.1135714043790	-79.92823960	1.94570268217949	0.45704333520328
0.8598477095319	-78.34574896	0.583314741445665	0.45889316095161
1.6576718789804	-76.76325831	1.3656154471962	0.456894269502821
2.726868031	-49.74203527	3.098861335	0.431934622364956
4.290996367	-49.00725948	4.9815063	0.449403831294678
1.50226051	-52.45505365	1.827641625	0.44197371118871
21.19317192	-53.81156280	22.839231737	0.437708553066464
7.42054892	-57.65500548	7.753698733	0.434598069628013
19.06533536	-61.84887970	20.434311606	0.427029787986314
2.016725257	-71.05053365	4.431886201	0.441505064141091
1.686275608	-82.41129804	2.066037611	0.423565835216421
1.463384081	-60.59410872	1.835377385	0.424383561142135
9.355299215	-75.68527336	9.94481607	0.434740129212076
2.463804193	-56.12893268	3.014477557	0.433702046778219
3.184314015	-53.03157004	3.310298542	0.442381497152026
1.705713823	-72.06791553	2.116550619	0.425913363685864
1.599451583	-80.43305548	2.033594473	0.421081472383637

5.389903433	-52.96374459	5.901733306	0.424840918993137
4.660322445	-73.763552	5.086696637	0.434170867043345
7.294848466	-72.63312770	7.868827925	0.436038445119537
5.759229511	-75.17658238	6.208909798	0.449887384773488
1.262377739	-188.2889791	2.975894855	0.453671214485438
1.380643977	-153.3825454	2.935162381	0.443552312496392
1.107505286	-139.5356464	1.787530773	0.45720365231239
1.27775560781	-140.6563187	1.17198074040641	0.450752206348253
3.44327835346	-146.7884700	3.25988616756238	0.443826257714449
1.99959652302	-128.5898274	2.01800936520588	0.447566214397485
4.290996367	-86.48082557	4.779520145	0.409567666294494
10.50215388	-79.58523723	11.884077957	0.428001711854019
13.58246295	-94.95900791	15.873324722	0.427221183881287
13.62133938	-78.17220685	15.492691446	0.427525396873818
3.416276709	-115.4762092	3.827113505	0.427879937070382
16.12886907	-96.31551710	20.921251678	0.420309728306368
2.901811962	-146.7324416	3.429179231	0.410453667475934
1.560575154	-93.48945634	1.963643251	0.41270210848098
2.386051335	-79.528716	2.812425527	0.42038951136304
22.12620622	-100.2720022	31.618651268	0.425209010161658
22.14564443	-89.41992876	29.63376533	0.426925821076346
22.47609408	-110.6153847	32.57449759	0.404988609659644
11.18249139	-42.78992574	11.803083038	0.434819451886738
15.14788717	-47.70727148	16.048151955	0.432039835125336
7.703050973	-30.92047042	8.199343449	0.440645588696233
1.54369699763	-79.33480564	1.32925788433648	0.458011881453765
3.12034952296	-119.2926948	3.10771543774947	0.450805806601701
2.60746255689	-110.7868075	2.45511729839327	0.448400318048293
4.349311011	-82.41129804	4.931059168	0.434827683697023
6.720773194	-54.94198714	7.286983955	0.430537205521806
7.285777299	-71.17488029	7.87788372	0.424533439107775
19.58887127	-82.91998897	23.666533639	0.421124737400432
9.666310649	-89.75905608	10.551038038	0.42892667362414
16.1677455	-121.5805005	18.396457686	0.422317035775168
-0.976759794	-72.57660646	1.298564581	0.423077610124565
1.161443814	-68.84620621	1.494593627	0.451624451591332
1.307878364	-82.29825557	1.602184686	0.42251878959594
23.62294874	-131.3021497	31.017845261	0.408433439812304
25.1585677	-113.837094	33.361408839	0.411857101946315
28.51101178	-114.2892637	41.18085441	0.411961760720294
19.46317082	-91.28512889	22.181310995	0.438323129314919
27.91879417	-95.69378374	35.98179874	0.420210704655273
24.05966063	-103.6067539	33.155902067	0.415611738868427
2.98737882806	-108.8086941	3.34730787159376	0.451738856670198
0.93583096376	-118.1058268	1.18709576141139	0.450865911444899
3.13934533652	-108.8086941	3.4527039889661	0.44860292450164
18.11156696	-69.52446080	30.19098852	0.393358100876891

