Genotypic and pheno-metabolomic characterization of a *Saccharomyces cerevisiae* strain collection

R Franco-Duarte [(1)*], I Mendes [(1)], CC Castro [(2)], JS Silva [(2)], A Xavier [(1)], J Drumonde-Neves [(1,3)], C Oliveira [(4,5)], RC Martins [(1)], JM Oliveira [(2)], AC Ferreira [(4)], D Schuller [(1)]

[(1)] CBMA - Centre of Molecular and Environmental Biology (CBMA) / Department of Biology / University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[(2)] IBB - Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Braga, Portugal
[(3)] Research Center for Agricultural Technology - Department of Agricultural Sciences, University of Azores
[(4)] Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal
[(5)] Department of Chemistry & QOPNA, University of Aveiro, Aveiro, Portugal
*ricardofilipeduarte@gmail.com

ABSTRACT

The objective of the present work was to gain a deeper understanding of the phenotypic diversity of natural isolates of *Saccharomyces cerevisiae*, using high-throughput methods in combination with bioanalytical data. A *S. cerevisiae* strain collection was constituted comprising 187 strains from different geographical origins and technological uses. Some microsatellite alleles and phenotypes were identified as responsible for the highest strain variability. All strains were used for fermentation with white grapes must, and final fermentation products were analysed using fiber optics spectroscopy and bioanalytical quantification. Inter-strain aromatic profiles (primary fermentation products, higher alcohols, esters, fatty acids) were discriminated by HPLC and GC-MS. Relating the metabolic signature of the strains with phenotypic and genetic data by computational analysis is a pre-requisite to obtain a holistic overview of yeast pheno-metabolomics.

RESUMO

O objectivo do presente trabalho consistiu em explorar a diversidade fenotípica de isolados naturais de *Saccharomyces cerevisiae*, através de métodos de alto débito combinados com dados bioanalíticos. Uma colecção de 187 estirpes de *S. cerevisiae* foi constituída com isolados de origens geográficas e aplicações tecnológicas diferentes. Alguns alelos de microssatélites e alguns fenótipos foram identificados como responsáveis pela grande variabilidade entre estirpes. Todas as estirpes foram usadas para fermentações em mosto de uvas brancas, e os produtos finais de fermentação foram analisados usando espectroscopia de fibra óptica e quantificação bioanalítica. Os perfis aromáticos entre estirpes (produtos de fermentação primários, álcoois superiores, esteres, ácidos gordos) foram descriminados por HPLC e GC-MS. A relação, através de abordagens computacionais, entre a assinatura metabólica das estirpes e os dados fenotípicos e genotípicos, é um pré-requisito para obter uma visão holística do feno-metaboloma da levedura.

INTRODUCTION

Nowadays, most of European wine producers use commercial starter yeasts to guarantee the reproducibility and the predictability of wine quality. The advantages of fermentations containing starter cultures are related to the fact that they are rapid and produce wine with more consistent and predictable quality through successive processes and harvests (Fleet, 2008; Schuller, 2010). In these fermentations the winemaker has control over the microbiology of the process, because the inoculated yeast strain predominates and suppresses the indigenous flora. The yeast diversity of many wine regions is still unexplored and it is a common practice among wineries to use commercial starter yeasts that were obtained in other winemaking regions. This arises questions regarding biodiversity preservation, one of the most important concerns of actual times, and ongoing public discussions reflect the benefits of biodiversity preservation.

Recent phylogenetic analyses of *S. cerevisiae* strains have found that the species as a whole consists of both "domesticated" and "wild" populations. Although the genomes of most *S. cerevisiae* strains with disparate ecological and geographic sources are mosaics, genealogical relationships from DNA sequence diversity showed that domesticated strains derived from two independent clades, corresponding to strains from winemaking and sake (Japanese rice wine). "Wild" populations are mostly associated with oak trees, nectars, or insects (e. g. *Drosophila* spp., honey bees and wasps) (Fay and Benavides, 2005; Greig and Leu, 2009; Liti, *et al.,* 2009; Schacherer, *et al*., 2009).

In a previous work (Franco-Duarte, *et. al.*, 2009) the phenotypic and genetic variability of 100 *Saccharomyces cerevisiae* strains from the *Vinhos Verdes* wine region (Northwest Portugal) was evaluated and an exploratory computational approach was established to estimate a strain´s phenotypic behaviour based on its genotypic data.

The objective of the present work is to gain a deeper understanding of the phenotypic diversity of a strain collection comprising 187 strains from different geographical origins and technological applications, using high-throughput quantitative and qualitative methods in combination with bioanalytical data. Only a holistic approach between molecular biology, analytical chemistry, signal processing and bioinformatics provides detailed information on the vast and dynamical relationships between genomics, phenomics and metabolomics.

