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Homogeneity of Interspecific Hybrids Between *Saccharomyces Cerevisiae* and *Saccharomyces Uvarum* by Phenotypic and Transcriptional Analysis

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Abstract

Oenological traits, such as temperature profile and production of certain metabolites, were tested for four interspecifc hybrids obtained by "spore to spore" crossing between *Saccharomyces cerevisiae* and *Saccharomyces uvarum* strains and uniformity of their inheritance was found. PCR/RFLP analysis of ITS regions was carried out to confirm the hybrid nature of the strains. They showed an additive profile with five bands of the respective 325, 230, 170 and 125 bp. Finally gene expression study was performed by comparative DNA macroarray analysis of the hybrids and the preliminary results showed that the global gene expression patterns of hybrids are remarkably similar to one another.
In conclusion, the data obtained by two different approaches, such as metabolic and transcriptomic strategies, suggest a large degree of homogeneity among interspecific hybrids between *S. cerevisiae* and *S. uvarum.* Moreover, the uniformity of F_1 hybrids advises that the oenological trait inheritance mechanism is highly constant and reproducible.

Keywords: interspecific hybrid, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, homogeneity.

Introduction

Saccharomyces cerevisiae wine strains have traditionally been developed by sampling and selecting wild yeasts from spontaneous fermenting musts or wines. In addition to

basic traits, such as fermentative vigour with high ethanol yield and without the development of off-flavours, yeast strains were selected on the basis of the other properties more specific to the type and style of wine to be made. A complete depiction of the ooenological traits employed in selection of wine yeasts is reported by Giudici and Zambonelli [1].

Rarely wilde strains show the complete traits profile desired for winemaking. For this reason, in the last few years there has been an ever-growing demand for new and improved wine yeast strains and an ever-growing application of genetic techniques to improve the winemaking properties.

Optimized strains can be obtained by selection of natural or induced mutants [2,3] sexual recombination methods or the technique of recombinant DNA. The sexual recombination methods are the best approach when the desired traits depend on a multitude of loci (Quantitative Traits Loci, or QTL). However QTLs are poorly understood and/or completely unknown and broadly distributed throughout the genome [4,5] and, for this reason, DNA recombinant techniques and random mutagenesis approach are often ineffective, whereas a “blind” strategy, such as the sexual recombination, allows to obtain quickly improved and recombiant strains [6].

As the wine strains are generally homothallic yeasts, the sexual recombination can be obtained by “spore to spore” crossing [7]. With this method, interspecific hybrids between cryotolerant *Saccharomyces uvarum* and non-cryotolerant *Saccharomyces cerevisiae* have been obtained in different laboratories [8-13]. They are extremely interesting in ooenological terms both for their fermentation competitiveness and for their secondary metabolism products. The interspecific hybrids show an additive combination of traits of the parents with a high degree of phenotypic homogeneity, in spite of variability at strain level [11]. Usually the growth optimal temperature profile is intermediate between those of the parental species and it is included in the wider range, between 27°C and 33°C. Their ability to quickly and efficiently ferment grape musts is higher than that of both the parents. Moreover they grow well both at low (6°C) and high temperature (37°C). The production of secondary fermentation compounds is always an average of that of the parent production [8,10,11].

In this work we tested the homogeneous metabolic behaviour of four interspecific hybrids between *S. cerevisiae* and *S. uvarum* for some important ooenological properties, such as temperature profile, fermentative vigour, ethanol and secondary compounds production. The continuous quantitative variation of these traits can be explained by their polygenic nature [5] in that each locus encodes only for small fraction of phenotypic variation. As each ooenological trait is determined by a multitude of genes, we evaluated the possibility that the metabolic uniformity of hybrids correlates with transcriptomic homogeneity.

Materials And Methods

Yeast strains and media

The yeast strains used in the present work are listed in Table 1. All of strains were maintained in YPD medium (1% yeast extract, 1% peptone, 2% D-glucose, 2% agar)

at 28°C. Sporulation was inducted by incubation of cells at 28°C for four-six days on acetate agar medium (0.5% $CH_3COONa \cdot 3\ H_2O$, 2% agar; final pH 6.5).