19.25842162	-104.9632631	25.961903997	0.415233086630739
20.71628772	-86.98951651	28.771523592	0.414832790836678
9.850325747	-72.57660646	10.898195799	0.423747171494602
1.424507652	-87.21560136	1.827575749	0.43202344119888
1.482822295	-75.00701876	1.994652168	0.428160469232943
5.525970936	-83.14607382	6.177637377	0.433970083581575
16.12886907	-88.34602570	19.056764098	0.436810987071916
21.3098012	-68.39403651	28.036589671	0.43270733310506
18.44331249	-78.29655353	21.863225074	0.431585906370203
23.79789268	-88.23298323	31.037415236	0.433136921942499
19.81305868	-86.87647408	25.560991172	0.433221856779106
1.03147699	-126.8795126	1.918200803	0.45163002989878
1.070899069	-171.8223963	1.976172731	0.452464761703872
0.806207966	-138.6850998	2.122228301	0.451937189424775
0.994870774	-122.6267794	1.709345982	0.45016662664339
4.238744716	-165.2901983	5.668714129	0.428519642843636
0.792128652	-132.2209454	1.713302185	0.446306573959258
1.943816536	-206.4906769	3.291636614	0.447052893128337
0.327511291	-98.53929896	0.215193195	0.442258105209598
1.141295639	-212.6146126	1.630522671	0.449591005600461
0.986423185	-191.6231218	1.637298908	0.436475276343146
1.659414394	-167.2634664	2.943634987	0.43792870146602
2.535147724	-159.9487654	3.771668703	0.436610764368616
0.6318979468323	-115.9299021	1.31781972802304	0.452898050573116
0.9358309637651	-113.9517888	0.628251068282828	0.459657300379779
1.4297221162808	-102.2809201	1.33947071257082	0.453830772269699
1.025376311	-102.9284993	1.560512279	0.428262909404073
7.528107041	-75.85483697	8.335147447	0.443225090644436
1.161443814	-87.15908017	1.696579782	0.428085575240162
3.008074202	-86.48082557	3.403373601	0.430699713223387
21.80482774	-104.4545722	30.403872489	0.410220661060007
7.245604989	-80.88522519	8.215788059	0.419215990414294
1.062451481	-172.8430523	1.14093184	0.442298024891849
2.549227038	-146.1018663	3.534000056	0.443833363422745
3.751600452	-126.8795126	4.97222156	0.436887344616857
1.206060483	-95.88559351	2.572430411	0.451184134124824
0.921658341	-120.3473145	1.474484858	0.447976941845928
0.851261771	-123.6474353	1.502137494	0.455290756608325
2.954711281	-152.8381956	3.732785974	0.435123300219493
1.476383312	-160.1869185	2.286257747	0.434997963987748
1.124400462	-98.70940831	1.756726335	0.448897000771977
0.913210753	-133.4117106	1.59323624	0.44599398217393
0.803392103	-114.2233787	1.515217332	0.44550649435343
0.865341085	-134.8066071	2.451659231	0.449161139467434
21.75688014	-114.6849122	30.472455363	0.401350822940787
19.47224198	-90.71991676	26.346635718	0.413000762159838
21.10505201	-120.7326823	27.699772611	0.412087464385301

1.425697782	-161.2075745	2.871567066	0.447916550270349
1.208876346	-144.0265326	2.000200931	0.441234419819886
0.702021043	-175.0544736	1.286647302	0.450250267052014
0.930105929	-199.5502164	1.721430514	0.446789523710765
2.166269697	-164.2014986	4.038785525	0.442348312066594
0.744258985	-114.2233787	1.376584858	0.456830658935701
1.225771523	-144.0265326	1.889897138	0.427331700241617
0.98079146	-139.9439088	1.660816947	0.448884026151349
1.090610109	-102.7920321	1.802435338	0.448814170978765
0.704836906	-157.4651693	2.084456726	0.446032640240357
1.163822542	-157.5332130	2.164495432	0.448186061956928
0.687941729	-143.5842483	1.972162322	0.459264745259169
1.03081003155	-164.5914899	2.15138879629175	0.450417822178417
2.66444999757	-167.9542826	2.54315166646419	0.446947917046666
2.94938720094	-76.96106960	2.78151847874464	0.439077221114454
12.21271676	-82.97651017	13.93646356	0.432029373953379
15.52628442	-86.31126191	18.531866431	0.426606839530508
24.32272447	-102.4763296	34.094842655	0.415908600373876
6.722069075	-60.83149782	7.314175496	0.445913456876031
15.34226932	-89.08080148	18.992653285	0.426774608296255
13.92327965	-85.35040123	17.356140064	0.43329233071997
2.68344581112	-114.3474114	2.5621474800225	0.44971406650731
2.15156303149	-125.2270348	2.04578816408815	0.448342675693627
2.30352953996	-141.6453753	2.30641891844065	0.446070998267391
20.62946369	-78.22872804	26.703681509	0.435718516888452
9.675381816	-86.48082557	10.894163229	0.432585029191714
8.402826699	-92.98076536	9.225404502	0.427440118100681
2.11357140437	-167.1630372	2.76844625817431	0.444175852219615
3.48126998057	-155.0965459	3.45311243165914	0.442994557012563
2.56947092977	-106.0393355	2.51026645346477	0.448719005342376
6.837402482	-122.7674461	7.590062001	0.420513604778623
11.42482113	-132.4325740	13.140799231	0.423793168536755
2.00765409	-104.8502207	2.379647394	0.426390971153291
14.72931762	-62.40278761	17.672750045	0.421607438286726
16.84808301	-88.51558931	20.102263364	0.429004015639672
20.86272227	-127.7413131	28.941264237	0.426177731373914
23.12662633	-81.90260706	30.156394033	0.419544029868852
11.27968246	-114.6849122	18.534742412	0.387384250174929
23.23418445	-102.6458932	29.805598956	0.421246904164486
3.65223230260	-167.5586599	3.51541050779772	0.445425542757173
2.11357140437	-127.7985821	2.55111776640209	0.446110039013919
2.79742069247	-143.8213000	2.6916458250703	0.45222459255931
18.94741019	-123.9543916	22.838623801	0.423138857287672
22.88429659	-93.31989268	32.283517259	0.424684904878878
21.06617558	-105.9806450	31.40540874	0.406236367835079
1.511044671	-17.43599123	1.014185517	0.46540933081345
0.316888864	-17.43599123	0.569553036	0.464618670778583