MATERIAL AND METHODS

Strain collection

A *Saccharomyces cerevisiae* collection was constituted, comprising 187 strains with different geographical origins and technological applications (Fig. 1). This collection includes strains used for winemaking (commercial and natural isolates), brewing, bakery, distillery (sake, cachaça) and ethanol production, laboratory strains, and also strains from particular environments (e.g. pathogenic strains, isolates from fruits, soil and oak exudates). The complete genome sequence of thirty strains is currently available, and the original strain code was used in the map of Fig. 1 (Liti, *et al*., 2009).

Phenotypic characterization

Phenotypic screening was performed considering a wide range of physiological traits that are important from an oenological point of view, such as ethanol resistance, growth in synthetic must media at various temperatures (18, 30 and 40 ºC), tolerance to

several stress conditions caused by extreme pH values (2 and 8), osmotic/saline stress (0.75M KCl and 1.5M NaCl), including also growth in finished wines supplemented with glucose (0.5 and 1%, w/v). Growth was also assessed in the presence of potassium bisulfite (150 and 300 mgL^{-1}) copper sulfate ($CuSO_4$, 5 mM), sodium dodecyl sulphate (SDS 0.01 % w/v), iprodion (0.05 and 0.1 mg mL^{-1}), procymidon (0.05 and 0.1 mg mL^{-1}) and cycloheximide (0.05 and 0.1 mg mL^{-1}). These experiments were carried out in white grape must with the addition of the mentioned compounds. After incubation (22 h, 30 ºC, 200 rpm), optical density (A_{640}) was determined and adjusted to 1.0. Fifteen μL of this suspension were then inoculated into replicate wells of 96-well microplates containing 135 μL of culture media, so that final cellular density was 5×10^6 cells mL^{-1}. Quadruplicate experiments were performed for each strain in each test and final A_{640} was determined after 22 h (30 ºC) in a microwell spectrophotometer.

Microsatellite amplification
Genetic characterization was performed using eleven highly polymorphic *S. cerevisiae* specific microsatellite loci (ScAAT1, ScAAT2, ScAAT3, ScAAT4, ScAAT5, ScAAT6, YPL009c, ScYOR267c, C4, C5 and C11) (Legras, *et al.*, 2005; Perez, *et al.,* 2001). Multiplex PCR mixtures and cycling conditions were optimized and performed in 96-well PCR plates as previously described (Franco-Duarte, *et al.,* 2009). For each microsatellite, the number of repeats for the alleles obtained was calculated by comparison with the sequenced strain S288c.

Must Fermentations
Individual fermentations of each of the 187 strains were carried out at 18 ºC using white grape must and Erlenmeyer flasks (100 mL) with rubber stoppers that were perforated with a syringe needle for CO_2 release. When glucose concentration was below 5 gL^{-1}, samples were collected and frozen (-20ºC) for fiber optics spectroscopy and metabolomic analysis. From the combined data of fiber optics spectroscopy, genetic and phenotypic data, a set of 28 most heterogeneous strains was constituted and used for additional fermentations. These fermentations were carried out under the same experimental conditions, and the fermentative profile of each strain was recorded by weight loss determination of the flasks due to CO_2 liberation. Samples were withdrawn and frozen when a constant weight was recorded.

Fiber optics spectroscopy
Spectral analysis of all finished fermentations was performed by transmittance fiber optics UV-VIS-SWNIR spectroscopy (200 to 1200 nm), using a highly sensitive scientific-grade spectrometer (Ocean Optics, QE65000) as previously described (Silva, *et al.,* 2008). Twenty spectra replicates were recorded for each sample.

Bioanalytical analysis
High-performance liquid chromatography with refractive index (HPLC-RI) was used to quantify fructose, glucose, ethanol, glycerol and organic acids (tartaric, malic, acetic and succinic), in a EX Chrome Elite HPLC, using a Rezex® Ion Exclusion column. Relevant metabolites that account for inter-strain differences and that are related to volatile compounds (higher alcohols, esters, fatty acids) were determined by GC-MS. Analyses were performed by solid phase microextraction (SPME), using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre, and 3-octanol (Sigma-Aldrich, 99% purity) as internal standard. Samples were analyzed using a Varian CP-3800 gas chromatography (Walnut Creek, CA, USA), equipped with a Varian Saturn 2000 mass selective detector, as previously described (Silva Ferreira and Pinho, 2003).

Statistical analysis
Principal Component Analysis (PCA), available in the Unscrambler X software was used for phenotypic, genetic, and metabolic variability analysis.

