Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

# Genomic and phenotypic comparison between similar wine yeast strains of *Saccharomyces cerevisiae* from different geographic origins

F. Salinas[1], D. Mandaković[1], U. Urzua[2], A. Massera[3], S. Miras[3], M. Combina[3], M. Angelica Ganga[1] and C. Martínez[1,4]

1 Departamento de Ciencia y Tecnología de los Alimentos, Universidad de Santiago de Chile (USACH), Santiago, Chile
2 Laboratorio de Genómica Aplicada, Programa de Biología Celular y Molecular-ICBM, Universidad de Chile, Santiago, Chile
3 Instituto Nacional de Tecnología Agropecuaria (INTA) and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mendoza, Argentina
4 Centro de Estudios en Ciencia y Tecnología de Alimentos (CECTA), Universidad de Santiago de Chile (USACH), Santiago, Chile

**Keywords**
commercial wine yeast, genomic and phenotypic comparison, microarray.

**Correspondence**
Claudio Martínez, Centro de Estudios en Ciencia y Tecnología de Alimentos (CECTA), Universidad de Santiago de Chile (USACH), Obispo Manuel Umaña 050, 9170201, Estación Central, Santiago, Chile.
E-mail: claudio.martinez@usach.cl

2009/1627: received 10 September 2009, revised 4 January 2010 and accepted 11 January 2010

doi:10.1111/j.1365-2672.2010.04689.x

## Abstract

**Aims:** To study genomic and phenotypic changes in wine yeasts produced in short time periods analysing yeast strains possibly derived from commercial strains recently dispersed.
**Methods and Results:** We conducted a genomic and phenotypic comparison between the commercial yeast strain EC1118 and two novel strains (LV CB and L-957) isolated from different wine areas industrially intervened <20 years ago. Molecular analysis by amplified fragment length polymorphism (AFLP) and RAPD-PCR was not able to distinguish between these strains. However, comparative genomic hybridization (aCGH) showed discrete DNA gains and losses that allowed unequivocal identification of the strains. Furthermore, analysis of aCGH data supports the hypothesis that strains LV CB and L-957 are derivatives from strain EC1118. Finally, scarce phenotypic differences in physiological and metabolic parameters were found among the strains.
**Conclusion:** The wine yeasts have a very dynamic genome that accumulates changes in short time periods. These changes permit the unique genomic identification of the strains.
**Significance and Impact of the Study:** This study permits the evaluation of microevolutive events in wine yeasts and its relationship with the phenotype in this species.

## Introduction

The transformation of grape must into wine is a complex microbiological process that involves different yeast and bacterial species. However, as alcohol concentration increases, the genus *Saccharomyces* becomes the dominating yeast where *S. cerevisiae* is the main species responsible for alcoholic fermentation (Pretorius 2000). The addition of dehydrated, active commercial wine yeast to the must as starter of fermentation has been common practice in wine-making for various decades (Querol *et al.* 1992). Commercial *S. cerevisiae* strains are derived from selected yeast isolates based on phenotypic characteristics such as alcohol tolerance (11–14%), reproducibility of the fermentation, low concentration of residual sugar (2–5 g $l^{-1}$), production of desirable esters, low production of volatile acids, high fermentative rate, ability to dominate diverse fermentation conditions, tolerance to other micro-organisms and minimal impact on grape varietal character (Bisson 2004; Cocolin *et al.* 2004). Hence, it is increasingly common to find that wild yeasts collected in different areas are identical to widely used commercial strains (Legras *et al.* 2005; Valero *et al.* 2005; Bradbury *et al.* 2006). Furthermore, our previous work has demonstrated that in regions with high industrial wine-making activity, the diversity of non-*Saccharomyces* yeasts is lower


 

than in regions where these practises are not occurring (Ganga and Martínez 2004). Additionally, yeast populations from nonindustrial areas have 40% higher genetic diversity than populations isolated from industrial areas, with no consensus with respect to the role that yeasts, introduced through industrial activity, play in the diversity of these ecosystems (Ganga and Martínez 2004; Valero *et al.* 2005; Cubillos *et al.* 2009). Hence, in this context, the release of commercial *S. cerevisiae* strains into the environment would, in time, result in genome changes that could correspond to adaptative mechanisms to the new environments encountered by the yeasts in nature (Schuller *et al.* 2007).

The *S. cerevisiae* wine strains are mostly diploid, homozygous and homothallic (Mortimer *et al.* 1994; Bradbury *et al.* 2006; Cubillos *et al.* 2009) with chromosome polymorphisms favoured by the recombination of *Ty* retrotransposons or repeated subtelomeric sequences (Querol *et al.* 2003). It has been described that some of these genomic rearrangements may confer an adaptative advantage to different environmental conditions (Bakalinsky and Snow 1990). Hence, genome changes that facilitate the adaptation of the yeasts have been described. An example is reciprocal translocation between chromosomes VIII and XVI that confer resistance to sulfite as a result of a change in regulation of the SS1 allele (Pérez-Ortín *et al.* 2002). Furthermore, frequency of homologous recombination during mitosis (Puig *et al.* 2000), changes in yeast ploidy and changes in gene copy number are mechanisms that favour environmental adaptation of the yeast (Bakalinsky and Snow 1990; Infante *et al.* 2003).