Strain hybridisation
The hybrid LS3 was performed by spore crossing, using gametes directly obtained by wild type strains with the "spore to spore" method of Winge [7,14]. The ascus wall were destroyed with Zymoliase 20T 20 mg/ml (Seikagaku Corporation, Japan).

Metabolic analysis of the hybrids
Temperature profile was determined as described by Zambonelli et al. [8] and Rainieri et al. [11]. The cell growth was expressed as measure of absorbance at 600 nm after 18 hours from inoculation in YPD medium.

Table 1. Yeast parental strains and interspecific hybrids used in the present work.

Strains	**Species**	**Collection or works**
7877	*S. uvuram*	DIPROVAL collection; [11]
3002	*S. cerevisiae*	[24]
LS3 (7877 x 3002)	Hybrid	This work
11204.1A	*S. uvarum*	DIPROVAL collection
7070.1A	*S. cerevisiae*	DIPROVAL collection
11204.1A x 7070.1A	Hybrid	DIPROVAL collection; [11]
12233	*S. uvarum*	DIPROVAL collection
6213.1A	*S. cerevisiae*	DIPROVAL collection
12233 x 6213.1A	Hybrid	DIPROVAL collection; [11]
11204	*S. uvarum*	DIPROVAL collection
11502.1A	*S. cerevisiae*	DIPROVAL collection
11204 x 11502.1A	Hybrid	DIPROVAL collection; [8]

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Grape juices were sterilized and used for fermentation tests, as described previously [8]. Fermentation progress was followed as weight loss determined by CO_2 release. The fermentation products were filtered and analysed for pH, total acidity, and ethanol by conventional procedures. The secondary compounds, such as glycerol, succinic acid, acetic acid, malic acid and 2-phenylethanol, were tested as reported by Zambonelli et al. [8]. For each strain, three indipendent replicas were performed. The obtained data were reported as mean values for the hybrids group, the *S. cerevisiae* parental strains group and the *S. uvarum* parental strains group. Differences among the groups were tested by one-way analysis of variance (Duncan's test), using the Statistical Analysis System Software (SAS Institute Inc. Cary NC).

PCR/RFLP analysis of its regions

The genomic DNA extraction was performed as described by Querol et al. [15]. The PCR reaction of ITS regions was conducted as described by Pulvirenti et al. [16]. The amplification program include 94°C for 3 min, 30 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min and 72°C for 5 min; the digestion reaction was carried out using 5-10 µl of the amplified DNA to a final volume of 20 µl with *Hae*III restriction enzyme (New England, Biolabs, Beverly, MA, USA). Finally the restricted fragments were separated for 2 h in 2% Nusieve 3: 1 agarose gel (FMC BioProduct, Rockland, ME, USA) using 0.5x Tris-borate-EDTA (TBE) buffer. The gels were stained with ethidium bromide, destained in sterile water and photographed using an UV-transilluminator.

Total RNA extraction

RNA was obtained from three independent cultures of each of the following strains: *S. cerevisiae* 3002 strain, *S. uvarum* 7877 strain and the hybrids LS3, 11204.1A x 7070.1A, 12233 x 6213.1A and 11204 x 11502.1A. After growing in 50 ml of YPD medium with orbital agitation to middle logarithmic phase (OD_{600} 0.5-0.6), cells were harvested by centrifugation and frozen at -80°C. The RNA extraction was performed as described in Ivorra et al. [17].

Labelling and Hybridisation

Both the labelling of total RNA by reverse transcription and the macrochips hybridisation with labelled cDNA were performed according to Alberola et al. [18].

Signal acquisition and statistical analysis

Digital images of radioactive signals were acquired with FujiFilm FLA3000 Phosphorimager and quantified using the ArrayVision software (Imaging Research Inc.) and taking the sARM density (intensity of each spot with the corresponding subtracted background) as signal. The normalization process and the measure of the significance level for each ORF were performed using ArrayStat software (Imaging Research Inc.). Hybridisations were subjected to double normalization, within each experiment replicate and between two conditions (hybrid *vs.* hybrid). Reproducibility of the replicates was tested considering the data as independent and allowing the program to take a minimum number of valid replicates of 2, in order to calculate the mean values for every gene. We applied the Z-test to normalize the data between conditions and the correlation coefficient was calculated by iterative median and corrected by the False Discovery Rate statistical test to estimate the statistical errors associated to each gene.