Fig.1: Geographical location and biological origin of yeast strains. Underline names indicate the original designation of sequenced strains (Liti, 2009)

RESULTS AND DISCUSSION

Genetic and phenotypic characterization

Genetic and phenotypic characterization of all 187 *S. cerevisiae* strains was performed with a set of eleven polymorphic microsatellites and 22 phenotypic tests. The results were visualized using PCA analysis (Fig. 2 and 3). PCA analysis of phenotypic data represent the segregation of all 187 strains (scores) and the discrimination of the phenotypic tests (loadings) for the same set of strains in the first two components of the PCA (Fig. 2a and b, respectively). The phenotypes that were responsible for the highest variance (strain variability) were associated with growth patterns (i) at 40 °C, (ii) in the presence of potassium bisulfite ($KHSO_3$), (iii) in a finished wine supplemented with glucose and (iv) resistance to ethanol. PC-1 (37%) and PC-2 (16%) explained 53% of strain variability and segregated strains by phenotypic behaviour into some notorious patterns. The group of sequenced strains (group 1, Liti, *et al.,* 2009) were separated by the second component of the PCA, indicating that they were influenced by the presence of ethanol in the media, and also by the growth in a stressful environment such as a glucose-supplemented finished wine. Strains from France (group 6) are mainly separated by growth in the presence of $KHSO_3$ and growth at 40 ºC, due to their occurrence in the PCA component 1. Commercial strains and strains from the *Vinhos Verdes* region show a heterogeneous phenotypic behaviour since they were dispersed in the PCA plots. These groups of strains show no discriminant results in any of the phenotypic tests.

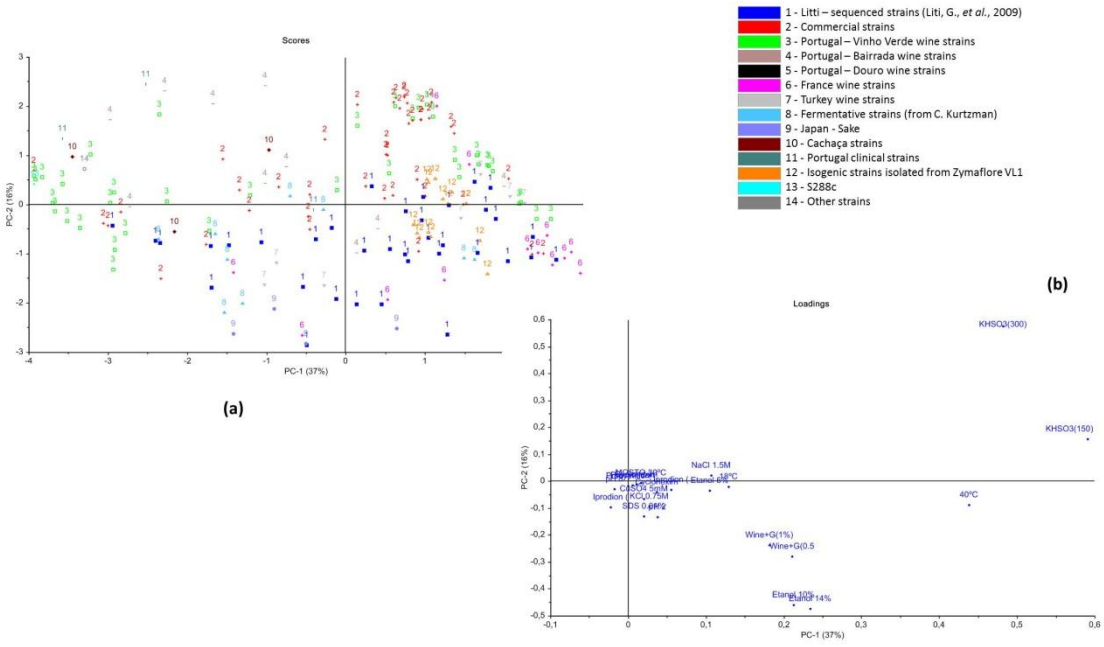

Fig. 2: PCA analysis of phenotypic data for 187 strains (scores, panel a) and 22 phenotypic tests (loadings, panel b)

Microsatellite analysis resulted in a total of 236 alleles. Thirty four alleles were identified by PCA analysis (data not shown) as significant variable between strains, as summarized in Tab. 1. However, the fact that the PCA components explain only a small part of the total variance (PC-1 - 8% and PC-2 - 6%) seems to indicate that all the

microsatellite alleles were important to characterize the genotypic differences among strains. Most polymorphic microsatellites (such as C5, YPL009c and YOR267c, with 44, 40 and 40 alleles, respectively) were associated with the smallest percentage of alleles that contributed to strain variability (4.55 – 7.5). This indicates that less polymorphic microsatellites might have a bigger impact in the global strain variability.