Genetic variability of wine yeasts has been demonstrated using various analysis tools at the molecular level (Schuller *et al.* 2004). This enabled characterization and discrimination of *S. cerevisiae* wine strains (Querol *et al.* 1992; Baleiras Couto *et al.* 1996). Amongst them, pulsed-field gel electrophoresis (PFGE) (Martínez *et al.* 2004), randomly amplified polymorphic DNA (RAPD-PCR) (Fernandez-Espinar *et al.* 2003), restriction analysis of the mitochondrial DNA (mtDNA-RFLP) (Fernandez-Espinar *et al.* 2001), amplified fragment length polymorphism (AFLP) (de Barros Lopes *et al.* 1999; Flores Berrios *et al.* 2005), amplification of interdelta regions by PCR (Legras and Karst 2003) and microarray comparative genomic hybridization (array CGH or aCGH) (Winzeler *et al.* 2003; Dunn *et al.* 2005; Carreto *et al.* 2008). The aCGH analysis has established that major differences between laboratory strains of *S. cerevisiae* are found in subtelomeric regions (Winzeler *et al.* 2003) and that the *S. cerevisiae* wine strains show a gene copy number variation that differentiate them from laboratory strains and strains of clinical origin. Differences were found in genes related to the fermentative process such as membrane transporters, ethanol metabolism and metal resistance (Dunn *et al.* 2005; Carreto *et al.* 2008).

The French commercial wine strain EC1118 is extensively used worldwide. In the regions of Casablanca (Chile) and Mendoza (Argentina), it has been used for the last two decades. Studies carried out in our laboratory using molecular markers have demonstrated that the commercial strain LV CB and the native strain L-957 isolated from Casablanca and Mendoza, respectively, show very similar molecular patterns. Additionally, studies using mtDNA-RFLP and PFGE showed a close phylogenetic relationship between strains EC1118 and LV CB, whilst having very different geographic origins (Martínez *et al.* 2007).

With the objective of studying genomic and phenotypic changes between similar yeast isolated from different origins, we carried out a genomic and phenotypic comparison of strains LV CB, L-957 and EC1118. AFLP and RAPD-PCR suggest that the three strains are closely related. In contrast, aCGH results indicate that LV CB and L-957 share amplifications and deletions supporting that strain EC1118 is a common ancestor. Various kinetic and fermentative parameters were evaluated and significant phenotypic differences were detected between strains, some of which may be explained by differences at the genomic level.

## Materials and methods

### Yeast strains and culture

Strains EC1118 and LV CB were commercially purchased, and strain L-957 was obtained from the collection of the Laboratorio de Biotecnología y Microbiología Aplicada of the Universidad de Santiago de Chile (Table 1). All strains were maintained in YPD media (2% glucose, 0·5% peptone and 0·5% yeast extract) at 4°C following growth.

### AFLP

The AFLP analysis was carried out according to the method described by de Barros Lopes *et al.* (1999). The amplification products were separated by polyacrylamide gel electrophoresis at 6% and visualized by silver staining

**Table 1** Strains used in this study

| Species | Strain | Origin |
|---|---|---|
| *Saccharomyces cerevisiae* | EC1118 | Champagne/France |
| *S. cerevisiae* | LV CB | Casablanca/Chile |
| *S. cerevisiae* | L-957 | Mendoza/Argentina |
| *S. cerevisiae* | S288c | California/USA |

 

(Silver Sequence DNA Sequencing System, Promega, USA).

### Microarray analysis

Whole genome yeast Y6·4K7 cDNA microarrays were purchased at the University Health Network Microarray Centre, Toronto, Canada. They consist of double-spotted slides containing 6240 yeast ORFs. All microarray experiments were conducted as dye-swap replicates resulting in a quadruplicate data set for each sample analysed. Genomic DNA was isolated with the Wizard® kit (Promega, USA). Briefly, 5 ml of culture was centrifuged at 16 000 ***g*** for 5 min. The pellet was washed with 285 $\mu$l of EDTA 50 mmol $l^{-1}$ followed by the addition of 15 $\mu$l of Zymoliase 100T 10 mg $ml^{-1}$ (Seikagaku Corporation, Japan) and incubation at 37°C for 2 h. After incubation, the cells were centrifuged at 16 000 ***g*** for 5 min. The pellet was washed with 400 $\mu$l of nucleolysis solution and treated with 133 $\mu$l of protein precipitation solution (Promega, USA) for 40 min on ice. The cell lysate was centrifuged at 13 000 ***g*** for 30 min at 4°C and the supernatant was transferred to an Eppendorf tube containing 300 $\mu$l of 2-propanol. This mixture was centrifuged at 14 000 ***g*** for 15 min; the pellet was washed with 300 $\mu$l of 70% ethanol and centrifuged at 14 000 ***g*** for 5 min. The DNA was finally resuspended in 50 $\mu$l of TE buffer. Genomic DNA was quantified by UV spectrophotometry and then digested with *Eco*RI (Fermentas, USA) using standard conditions. One microgram of digested DNA was employed in the labelling-amplification reaction with the Bioprime Array CGH Genomic Labeling System (Invitrogen, USA). The fluorescent Alexa Fluor 647 dUTP and Alexa Fluor 555 dUTP nucleotides were used (Invitrogen, USA). Clean-up of labelling reactions was done with the MiniElute PCR Purification Kit (Quiagen, USA). Labelled DNA was combined to a final volume of 65 $\mu$l hybridization solution consisting of 25% deionised formamide, 5× SSC, 0·1% SDS and 15 $\mu$g of denatured–sonicated fish sperm DNA. The hybridization mixture was denatured at 99°C for 3 min, pre-incubated at 37°C for 2 h and then deposited on the microarray surface. Slides were enclosed in individual hybridization chambers (Telechem, USA) and incubated at 42°C for 24 h. Washes were performed sequentially as follows: 5 min in a 2× SSC–0·1% SDS solution, 5 min in a 1× SSC solution, 1 min in a 0·2× SSC solution, and 1 min in 0·05× SSC solution. Slides were dried by centrifugation at 1000 ***g*** for 10 min and immediately scanned in a ScanArray Lite fluorescence scanner (PerkingElmer, USA). Images were saved in tiff-format and analysed with the GenePixPro 6.0 software (Molecular Devices, USA). Data normalization was performed with the DMAD tool and filtered with the preP tool at Asterias website (Diaz-Uriarte and Rueda 2007). Detection of DNA gains and losses was performed with the ADaCGH software, also part of Asterias (Diaz-Uriarte and Rueda 2007). Cluster analysis was done with the MeV software (Saeed *et al.* 2003). Raw and processed data were deposited on the Gene Expression Omnibus database, accession number GSE 16941.