Results

Metabolic characterization

All of the parental strains belonging to *S. cerevisiae* and *S. uvarum* reported in Table 1 were previously tested for fermentation ability to produce the following compounds: acetic acid, malic acid, succinic acid, glycerol, ethanol and

2-phenylethanol. In Table 2 we reported the mean values of *S. cerevisiae* and *S. uvarum* parental strains. The cryotolerant strains belonging to *S. uvarum* always presented a higher capacity to synthesize malic acid, glycerol, succinic acid than the *S. cerevisiae* strains, while their acetic acid production and ethanol yields were lower.

Table 2. Wine properties and secondary compounds production for the four hybrids and their parental strains in grape juice (expressed as average values)

	pH	**Total acidity**[a]	**EtOH**	**Glycerol**	**Succinic Acid**	**Acetic Acid**	**Malic Acid**	**2-phenyl-ethanol**
S. cerevisiae	3.35	7.62 B[b]	10.07 A	7.22 C	0.45 C	0.27 A	1.48 C	0.04 C
S. uvarum	3.25	8.22 A	10.84 B	10.89 A	1.04 A	0,08 B	2.67 A	0.18 A
Hybrids	3.3	7.62 C	10.48 AB	9.25 B	0.77 B	0,15 AB	1.92 B	0.11 B

[a] As tartaric acid;
[b] The values followed different letters are statistically different (Duncan's test, $P < 0.01$).

The hybrids LS3, 11204.1A x 7070.1A, 12233 x 6213.1A and 11204 x 11502.1A showed an optimal growth temperature (Topt) range included between the Topts of both parental strains, that is from 27°C to 30°C. Sugar fermentation process was conducted very well both at 6C° and 36°C, always with higher performance and vigour than the parental strains. The data are in agreement with Rainieri et al. [11].

Finally the secondary metabolic profile of the hybrids was monitored as production of acetic acid, malic acid, succinic acid, glycerol and 2-phenylethanol. As expected, the amount of metabolites produced by four interspecific hybrids was an average of those produced by the parental strains (Table 2). The results are in agreement with the previous data [8,10,11].

PCR/RFLP analysis of its regions

When two yeast spores were subjected to crossing, the result was a zygote that showed a nucleus as a sum of the genetic material of two haploid parental strains. According to Pulvirenti et al. [19,20], ITS regions PCR/RFLP analysis can differentiate two very strongly correlated species such as *S. cerevisiae* and *S. uvarum*, when the amplified fragment of 830 bp was digested by *Hae*III restriction enzyme. The parental strains belonging to *S. uvarum* and their monosporic cultures showed three bands respectively of 495, 230, 125 bp, whereas for the *S. cerevisiae* parental strains or their monosporic clones the restriction pattern shows four bands of 325, 230, 170 and 125 bp. The additive restriction profile with 5 bands of LS3, 11204.1A x 7070.1A, 12233 x 6213.1A and 11204 x 11502.1A confirmed the hybrid nature of all of strains (Figure 1).

Preliminary trascriptome analysis

The transcriptomic profiles were obtained by cDNA hybridization of the two parental strains and four hybrids described before. On the basis of these hybridizations, a preliminary analysis was performed to examine the possible use of

yeast array, generated with the reference strain *S. cerevisiae* S288c [21] DNA sequence, to detect genes of other microrganisms, such as interspecific hybrids between *S. cerevisiae* and *S. uvarum*. Using DNA macroarrays for *S. cerevisiae* genes [18], 70.3% of the 3002 *S. cerevisiae* strain genes were over the threshold value, whereas the hybridization pattern of the *S. uvarum* strain was significantly differenent, with only 48% (2970) of its genes over the threshold, reflecting both genomic and transcriptional differences from *S. cerevisiae* strain (Table 3). Of last these, 2919 ORFs are shared by both the parental species, whereas 51 ORFs are detected only in *S. uvarum* parental species. This can be due to an increased expression of these genes that are, only in *S. uvarum*, above the imposed threshold. In contrast, logically, the number of the ORFs detected only by *S. cerevisiae* parental species is greater (1406). The hybrid LS3, achieved by crossing between those two strains, showed the same percentage of expressed ORFs of the parental strain 3002. A similar percentage of expressed ORFs was found for the remaining hybrids (Table 3). This demonstrates a high degree of homogeneity within the hybrids and also means that there is high homogeneity between the *S. cerevisiae* parental strains.