Tab. 1: Summary of alleles for all microsatellite markers from 187 strains, including alleles that most contributed to strain variability and their percentage of occurrence among all alleles for each microsatellite

Microsatellite	Total number of alleles	Most variable alleles (bp)	Percentage of most variable alleles among the total number of alleles
C5	44	115, 211	4.55
YPL009c	40	256, 298, 301	7.50
YOR267c	40	278, 290	5.00
C11	32	189, 191, 209, 211, 213	15.63
ScAAT1	31	171, 174, 201, 204, 219	16.13
ScAAT3	24	232, 241, 247, 262, 265	20.83
C4	23	245, 260	8.70
ScAAT4	21	302, 332	9.52
ScAAT2	18	372, 375, 378, 381	22.22
ScAAT6	15	256, 259	13.33
ScAAT5	13	219, 222	15.38

Must Fermentations

Fermentations were carried out with each of the 187 strains. When glucose concentration was below 5 g/L, samples were frozen and used for further analysis. Wines obtained from ninety one strains that completed fermentation were analysed by transmittance fiber optics UV-VIS-SWNIR spectroscopy and the respective PCA plots are shown in Fig. 3. Almost 100 % of strain variance is explained by PC-1 (97.4%) and PC-2 (2.1%). Strains were segregated into groups according to their geographical origin (and technological application), with the exception of strains from the *Vinhos Verdes* region, that formed two groups.

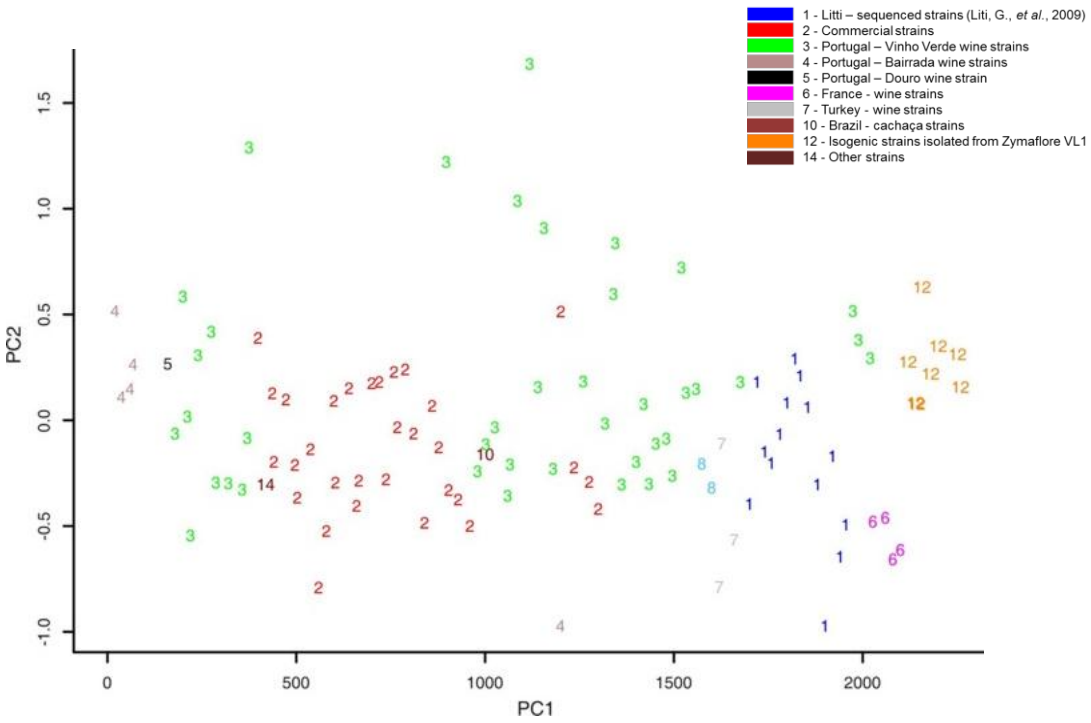

Fig. 3: PCA plot of fiber optics UV-VIS-SWNIR spectroscopy data for 91 strains (PC-1 (97.4 %), PC-2 (2.1%))

From the spectroscopy data obtained, and in combination with microsatellite and phenotypic data, a more restricted set of 28 most heterogeneous strains was chosen. Triplicate fermentations were carried out with all strains, using white grape must. HPLC and GC-MS analysis was performed with samples obtained from the end of fermentation (constant weight), to evaluate the chemical compounds that might be associated with the differences observed in the previous analysis.

Globally, there was a rather poor reproducibility between the triplicate fermentations for each strain. However, strain-dependent differences could be observed concerning organic acids (malic, tartaric, succinic and acetic), glycerol, fructose and ethanol. Tartaric acid concentration ranged, for most strains, between 0.5 and 1.4 g/L, whereas malic, acetic and succinic acids ranged between 4.7 – 8.2 g L^{-1}, 0 - 1.2 g L^{-1}, and 0.3 – 1.3 g L^{-1}, respectively. Final concentrations of ethanol, glycerol and fructose ranged between 80 - 138 g L^{-1}, 5 - 9.75 g L^{-1} and 0 - 9 g L^{-1}, respectively. PCA plots of HPLC data (data not shown) explained 98% of strain variance in the first two components (PC-1 - 73%, PC-2 - 25%), and showed that strain variability was mainly influenced by ethanol and fructose concentrations. This analysis also showed that commercial strains provided the most variable results regarding the concentrations of these two compounds.