### Growth rate and biomass

Growth was assessed with a synthetic must of the following composition: tartaric acid 5 g $l^{-1}$, malic acid 5 g $l^{-1}$, calcium chloride (dihydrate) 0·3 g $l^{-1}$, magnesium sulfate 1·3 g $l^{-1}$, ammonium phosphate 1·2 g $l^{-1}$, fructose 100 g $l^{-1}$, sucrose 5 g $l^{-1}$, glucose 100 g $l^{-1}$, potassium hydroxide 2·5 g $l^{-1}$, vitamin solution 2 ml $l^{-1}$. The must was autoclaved for 21 min at 15 psi and the vitamin

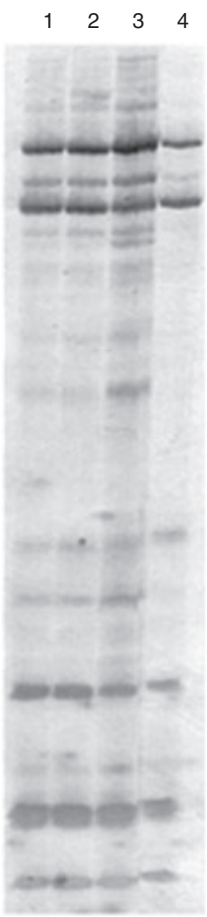


**Figure 1** Amplified fragment length polymorphism (AFLP) analysis of wine yeasts. Lanes: 1; EC1118, 2; LV CB, 3; L-957, 4; S288C.


 

solution added. The vitamin solution contains the following: thiamine 1·152 g $l^{-1}$, biotin $4{\cdot}8 \times 10^{-3}$ g $l^{-1}$, nicotinic acid 2·3 g $l^{-1}$, pyridoxine hydrochloride 0·23 g $l^{-1}$, calcium pantoneate 1·152 g $l^{-1}$ and sulfuric acid 0·25 mol $l^{-1}$. Growth curves were obtained with initial inoculums of $1{\cdot}5 \times 10^6$ cells per ml in 200 ml of synthetic must at 28°C. Absorbance of cultures was measured at 600 nm every hour up to 35 h. Biomass was determined in the same culture conditions up to 30 h of incubation. Cells were recovered by centrifugation at 15 700 ***g*** for 15 min, dried, weighed and diluted in 10 ml of synthetic must. The absorbance of each dilution was measured at 600 nm; therefore, biomass (mg $ml^{-1}$) *vs* time curves were constructed, calculating the maximum growth rate ($\mu_{máx}$) with the slope of the curve situated on the points where the yeasts were in exponential phase.

### Physiological characterization

Strains were characterized for their ability to ferment D-glucose, galactose, melibiose, maltose and sucrose, as well as their ability to use D-glucose, galactose, D-xylose, sucrose, fructose, maltose, raffinose, melezitose, sorbitol, D-mannitol, malic acid, citric acid, tartaric acid and ethanol as sole carbon source for aerobic growth and cycloheximide (actidione) resistance (0·01 and 0·1%) using YNB (Sigma, USA) as nitrogen basal medium (Kurtzman and Fell 1998; Combina *et al.* 2005). Prior to the evaluation, the strains were cultured in a starvation medium to avoid false positives as suggested by Kurtzman and Fell (1998). All the assays were done in triplicate. The carbon sources evaluated were based on the composition of grapes and wines (Flanzy 2000).

### Fermentation in natural must

Fermentation was carried out in triplicate 500-ml Erlenmeyer flasks containing 300 ml of the Bonarda variety must with 240 g $l^{-1}$ of reduction sugars, 7 g $l^{-1}$ of tartaric acid and pH 3·5. The must was individually inoculated with each strain at $2 \times 10^6$ cells per ml. Flasks were kept at 25°C without agitation and plugged with glass fermentation traps containing sulfuric acid to allow only $CO_2$ to evolve from the system. The fermentation evolution was followed daily by loss of weight (until constant weight) (Schuller *et al.* 2004). Volatile acidity, pH, ethanol and residual sugar concentrations were determined by standard methods (Nelson 1944; Somogyi 1945; Zoecklein *et al.* 1995). Fermentation rate was calculated as the