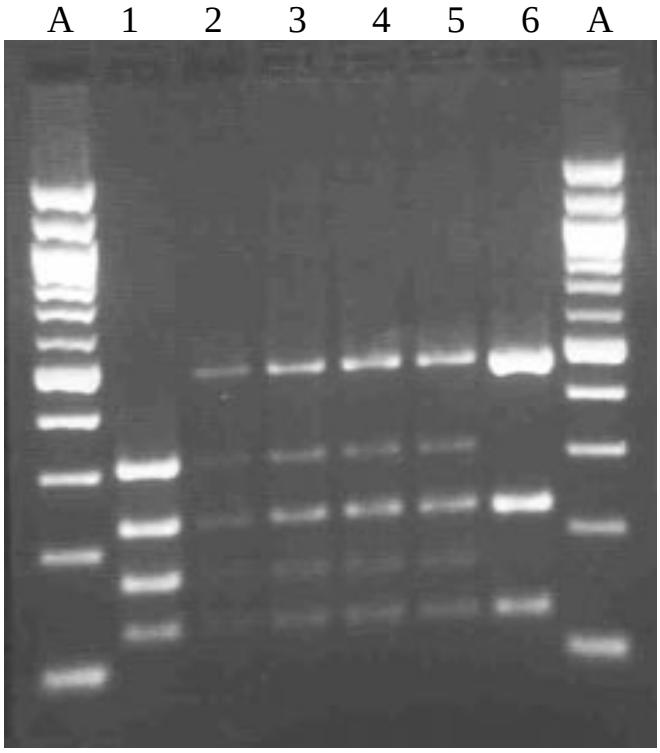

Figure 1. PCR/RFLP analysis with *Hae*III restriction enzyme of ITS regions. From left to right. A: marker 100 kb ladder; 1: 3002 *S. cerevisiae* parental strain; 2: LS3 hybrid strain; 3: 11204.1Ax7070.1A hybrid strain; 4: 12233x6213.1A hybrid strain; 5: 11204x11502.1A hybrid strain; 6: 7877 *S. uvarum* parental strain.

According to the previous results, the ORFs present on the macroarray were classified as follows: i) “common ORFs”: ORFs detected in both parental strains and in the hybrids; ii) “*S. cerevisiae* ORFs”: ORFs detected in both *S. cerevisiae* 3002 strain and

in the hybrids; iii)"*S. uvarum* ORFs": ORFs detected in both *S. uvarum* 7877 strain and in the hybrids; iv) "specific ORFs": ORFs detected only in any of the interspecific hybrids, but not in parental strains. A high similarity was found between the hybrids for the number and the identity of the ORFs. As reported in Table 3, "common ORFs" were more than half of the detected ORFs with a number very similar for all of the interspecific hybrids. A very low number of detected ORFs were shared with *S. uvarum*, within values from 20 to 36 ORFs, whereas the number of detected ORFs shared with *S. cerevisiae* is higher, from 1024 to 1160. For all of the hybrids a similar low number of "specific ORFs" was detected. Comparing the hybrids for each single ORFs group, the homogeneity of results is confirmed because in all of the hybrids the detected ORFs are approximately the same for each category.

Table 3. Detected ORFs distribution in interspecific hybrids.