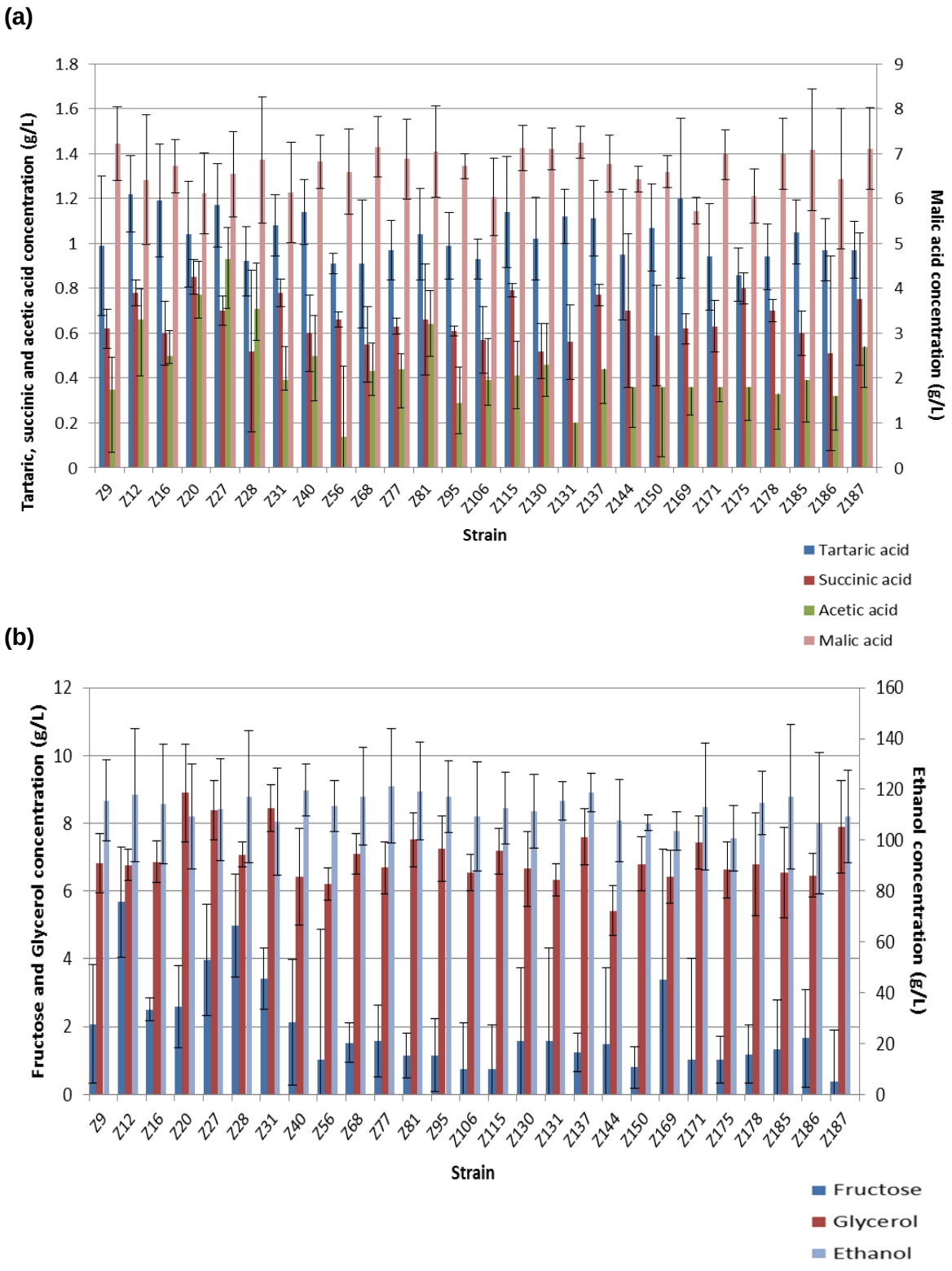

Fig. 4: Concentration of (a) tartaric, succinic, acetic and malic acids; (b) fructose, glycerol and ethanol from the end of fermentations performed with 28 *S. cerevisiae* strains. Determinations were carried out by HPLC analysis.