**Table 2** Gene copy number variations in yeast strains EC1118, LV CB and L-957

| Strains | Changes* | ORFs† |
|---|---|---|
| EC1118 | Amplifications Chromosome I | YAL068C, YAL069W, YAR002W, YAR007C, YAR008W, YAR014C, YAR020C, YAR031W, YAR033W, YAR035W, YAR042W, YAR062W, YAR066W, YAR069C, YAR071W, YAR073W. |
| EC1118 | Amplifications Chromosome III | YCR027C, YCR028C, YCR032W, YCR033W, YCR034W, YCR035C, YCR036W, YCR037C, YCR040W, YCR042C, YCR045C, YCR047C, YCR048W, YCR052W. |
| EC1118 | Amplifications Chromosome XII | YLR003C, YLR004C, YLR005W, YLR007W, YLR009W, YLR011W, YLR014C, YLR015W, YLR016C, YLR018C, YLR019W, YLR020C, YLR021W, YLR022C, YLR023C, YLR025W, YLR026C, YLR027C, YLR028C, YLR029C. |
| LV CB | Deletions Chromosome IV | YDL242W, YDL243C, YDL244W, YDL245C, YDL246C, YDL247W, YDL248W. |
| LV CB | Deletions Chromosome X | YJR025C, YJR026W, YJR028W, YJR030C, YJR032W. |
| LV CB | Deletions Chromosome XV | YOL161C, YOL162W, YOL163W, YOL164W, YOL165C, YOL166C. |
| LV CB | Amplifications Chromosome XII | YLR162W, YLR163C, YLR164W, YLR165C, YLR166C, YLR168C, YLR170C, YLR172C, YLR173W, YLR174W, YLR175W, YLR176C, YLR177W, YLR178C, YLR179C, YLR180W, YLR181C, YLR182W, YLR183C, YLR184W, YLR185W, YLR187W, YLR189C, YLR191W, YLR192C, YLR193C, YLR194C, YLR195C, YLR196W, YLR197W, YLR199C, YLR201C, YLR202C, YLR203C, YLR204W, YLR205C, YLR206W, YLR207W, YLR208W, YLR209C, YLR210W, YLR212C, YLR213C, YLR214W, YLR215C, YLR216C, YLR218C, YLR219W, YLR220W, YLR221C, YLR222C, YLR224W, YLR225C, YLR226W, YLR227C. |
| L-957/LV CB | Amplifications Chromosome XVI | YPL272C, YPL273W, YPL274W, YPL275W, YPL276W, YPL277C, YPL278C, YPL279C, YPL280W, YPL281C. |

*Microarray CGH data were analysed with the online tool ADaCGH (11). Median Centering and circular binary segmentation were used to define gene amplifications and deletions.

†ORFs name from *Saccharomyces* genome database.

 

amount of $CO_2$ produced after 3 days of fermentation ($CO_2$ $day^{-1}$). Efficiency in conversion of sugar to ethanol was calculated as the amount of sugar concentration required to produce 1 alcoholic degree (Marullo *et al.* 2006).

### Statistical analysis

The *t*-student statistical analysis was carried out using the software STATGRAPHIC 4·0 (Statistical Graphics, Cheshire, CT). Gaussian distribution of fermentative data and variance homogeneity were checked by standardized Skewness and Cochran's tests, respectively. According to these results, parametric tests (ANOVA following LSD Fisher test) or nonparametric tests (Kruskall–Wallis) were applied to find significant differences between means of fermentative data. Statistical significance was determined at the level $P < 0{\cdot}05$ using STATGRAPHIC 4.0 (Statistical Graphics).

## Results

### Genomic comparison between wine yeasts

With the objective of differentiating the wine strains EC1118, LV CB and L-957, their genomes were analysed by AFLP (Fig. 1). The AFLP analysis did not show differences between these wine strains obtaining similar amplification profiles for all of them (Fig. 1). Moreover, RAPD-PCR analysis shows little difference between these