0.304185079	-23.05294737	0.328181118	0.464947624604448
17.79148436	-128.9282586	22.933458389	0.400202662944305
19.39448912	-130.7369375	22.866193025	0.423006536331627
20.54134379	-116.1544638	27.073914805	0.409382187640809
0.879420399	-79.52107638	1.416347045	0.4650152870366
1.268009465	-117.4894778	2.138833406	0.466062574700659
-0.021655696	-108.4736835	0	0.451139240506329
1.561859812	-51.13772826	1.814523984	0.464909661311575
1.358599249	-60.49932193	1.941561836	0.46350176088546
1.892158227	-66.11627811	2.19563754	0.462768233356443
1.371303034	-60.49932193	0	0.457848101265823
1.180746256	-23.05294737	1.306372576	0.468022836279567
1.714305234	-47.39309084	1.941561836	0.459204957044301
0.668230689	-1.134699158	1.634453858	0.466508381535825
1.298983956	-11.37528059	2.569954656	0.468497968504151
0.935737655	-17.90747866	1.761511961	0.46565509289381
0.682310003	-53.01804346	1.364985468	0.470133864527828
1.282088779	-86.90382108	2.028363729	0.464822059944432
0.665414826	-77.30965514	1.236791193	0.467375722266333
0.659891064	-51.13772826	1.052296873	0.466289769054379
0.266073723	-54.88236574	0.150328125	0.459109776843553
1.75241659	-54.88236574	1.052296873	0.467518854975355
1.511044671	-75.47787173	1.674782347	0.471262710839295
1.701601449	-53.01004702	1.763708843	0.469570402834984
1.030810031556	-67.86174831	0.847417845658444	0.462878699029251
0.346960743457	-61.72959702	0.442990904123971	0.464200999241981
1.315747234931	-77.15888094	1.05473773054285	0.465828383002152
1.142005599	-26.28573072	1.614991981	0.439577698132958
1.005938097	-43.58122276	1.432312289	0.436435068828836
13.44639545	-32.05089472	14.665176863	0.435244459840515
0.977485694	-51.13772826	1.179334725	0.450172591431724
0.418519145	-28.66990356	0.798221169	0.469259802142747
1.472933316	-32.41454103	1.725597488	0.470854452576284
0.43122293	-24.92526613	0.226550835	0.468253515717204
1.129931116	-30.54222227	1.306372577	0.469719954716079
0.456630501	-21.18062866	0.086809199	0.4663733565296
1.614360589	-80.74586351	2.342085689	0.459221673031586
0.868156948	-103.6425787	1.582632156	0.459396861796815
-0.351111644	-97.45059930	0	0.459620253164557
1.460229531	-47.39309084	1.814523985	0.458574236077067
1.587267382	-64.24395935	1.522336924	0.458599760968183
0.355000219	-54.88236574	0.50603411	0.460688286865956
1.371303034	-23.05294737	1.56044828	0.464917636821603
1.968380938	-49.26540955	2.208341325	0.469991142882751
0.888559197	-45.52077212	1.052296873	0.472859021390629
1.346754793	-22.10316072	1.765360287	0.447340579297278
1.307878364	-31.76828863	1.710946461	0.445579589968568

1.327316579	-17.86406957	1.745922073		0.449854051128667
0.48801547	-33.21731797	0.757294283		0.448394796692245
0.659783101	-31.75437777	1.180809875		0.450048191344156
0.665414826	-25.86859509	1.268590935		0.447670509494564
-0.013409551	12.52110841	0.18843948		0.471556370621395
0.812336486	23.75502074	1.052296873		0.471545170923379
-0.699413951	14.39342712		0	0.468143459915612
0.532853212	10.64878965		0	0.472194092827004
0.329592649	-4.329760148	0.658479532		0.474017429430765
1.22076816713	-7.133669207	1.09946983603349		0.467442781596782
0.32796492989	11.26278470	0.67237050973392		0.468248254504486
0.59390631971	2.954708762	0.922788435852086		0.466245764556107
1.083690955	19.15732680	1.494527751		0.436635289269533
1.180882028	14.40954468	1.545106634		0.442929847450177
1.346754793	5.874841106	1.74982289		0.42979776356437

Declaration of interests

☐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.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Marullo Philippe reports financial support was provided by Biolaffort. Vion Charlotte reports financial support was provided by Biolaffort. Maitena Muro reports financial support was provided by Biolaffort. Margaux Bernanrd reports financial support was provided by Biolaffort.