Aromatic compounds from the final fermentation stage were quantified by GC-MS after solid phase microextraction. Tab. 2 shows the concentration of the quantified aromatic compounds, including also the respective sensorial thresholds and odor descriptors. Ethyl butanoate and isoamyl acetate occurred in concentrations above the sensorial detection threshold in fermentations of 11 and 20 strains, respectively. The concentration range of these compounds was very broad (6.6 – 43.3 μg L^{-1} and 12.7 – 339.9 μg L^{-1} (ethyl butanoate and isoamyl acetate, respectively. A large variance between strains was also observed for other compounds, being some of them produced (in detectable quantities) by a small number of strains, such as isobutyl acetate (14 strains), 2-methy-1-butanol (3 strains), ethyl lactate (8 strains) and 2-phenylethyl acetate (15 strains). PCA analysis (data not shown) clearly separated all strains in both PCA components (PC-1 (58%) and PC-2 (22%)). This analysis revealed ethyl acetate and ethyl lactate as the ones with highest weight in strains variability, followed by isoamyl acetate, 2-methyl-1-propanol and 2-phenylethanol. More aromatic compounds are being identified and quantified in these samples. Computational analyses are underway to relate the metabolic signature of these strains with previously shown phenotypic, genetic and spectroscopic data.

CONCLUSIONS

The present work contributes to a better understanding of intra-strain differences regarding the pheno-metabolomic characterization of *S. cerevisiae* isolates. A set of 11 microsatellite markers and 22 phenotypic characteristics explained large part of strain diversity. For few phenotypes a high variability was observed among strains that were, to some extent, grouped according to their geographical origin. PCA analysis of microsatellites revealed a group of 34 alleles that were most significantly contributing to strain variability. PCA analysis of fiber optics spectroscopy data separated some groups of strains according to their geographic origin and technological use (commercial wine strains). Aromatic profiles of final fermentations from 28 strains, obtained by GC-MS analysis, revealed a group of relevant metabolites (ethyl butanoate, isoamyl acetate, ethyl acetate and ethyl lactate) that mostly account for inter-strain variability. Strain-dependent differences, although to a lower extent, were also obtained for the concentrations of several primary fermentation products. The acquired data will contribute to obtain a deeper insight into the ecology and biogeography of *Saccharomyces cerevisiae* strains. Additional data mining is currently underway to explore relations between the genotype and phenotype of the strains, which may contribute to the establishment of new tools for strain selection and improvement approaches.

Tab. 2: concentration (μg L^{-1}) of aromatic compounds determined by GC-MS in the 28 strains