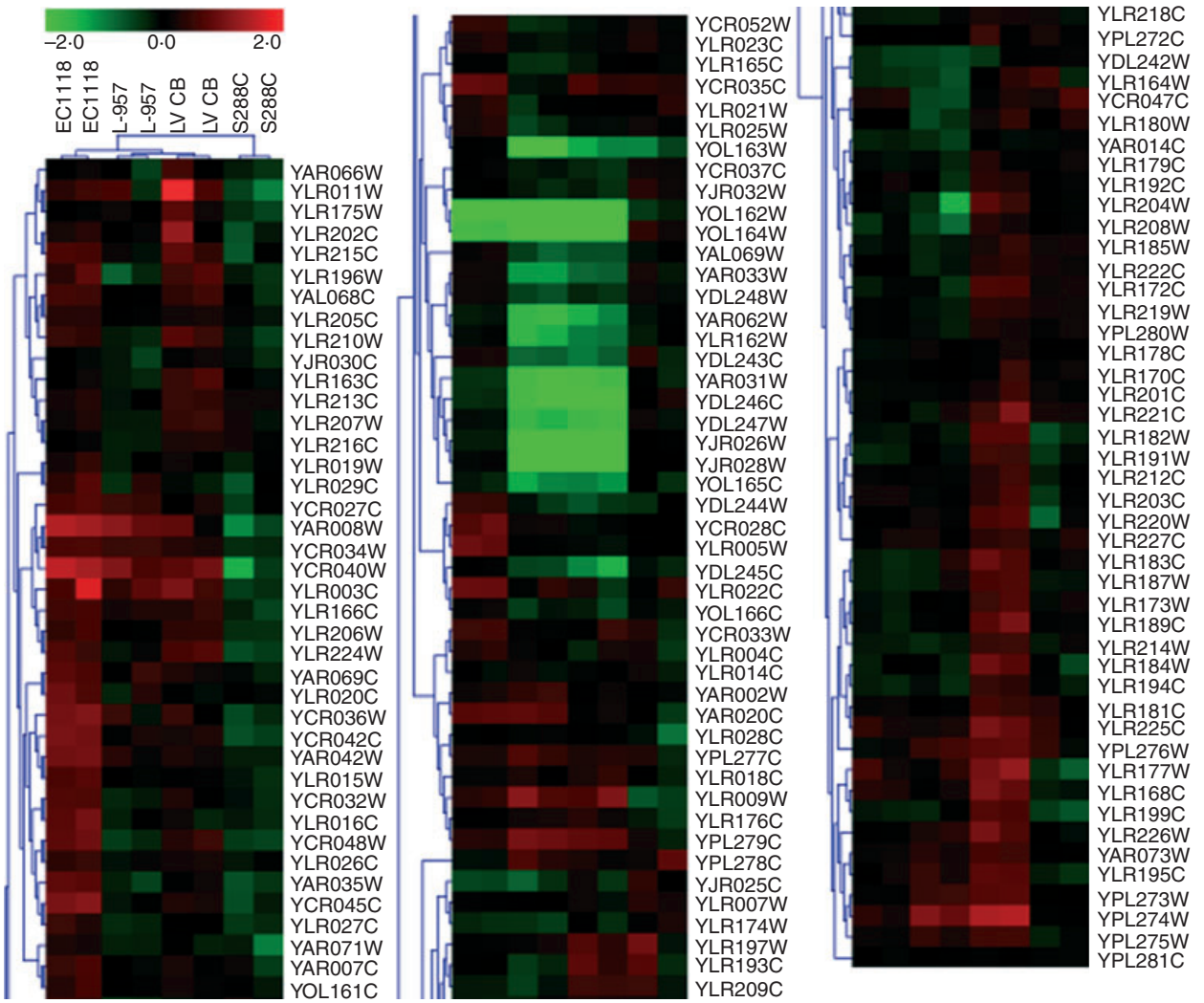


**Figure 2** Comparison of aCGH profiles among strains. Significantly altered regions were subjected to hierarchical clustering with the MeV tool (32). Pearson correlation was the metric distance used. Gene and sample dendrogram trees are shown. Each column corresponds to the average of two values from a single array.


 

strains, and the amplification of delta sequences did not discriminate between strains EC1118 and LV CB; however, strain L-957 lacked a band of *c.* 160 bp present in strains EC1118 and LV CB (data not shown).

Given that the results obtained using molecular markers suggested that the three wine strains have very similar genomes, we decided to apply a more sensitive approach, namely aCGH. For this purpose, the genome of each yeast strain was hybridized against the laboratory type strain S288C as reference DNA. In addition, a control, 'self-to-self' microarray experiment was conducted with genomic DNA of strain S288C. Overall, the results obtained suggest a close phylogenetic relationship among the three strains. However, characteristic amplifications and deletions allowed their discrimination. Strain EC1118 showed amplifications in chromosomes I, III and XII (Table 2) with approximate sizes of 44, 59 and 46 kbp, respectively. Strain LB CV displays discrete deletions located in chromosomes IV, X and XV (Table 2) with approximate sizes of 18, 21 and 10 kbp, respectively, in addition to amplifications located in chromosomes XII and XVI (Table 2) with approximate sizes of 102 and 17 Kbp. Strain L-957 showed an amplification in chromosome XVI similar to that found in strain LB CV that spans over 10 genes (Table 2). Microarray data of significantly altered regions in the whole genome of the three wine strains were subjected to a hierarchical clustering analysis (Fig. 2); this result in addition to the history of commercial wine yeast strain use in South America suggests that strains LV CB and L-957 are derived from the commercial strain EC1118.

### Phenotypic comparison of yeast strains

Because the three yeast strains are genetically related and the differences at the genome level could be related with phenotypic changes, the metabolism of some carbonated compounds and various kinetic and fermentative parameters were evaluated. The growth curves of the three strains showed similar kinetic parameters, without significant differences (Student's *t*-test, $P < 0{\cdot}05$) for maximum growth rate and production of biomass in synthetic medium (Fig. 3). Furthermore, no differences were found in generational time, lag phase time and exponential phase time in the three strains (data not shown).

The assimilation and fermentation profiles of different carbon sources were determined for the three strains. As with the kinetic parameters, the strains show very similar phenotypes for assimilation and fermentation profiles, only strain LV CB showed the ability to ferment galactose as laboratory strain S288C does (Table 3).

Fermentation in natural must provided insights on strain behaviour under similar conditions found in winemaking and evaluated fermentative parameters (Table 4). The chemical composition of the wines obtained with strains LV CB and L-957 show significant differences in the percentage of ethanol, volatile acidity and efficiency, with no differences in residual sugar and volatile acidity when individually compared to strain EC1118. On the other hand, strain L-957 showed a higher fermentative

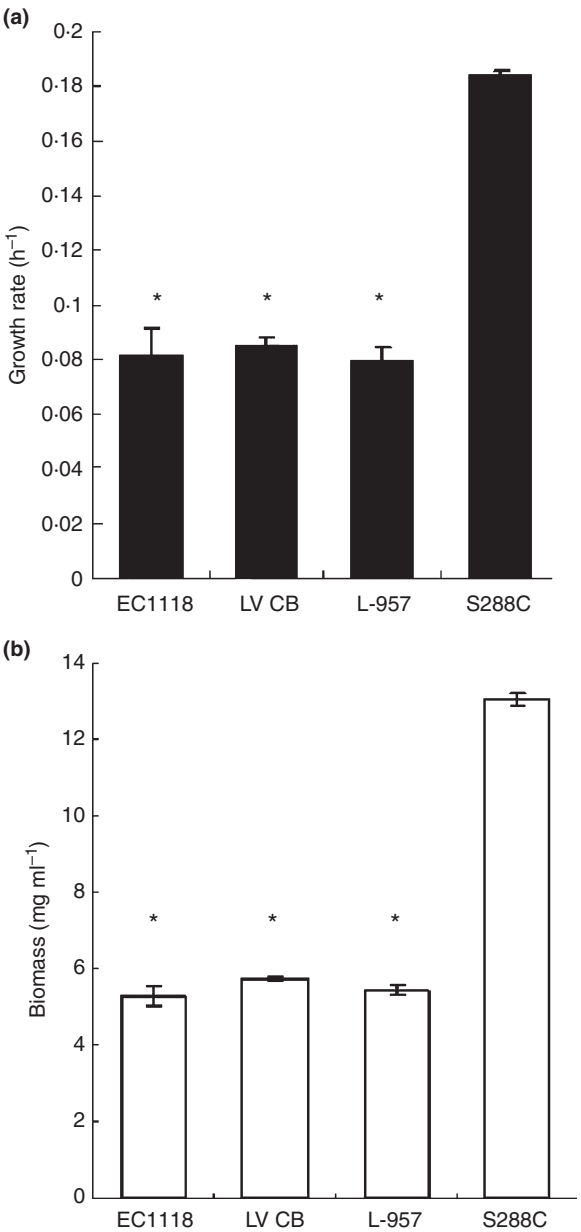


**Figure 3** Growth rate and biomass production of wine yeasts. (a) Maximum growth rate ($\mu_{max}$) in synthetic must. (b) Biomass production in synthetic must. The average of triplicates with their SD is shown. The asterisk depicts significant differences with respect to strain S288C (*t*-student $P < 0{\cdot}05$).