Hybrid and parental strains	**Detected ORFs**	**common ORFs[a]**	***S. cerevisiae* ORFs[b]**	***S. uvarum* – ORFs[c]**	**Specific ORFs[d]**
LS3	4135 (67.3%[1])	2855	1014	20	246
11204.1A x 7070.1A	4375 (71.2%)	2882	1160	24	309
11204 x 11502.1A	4124 (67.1%)	2838	1003	22	261
12233 x 6213.1A	4399 (71.6%)	2888	1115	36	360
3002[2]	4325 (70.3%)	2919	1406	-	-
7877	2970 (48.3%)	2919	-	51	-

[a] Hybrids detected ORFs shared with both parental species;
[b] Hybrids detected ORFs shared with *S. cerevisiae* parental species;
[c] Hybrids detected ORFs shared with *S. uvarum* parental species;
[d] ORFs detected only in hybrids.
[1]Percentage of spots present on macrochip and detected.
[2]The number of detected ORFs in parental strains, 3002 and 7877, is given as a reference.

This preliminary analysis was performed to verify the possible use of yeast array, generated with S288c DNA sequence, for the study of interspecific hybrids between *S. cerevisiae* and *S. uvarum*. All of the interspecific hybrids had a number of detected ORFs very similar to the *S. cerevisiae* parental species and the distribution profiles were very homogeneous among the hybrids.

Hybrids *vs* Hybrids Comparison

Successfully, we performed a comparison analysis between transcript levels of different hybrids, after normalization of the signal data was achieved by three repeated hybridisations as reported in the Materials and Methods. When each hybrid was compared in turn with the other hybrid, the global gene expression patterns of hybrids grown to OD = 0.5 in YPD were remarkably similar to one another, because the large majority of ORFs did not show relative expression changes (Table 4). In all of comparisons among interspecific hybrids only a very low amount of genes on the

array showed statistically different changes of at least 2.5-fold in transcript abundance, which is the highest number of differentially expressed ORFs 357.

Table 4. Differential expression profile analysis in hybrids.

Hybrid strains comparison	**Commonly[a] expressed ORFs**	**Differentially[a] expressed ORFs**
LS3 *vs.* 11204 x 11502.1A	5789	260
LS3 *vs.* 12233 x 6213.1A	5692	357
LS3 *vs.* 11204.1A x 7070.1A	6048	1
11204.1A x 7070.1A *vs.* 11204 x 11502.1A	5868	181
11204.1A x 7070.1A *vs.*12233 x 6213.1A	5793	256
11204 x 11502.1A *vs.* 12233 x 6213.1A	5778	271

[a] Considered as statistically differently expressed by ArrayStat analysis as described in Material and Methods.

Discussion

All of analysed hybrids showed a very similar metabolic behaviour for the considered properties: the additive temperature profile; a more fermentative competitiveness than parental strains fermentation performance; an intermediate secondary metabolite production compared to parents. This uniformity of results was in agreement with which reported by several authors [8,10,11]. These analysed traits are continuously distributed phenotypes, determined by the cumulative action of multiple loci, defined QTLs. For example, it is speculated that the genetic of the ethanol tolerance basic trait involves more than 250 genes [3]. Moreover, each QTL is a polymorphic gene, which contains alleles that differentially affect the quantitative trait expression. As each quantitative ooenological property involves a large number of polymorphic genes, the hybrids metabolic similarity for many quantitative ooenological traits, underlined in this work, suggests an analogous expressed genetic background. This hypothesis was investigated by transcriptomic approach for 4 hybrids and two parental species.

DNA macroarrays were used to compare genome-wide patterns of gene expression among hybrids. The application of yeast arrays generated with laboratory strain S288c DNA sequence for the analysis of industrial strains has been previously demonstrated in the literature [22,23]. In this work, we have evaluated the possibility of their use in the transcriptomic study of interspecific hybrids between *S. cerevisiae* and *S. uvarum*, on the basis that *S. uvarum* is the species phylogenetically closest to *S. cerevisiae* with 85% sequence homology [24]. Our conclusion is that *S. cerevisiae* membrane arrays are suitable to compare close species such as *S. uvarum* and their interspecific hybrids. A detailed analysis of the transcriptomes will be presented elsewhere (Solieri et al. in preparation), but here we show that the number of detectable spots in the macroarray and the general transcriptome pattern are useful

tools for the study of the physiological similarity of the hybrids. *S. uvarum* shows a high percentage of genes detected by the macroarrays. The macrochip pattern obtained for this species and for the hybrids does not reflect, however, the real transcriptome. It is necessary to consider that, for a *S. uvarum* gene detection, both the homology to the *S. cerevisiae* probe of the macroarray and the expression level have independent and additive contributions. Nevertheless, as both the expression level and the precise sequence of the alleles present are characteristic of every hybrid, the results obtained reflect the distinctive physiology of each one. The interspecific hybrids showed a number of detected ORFs to be very homogeneous when compared to both the *S. cerevisiae* parental species and to each other. Moreover, a strong similarity in global transcriptional patterns was found, although the interspecific hybrids compared were achieved by crossing between different strains.