Compounds	**Ethyl acetate**	**Isobutyl acetate**	**Ethyl butanoate**	**2-methyl-1-propanol**	**Isoamyl acetate**	**2-Methy-1-butanol**	**Ethyl hexanoate**	**Hexyl acetate**	**Ethyl lactate**	**1-Hexanol**	**cis-hex-3-en-1-ol**	**2-Phenylethyl acetate**	**2-Phenyl ethanol**
Sensorial threshold $\mu g\ L^{-1}$ [a] $mg\ L^{-1}$ [b]	12.3 [b] &	1.6 [b] &	20 [a] $	75 [b] $	30 [a] $	30 [b] $	14 [a] &	1 [b] $	14 [b] $	5.2 [b] $	400 [a] $	650 [a] $	14 [b] &
Odor description	Solvent, fruity, nail polish [1,2,3]	Banana, sweet, fruity[1,2]	Papaya, butter, sweet, apple, fragrant, fruity[1,4,5,2,6]	alchool[1,8]	Banana, apple, solvent[1,2,4,5]	alcohol, banana, sweet, aromatic, cheese[1,5,7]	apple, fruity, sweet, aniseed-flavored[1,5]	sweet, aromatic, fragrant[1,8]	Strawberry, raspberry[1,2,9]	coconut, green leefs[1,9,10]	green leafs, banana, sweet, herb[1,4,5,11]	roses, honey, apple, sweet, floral[1,5,9]	Roses, sweet, fragrant, flowery, honey-like[1,2,4,5,6,12]
Z9	3970.4	1.8	38.9	666.1	260.9	128.9	0.5	14.9	*nd*	227.1	55.8	6.5	2820.1
Z12	3213.3	2.6	38.2	575.0	266.6	*nd*	0.6	16.2	*nd*	188.4	*nd*	*nd*	735.0
Z16	4390.1	1.7	41.2	473.9	339.9	*nd*	*nd*	11.9	*nd*	153.8	*nd*	*nd*	446.3
Z20	2600.4	*nd*	16.8	1754.7	65.0	*nd*	0.6	5.0	*nd*	181.7	34.4	1.6	965.7
Z27	714.0	*nd*	13.1	1178.0	27.0	*nd*	*nd*	3.4	*nd*	125.5	*nd*	6.7	799.2
Z28	2785.5	*nd*	15.5	337.4	102.9	*nd*	0.6	10.9	*nd*	181.4	36.2	1.9	614.9
Z31	3112.6	0.4	24.0	476.0	98.1	*nd*	0.6	6.8	735.9	135.8	27.4	0.9	810.8
Z40	2614.4	0.8	16.0	789.3	67.4	*nd*	0.5	10.6	*nd*	177.1	37.9	*nd*	606.7
Z63	2430.9	*nd*	16.6	840.9	24.9	*nd*	0.7	5.9	*nd*	209.4	*nd*	*nd*	414.6
Z77	3699.9	2.2	43.3	920.9	306.3	*nd*	0.6	13.0	889.0	105.4	29.2	3.6	555.6
Z81	1583.1	*nd*	7.4	1086.1	13.2	*nd*	0.5	2.5	952.0	111.3	34.1	*nd*	*nd*
Z89	2008.7	0.9	10.8	284.1	54.9	*nd*	0.5	10.0	*nd*	143.0	30.6	1.2	194.9
Z95	2534.8	0.7	23.5	218.9	44.3	96.3	0.5	8.8	*nd*	150.8	33.7	*nd*	560.1
Z103	2231.2	*nd*	29.1	354.7	152.5	*nd*	0.5	18.3	*nd*	154.0	33.7	5.2	773.4
Z115	3565.2	2.5	32.3	401.8	242.2	*nd*	0.6	21.2	*nd*	192.0	37.1	2.4	454.2
Z127	2949.4	*nd*	26.4	329.2	154.9	*nd*	*nd*	10.3	*nd*	177.4	34.0	*nd*	605.1
Z131	*nd*	2.3	34.7	1790.4	256.9	*nd*	0.6	15.4	*nd*	163.8	*nd*	2.3	897.9
Z137	1462.6	*nd*	9.7	744.4	44.8	*nd*	0.6	8.8	1395.0	146.9	36.2	1.7	850.8
Z169	6381.6	1.0	26.6	526.2	105.9	*nd*	0.7	11.6	1919.6	185.7	36.7	1.1	650.0
Z171	732.3	0.2	6.6	549.3	12.7	*nd*	0.6	3.5	*nd*	123.3	31.2	*nd*	595.8
Z178	1747.6	*nd*	14.3	467.9	102.9	*nd*	0.6	12.6	1353.2	127.4	31.8	1.8	909.2
Z185	1097.5	1.2	11.4	609.8	107.8	*nd*	0.7	12.4	196.9	186.3	37.2	1.5	668.3
Z186	646.5	0.6	9.1	121.0	80.3	*nd*	0.6	12.9	401.3	151.4	30.8	1.0	364.9
Z187	1821.1	*nd*	11.5	214.8	106.0	*nd*	*nd*	15.5	*nd*	171.6	*nd*	*nd*	502.4
non-fermented must	*nd*	*nd*	*nd*	*nd*	*nd*	48.6	0.6	*nd*	*nd*	97.8	43.0	*nd*	*nd*

[&] perception threshold obtained in a model wine solution
[$] perception threshold obtained in a hydroalcoholic solution
[#] aromatic threshold of difference in wine
nd - peak not detected
underlined numbers refer to concentrations above the sensorial threshold

1- (Meilgaard, 1975); 2- (Siebert, et al., 2005); 3- (Boutou and Chatonnet, 2007); 4- (Cullere, et al., 2004)
5- (Escudero, et al., 2004); 6- (Czerny, et al., 2008); 7- (Moreno, et al., 2005); 8- (Etievant, et al., 1991)
9- (Salo, 1970); 10- (Simpson, 1979); 11- (Guth, 1997); 12- (Ferreira, et al., 2000)

ACKNOWLEDGMENTS

R. Duarte, C. Castro, J. Silva and I. Mendes are recipients of a fellowship from the Portuguese Science Foundation (FCT, SFRH/BD/48591/2008, SFRH/BD/46737/2008, SFRH/BD/48616/2008 and SFRH/BD/74798/2010, respectively). This work was financially supported by the Portuguese Science Foundation (Fundação para a Ciência e Tecnologia, FCT, within the project AGR-ALI/103392/2008 and the European Community´s Seventh Framework Program (FP7/2007-2013) under grant agreement nº 232454.

BIBLIOGRAPHY

Boutou, S. and Chatonnet, P. (2007). Rapid headspace solid-phase microextraction/gas chromatographic/mass spectrometric assay for the quantitative determination of some of the main odorants causing off-flavours in wine. J Chromatogr A 1141, 1-9.

Cullere, L., Escudero, A., Cacho, J. and Ferreira, V. (2004). Gas chromatography-olfactometry and chemical quantitative study of the aroma of six premium quality spanish aged red wines. J Agric Food Chem 52, 1653-60.

Czerny, M., Christbauer, M., Christbauer, M., Fischer, A., Granvogl, M., Hammer, M., Harti, C., Hernandez, N. M. and Schieberle, P. (2008). Analytical Method for Determination of Some Aroma Compounds on White Wines by Solid Phase Microextraction and Gas Chromatography. Eur Food Res Technol 228, 265-273.