 

**Table 3** Carbon source usage of four yeast strains

| Compounds | Yeast strains | | | |
|---|---|---|---|---|
| | L-957 | EC1118 | LV CB | S288c |
| Assimilation | | | | |
| D-glucose | + | + | + | + |
| Galactose | – | – | + | – |
| Melezitose | – | – | – | – |
| Maltose | + | + | + | + |
| Sucrose | + | + | + | + |
| Fructose | + | + | + | + |
| Raffinose | + | + | + | + |
| D-xylose | – | – | – | – |
| Malic acid | – | – | – | – |
| Citric acid | – | – | – | – |
| Tartaric acid | – | – | – | – |
| D-mannitol | – | – | – | – |
| Sorbitol | – | – | – | – |
| Ethanol | + | + | + | + |
| Fermentation | | | | |
| D-glucose | + | + | + | + |
| Galactose | – | – | +D* | + |
| Melibiose | – | – | – | – |
| Maltose | + | + | + | + |
| Sucrose | + | + | + | + |
| Resistance | | | | |
| Cycloheximide 0·1% | – | – | – | – |
| Cycloheximide 0·01% | – | – | – | – |

*D = positive delay (positive after 7 days).

rate and strain LV CB a lower efficiency in conversion of sugars to ethanol, when compared to strain EC1118 in the conditions evaluated (Table 4). The results of the comparison between kinetic and fermentative parameters of the three wine strains showed differences in the fermentative phenotype.

## Discussion

The impact of introducing new strains on yeast population in regions intervened by the wine-making industry has been recently assessed. Biodiversity of yeast is low in industrialized areas, both at the species (Ganga and Martínez 2004) and at the strain (Cubillos *et al.* 2009) levels, compared to regions where oenological practices do not use commercial yeasts.

Strains LV CB and L-957 were isolated in Casablanca (Chile) and Mendoza (Argentina), respectively. In these regions, the commercial strain EC1118, of French origin, has been intensively used for the past two decades. Previous studies showed that strains EC1118 and LV CB are phylogenetically related even though they have different geographic origins and both strains are genetically different to strains isolated in Chile as shown by cluster analyses (Martínez *et al.* 2007). This evidence suggests that strain LV CB derived from EC1118 in the last two decades. Here, we report evidence to extend a similar conclusion about strain L-957. Furthermore, we show that strains EC1118, LV CB and L-957 display similar genomes with small DNA copy number alterations which permit their discrimination. Methodologies widely used to differentiate strains used by us are in agreement with data previously published (Martínez *et al.* 2007) indicating a tight genetic relationship between the three strains (Fig. 1 and data not shown). Hierarchical clustering analysis (Fig. 2) of aCGH data suggests that strains LV CB and L-957 are derived from the commercial strain EC1118. This is supported by the history of use of strain EC1118 in this region of South America which has undergone a recent industrialization of the wine-making activity.

The high genetic diversity of *S. cerevisiae* wine strains has been shown through multiple analyses at the molecular level (Schuller *et al.* 2004); and recently, diversity in yeast populations was demonstrated by genome sequencing of yeasts from different geographic origins (Liti *et al.* 2009). However, the aCGH analysis is useful and accurate to understand the genetic diversity in natural populations of yeast (Carreto *et al.* 2008). Using aCGH, Dunn *et al.* (2005) determined that copy number variations between yeast strains are moderate and correspond to hexose transporters and metal resistance genes. Comparisons of laboratory, clinical and wine *S. cerevisiae* strains using aCGH demonstrated the existence of characteristic gene copy number variations in wine-related strains that

**Table 4** Chemical and fermentative data of the four strains in natural must

| Strain | Parameters | | | | |
|---|---|---|---|---|---|
| | Ethanol (%v $v^{-1}$) | Residual sugars (g $l^{-1}$) | Volatile acidity (g $l^{-1}$) | Fermentation rate (g $CO_2$ $d^{-1}$) | Efficiency (g $l^{-1}$ sugar/% v $v^{-1}$ ethanol) |
| L-957 | 13·67 ± 0·03$^{c}$ | 2·85 ± 0·04$^{a}$ | 0·84 ± 0·10$^{a}$ | 6·12 ± 0·39$^{c}$ | 17·21 ± 0·04$^{a}$ |
| LV CB | 13·07 ± 0·18$^{ab}$ | 2·81 ± 0·05$^{a}$ | 0·97 ± 0·01$^{ab}$ | 5·75 ± 0·12$^{bc}$ | 18·01 ± 0·24$^{b}$ |
| EC1118 | 13·57 ± 0·20$^{bc}$ | 2·72 ± 0·06$^{a}$ | 0·85 ± 0·03$^{a}$ | 5·24 ± 0·21$^{ab}$ | 17·35 ± 0·26$^{a}$ |
| S288c | 12·90 ± 0·15$^{a}$ | 13·35 ± 2·35$^{b}$ | 1·11 ± 0·01$^{b}$ | 4·72 ± 0·09$^{a}$ | 17·42 ± 0·03$^{a}$ |

Data are means of triplicates. ±SD is indicated. Number with no shared superscript letters within the same column is statistically significant difference ($P < 0·05$).