Escudero, A., Gogorza, B., Melus, M. A., Ortin, N., Cacho, J. and Ferreira, V. (2004). Characterization of the aroma of a wine from maccabeo. Key role played by compounds with low odor activity values. J Agric Food Chem 52, 3516-24.

Etievant, C., Kruczynski, A., Pauwels, O. and Kiss, R. (1991). The combination of the tetrazolium derivative reduction (MTT) and digital cell image analysis to monitor in vitro the cytotoxicity of anti-neoplastic drugs. Anticancer Res 11, 305-11.

Fay, J. C. and Benavides, J. A. (2005). Evidence for domesticated and wild populations of Saccharomyces cerevisiae. PLoS Genetics 1, 66-71.

Ferreira, V., Lopez, R. and Cacho, J. F. (2000). Quantitative determination of the odorants of young red wines from different grape varieties. Journal of the Science of Food and Agriculture 80, 1659-1667.

Fleet, G. H. (2008). Wine yeasts for the future. FEMS Yeast Res 8, 979-95.

Franco-Duarte, R., Umek, L., Zupan, B. and Schuller, D. (2009). Computational approaches for the genetic and phenotypic characterization of a Saccharomyces cerevisiae wine yeast collection. Yeast 26, 675-692.

Greig, D. and Leu, J. Y. (2009). Natural history of budding yeast. Current Biology 19, R886-R890.

Guth, H. (1997). Quantitation and sensory studies of character impact odorants of different white wines varieties. J Agric Food Chem 45, 3027-3032.

Legras, J. L., Ruh, O., Merdinoglu, D. and Karst, F. (2005). Selection of hypervariable microsatellite loci for the characterization of Saccharomyces cerevisiae strains. Int J Food Microbiol 102, 73-83.

Liti, G., Carter, D. M., Moses, A. M., Warringer, J., Parts, L., James, S. A., Davey, R. P., Roberts, I. N., Burt, A., Koufopanou, V., Tsai, I. J., Bergman, C. M., Bensasson, D., O'Kelly, M. J. T., van Oudenaarden, A., Barton, D. B. H., Bailes, E., Ba, A. N. N., Jones, M., Quail, M. A., Goodhead, I., Sims, S., Smith, F., Blomberg, A., Durbin, R. and Louis, E. J. (2009). Population genomics of domestic and wild yeasts. Nature 458, 337-341.

Meilgaard, M. C. (1975). Flavor chemistry of beer: Part II: Flavor and threshold of 239 aroma volatiles. MBAA Technical Quarterly 12, 151:168.

Moreno, J. A., Zea, L., Moyano, L. and Medina, M. (2005). Aroma compounds as markers of the changes in sherry wines subjected to biological ageing. Food Control 16, 333-338.

Perez, M. A., Gallego, F. J., Martinez, I. and Hidalgo, P. (2001). Detection, distribution and selection of microsatellites (SSRs) in the genome of the yeast Saccharomyces cerevisiae as molecular markers. Lett Appl Microbiol 33, 461-6.

Salo, P. (1970). Determining the odor thresholds for some compounds in alcoholic beverages. J Food Sci. 35, 95-99.

Schacherer, J., Shapiro, J. A., Ruderfer, D. M. and Kruglyak, L. (2009). Comprehensive polymorphism survey elucidates population structure of Saccharomyces cerevisiae. Nature 458, 342-U103.

Schuller, D. (2010). Better yeast for better wine - Genetic improvement of Saccharomyces cerevisiae wine strains in Rai, M. and Koevics, G. (Eds), Progress in Mycology, Scientific Publishers (India), pp. 1-51.

Siebert, T. E., Smyth, H. E., Capone, D. L., Neuwohner, C., Pardon, K. H., Skouroumounis, G. K., Herderich, M. J., Sefton, M. A. and Pollnitz, A. P. (2005). Stable isotope dilution analysis of wine fermentation products by HS-SPME-GC-MS. Anal Bioanal Chem 381, 937-47.

Silva, J. S., Martins, R. C., Vicente, A. A. and Teixeira, J. A. C. (2008). Feasability of Yeast and Bacteria Identification using UV-VIS-SWNIR Difusive Reflectance Spectroscopy. In Proceedings of First International Conference on Biomedical Electronics and Devices, BIOSIGNALS 2008, Volume 1: 25-32.

Silva-Ferreira A. C., Guedes de Pinho P. (2003) Analytical method for the determination of some aroma compounds on white wines by Solid Phase MicroExtraction and gas chromatography. J. of Food Science 68 (9), 2817-2820

Simpson, R. F. (1979). Some important aroma components of white wine. Food Technology in Australia, 516-622.