 

differentiate them from strains of clinical origin or from the laboratory (Carreto *et al.* 2008). Our aCGH results showed genome changes in the strains analysed that allow their discrimination. The observed rearrangements include copy number variation of genes related to the fermentative process, such as gene PAU7 which is active only during fermentation and is regulated by anaerobiosis, and genes coding for transcription factors as well as other unknown functions (Table 2). The PAU genes are related to the adaptation of yeast to the stress conditions in wine production, increasing the transcription of these genes in alcoholic fermentation (Rachidi *et al.* 1999, 2000). In this sense, the amplification of the PAU7 gene in chromosome I of strain EC1118 could be related to the adaptation of this yeast to the fermentation process.

Because the three yeast strains are genetically related and the differences at the genome level could be related to phenotypic changes, we carried out a phenotypic analysis using assimilation profiles and fermentation in natural must.

Scarce differences were found between the strains. Comparison of maximum growth rates and biomass production between wine yeast strains did not show significant differences in synthetic must (Fig. 3). It has been described that fermentation in diverse carbon sources allows discrimination of *S. cerevisiae* wine strains (Combina *et al.* 2005). Our results are in agreement with metabolic profiles described for this species (Kurtzman and Fell 1998).

On the other hand, fermentative variables evaluated in natural must showed significant differences between strains (Table 4). This fact could be related to a differential phenotype associated to its adaptation to the wine-making environment which may be explained by changes in gene expression patterns during the fermentative process (Cavalieri *et al.* 2000; Zuzuarregui *et al.* 2006). Knowledge of the genes involved in the DNA copy alterations detected in our study, particularly those with unknown function, could explain the differences found in the fermentative phenotype of the strains evaluated. Moreover, regression analysis between fermentation rate (Table 4) and the copy number variation by aCGH (Table 2) show genes with positive correlation (YAR073W chromosome I; YPL273W, YPL275W, YPL279C; chromosome XVI). This means that gaining a copy of the four genes increases the fermentation rate (data not shown). The genes YAR073W, YPL275W and YPL275c correspond to a dubious ORF, pseudogene and uncharacterized ORF respectively; only the gene YPL273W corresponds to S-adenosylmethionine–homocysteine methyltransferase involved in methionine biosynthesis (Thomas *et al.* 2000).

Finally, the results obtained suggest that yeasts commercially disseminated in the environment can accumulate changes in the genome in short periods of time, generating new genotypes that modify aspects such as their fermentative phenotype.

## Acknowledgement

This research was supported by FONDECYT 1070154 and Mincyt-Conicyt CH/08/02.

## References

Bakalinsky, A.T. and Snow, R. (1990) The chromosomal constitution of wine strains of *Saccharomyces cerevisiae*. *Yeast* **6**, 367–382.

Baleiras Couto, M.M., Eijsma, B., Hofstra, H., Huis in't Veld, J.H. and van der Vossen, J.M. (1996) Evaluation of molecular typing techniques to assign genetic diversity among *Saccharomyces cerevisiae* strains. *Appl Environ Microbiol* **62**, 41–46.

de Barros Lopes, M., Rainieri, S., Henschke, P.A. and Langridge, P. (1999) AFLP fingerprinting for analysis of yeast genetic variation. *Int J Syst Bacteriol* **2**, 915–924.

Bisson, L. (2004) The biotechnology of wine yeast. *Food Biotechnol* **18**, 63–69.

Bradbury, J.E., Richards, K.D., Niederer, H.A., Lee, S.A., Rod Dunbar, P. and Gardner, R.C. (2006) A homozygous diploid subset of commercial wine yeast strains. *Antonie Van Leeuwenhoek* **89**, 27–37.

Carreto, L., Eiriz, M.F., Gomes, A.C., Pereira, P.M., Schuller, D. and Santos, M.A. (2008) Comparative genomics of wild type yeast strains unveils important genome diversity. *BMC Genomics* **9**, 524–541.

Cavalieri, D., Townsend, J.P. and Hartl, D.L. (2000) Manifold anomalies in gene expression in a vineyard isolate of *Saccharomyces cerevisiae* revealed by DNA microarray analysis. *Proc Natl Acad Sci USA* **97**, 12369–12374.

Cocolin, L., Pepe, V., Comitini, F., Comi, G. and Ciani, M. (2004) Enological and genetic traits of *Saccharomyces cerevisiae* isolated from former and modern wineries. *FEMS Yeast Res* **5**, 237–245.

Combina, M., Elía, A., Mercado, L., Catania, C., Ganga, A. and Martínez, C. (2005) Dynamics of indigenous yeast populations during spontaneous fermentation of wines from Mendoza, Argentina. *Int J Food Microbiol* **99**, 237–243.

Cubillos, F.A., Vásquez, C., Faugeron, S., Ganga, A. and Martínez, C. (2009) Self-fertilization is the main sexual reproduction mechanism in native wine yeast populations. *FEMS Microbiol Ecol* **67**, 162–170.

Diaz-Uriarte, R. and Rueda, O.M. (2007) ADaCGH: a parallelized web-based application and R package for the analysis of aCGH Data. *PLoS ONE* **2**, e737.

Dunn, B., Levine, R.P. and Sherlock, G. (2005) Microarray karyotyping of commercial wine yeast strains reveals shared, as well as unique, genomic signatures. *BMC Genomics* **16**, 6–53.

 

Fernandez-Espinar, M.T., Lopez, V., Ramon, D., Bartra, E. and Querol, A. (2001) Study of the authenticity of commercial wine yeast strains by molecular techniques. *Int J Food Microbiol* **70**, 1–10.

Fernandez-Espinar, M., Barrio, E. and Querol, A. (2003) Analysis of the genetic variability in the species of the *Saccharomyces* sensu stricto complex. *Yeast* **20**, 1213–1226.

Flanzy, C. (2000) *Enología: Fundamentos científicos y tecnológicos* ed. Madrid, España: AMV, Mundi Prensa.

Flores Berrios, E.P., Alba González, J.F., Arrizon Gaviño, J.P., Romano, P., Capece, A. and Gschaedler Mathis, A. (2005) The uses of AFLP for detecting DNA polymorphism, genotype identification and genetic diversity between yeasts isolated from Mexican agave-distilled beverages and from grape musts. *Lett Appl Microbiol* **41**, 147–152.

Ganga, M.A. and Martínez, C. (2004) Effect of wine yeast monoculture practice on the biodiversity of non-*Saccharomyces* yeast. *J Appl Microbiol* **96**, 76–83.

Infante, J.J., Dombek, K.M., Rebordinos, L., Cantoral, J.M. and Young, E.T. (2003) Genome-wide amplifications caused by chromosomal rearrangements play a major role in the adaptive evolution of natural yeast. *Genetics* **165**, 1745–1759.

Kurtzman, C.P. and Fell, J.W. (1998) *The Yeasts: A Taxonomic Study* ed, 4th edn-Fourth revised and enlarged Edition. Elsevier Science Publisher: Amsterdam.

Legras, J.L. and Karst, F. (2003) Optimisation of interdelta analysis for *Saccharomyces cerevisiae* strain characterisation. *FEMS Microbiol Lett* **221**, 249–255.

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. *et al.* (2009) Population genomics of domestic and wild yeasts. *Nature* **458**, 337–341.

Martínez, C., Gac, S., Lavin, A. and Ganga, M. (2004) Genomic characterization of *Saccharomyces cerevisiae* strains isolated from wine-producing areas in South America. *J Appl Microbiol* **96**, 1161–1168.

Martínez, C., Cosgaya, P., Vásquez, C., Gac, S. and Ganga, A. (2007) High degree of correlation between molecular polymorphism and geographic origin of wine yeast strains. *J Appl Microbiol* **103**, 2185–2195.

Marullo, P., Bely, M., Masneuf-Pomarède, I., Pons, M., Aigle, M. and Dubourdieu, D. (2006) Breeding strategies for combining fermentative qualities and reducing off-flavor production in a wine yeast model. *FEMS Yeast Res* **6**, 268–279.

Mortimer, R.K., Romano, P., Suzzi, G. and Polsinelli, M. (1994) Genome renewal: a new phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from natural fermentation of grape musts. *Yeast* **10**, 1543–1552.

Nelson, N. (1944) A photometic adaptation of the Somogyi method for the determination of glucose. *J Biol Chem* **153**, 375–380.

Pérez-Ortín, J.E., Querol, A., Puig, S. and Barrio, E. (2002) Molecular characterization of a chromosomal rearrangement involved in the adaptive evolution of yeast strains. *Genome Res* **12**, 1533–1539.

Pretorius, I.S. (2000) Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast* **16**, 675–729.

Puig, S., Querol, A., Barrio, E. and Pérez-Ortín, J.E. (2000) Mitotic recombination and genetic changes in *Saccharomyces cerevisiae* during wine fermentation. *Appl Environ Microbiol* **66**, 2057–2061.

Querol, A., Barrios, E., Huerta, T. and Ramón, D. (1992) Molecular monitoring of the wine fermentation conducted by active dry yeast strain. *Appl Environ Microbiol* **58**, 2948–2953.

Querol, A., Fernandez-Espinar, M.T., del Olmo, M. and Barrio, E. (2003) Adaptive evolution of wine yeast. *Int J Food Microbiol* **86**, 3–10.

Rachidi, N., Barre, P. and Blondin, B. (1999) Examination of the transcriptional specificity of an enological yeast. A pilot experiment on the chromosome-III right arm. *Curr Genet* **37**, 1–11.

Rachidi, N., Martinez, M.J., Barre, P. and Blondin, B. (2000) *Saccharomyces cerevisiae* PAU genes are induced by anaerobiosis. *Mol Microbiol* **35**, 1421–1430.

Saeed, A.I., Sharov, V., White, J., Li, J., Liang, W., Bhagabati, N., Braisted, J., Klapa, M. *et al.* (2003) TM4: a free, open-source system for microarray data management and analysis. *BioTechniques* **34**, 374–378.

Schuller, D., Valero, E., Dequin, S. and Casal, M. (2004) Survey of molecular methods for the typing of wine yeast strains. *FEMS Microbiol Lett* **231**, 19–26.

Schuller, D., Pereira, L., Alves, H., Cambon, B., Dequin, S. and Casal, M. (2007) Genetic characterization of commercial *Saccharomyces cerevisiae* isolates recovered from vineyard environments. *Yeast* **24**, 625–636.

Somogyi, M. (1945) A new reagent for the determination of sugars. *J Biol Chem* **160**, 61–68.

Thomas, D., Becker, A. and Surdin-Kerjan, Y. (2000) Reverse methionine biosynthesis from S-adenosylmethionine in eukaryotic cells. *J Biol Chem* **275**, 40718–40724.

Valero, E., Schuller, D., Cambon, B., Casal, M. and Dequin, S. (2005) Dissemination and survival of commercial wine yeast in the vineyard: a large-scale, three-year study. *FEMS Yeast Res* **5**, 959–969.

Winzeler, E.A., Castillo-Davis, C.I., Oshiro, G., Liang, D., Richards, D.R., Zhou, Y. and Hartl, D.L. (2003) Genetic diversity in yeast assessed with whole-genome oligonucleotide arrays. *Genetics* **163**, 79–89.

Zoecklein, B.W., Fugelsang, K., Gump, B. and Nury, F. (1995) *Wine Analysis and Production* (ed.). NY, USA: Chapman and Hall.

Zuzuarregui, A., Monteoliva, L., Gil, C. and Del Olmo, M. (2006) Transcriptomic and proteomic approach for understanding the molecular basis of adaptation of *Saccharomyces cerevisiae* to wine fermentation. *Appl Environ Microbiol* **72**, 836–847.