On the basis of data obtained by different approaches, such as metabolic and transcriptomic analysis, it became clear that the interspecific hybrids between *S. cerevisiae* and *S. uvarum* are a group of very homogeneous strains. On the metabolic hand, a high homogeneity degree was found for all of the considered oenological traits. The molecular basis of these oenological traits inheritance in interspecific hybrids is not completely clear because of complex polygenic regulation. Nevertheless the result of their inheritance mechanism is highly constant and reproducible in interspecific hybrids and that affect trascriptional similarity. The uniformity of F_1 hybrids, obtained by crossing between *S. cerevisiae* and *S. uvarum* species, suggests to perfom a genetic improvement plan of wine yeasts on the basis of knowledge of the oenological traits of the parental strains.

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References

[1] Giudici, P. and Zambonelli, C., 1992, “Criteri di selezione dei lieviti per enologia” Vignevini, 9, pp. 29-34.

[2] Volschenk, H., Voljon, M., Grobler, J., Petzold, B., Bauer, F., Subden, R.E., Young, R.A., Lonvaurd, A., Denayrolles, M. and van Vuuren, H.J., 1997, “Engineering pathways for malate degradation in *Saccharomyces cerevisiae*” Nat. Biotechnol., 15**,** pp. 253-257.

[3] Pretorius, I.S., 2000, “Tailoring wine yeast for the new millenium: novel approaches to the ancient art of winemaking” Yeast, 16, pp. 675-729.

[4] Steinmetz, L.M., Sinha, H., Richards, D.R., Spiegelman, J.I., Oefner, P.J., McCusker, J.H. and Davis, R.D., 2002, “Dissecting the architecture of a quantitative trait locus in yeast” Nature, 416, pp. 326-330.

[5] Marullo, P., Bely, M., Masneuf-Pomarede, I., Aigle, M. and Dubourdieu, D., 2004, “Inheritable nature of oenological quantitative traits is demonstrated by meiotic segregation wine yeast strains” FEMS Yeast Res, 4, pp. 711-719.

[6] Giudici, P., Solieri, L., Pulvirenti, A. and Cassanelli, S., 2005, "Strategies and perspectives for genetic improvement of wine yeasts" Appl. Microbiol. Biotechnol., 66(6), pp.622-8.

[7] Winge, O. and Lausten, O., 1938, "Artificial species hybridisation in yeast" Comptes Rendus des Travaux u Laboratoire Carlsberg-Serie Physiologique, 22, pp. 235-244.

[8] Zambonelli, C., Passarelli, P., Rainieri, S. and Giudici, P., 1993, "Taxonomic and technological implications of sterility in hybrids from cryotollerant and non cryotollerant *Saccharomyces* strains" Ann. Microbiol., 43, pp. 217-223.

[9] Kishimoto, M., 1994, "Fermentation characteristics of hybrids between the cryophilic wine yeast *Saccharomyces bayanus* and mesophilic wine yeast *Saccharomyces cerevisiae*" J. Ferment. Bioeng., 77, pp. 432-435.

[10] Zambonelli, C., Passarelli, P., Rainieri, S., Bertolini, L., Giudici, P. and Castellari, L., 1997, "Technological properties and temperature response of interspecific *Saccharomyces* hybrids" J. Sci. Food Agric., 78, pp. 7-12.

[11] Rainieri, S., Giudici, P. and Zambonelli, C., 1998, "Oenological properties of *Saccharomyces bayanus* and *Saccharomyces cerevisiae* interspecific hybrids" Food Technol. Biotech., 36, pp. 51-53.

[12] Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T. and Dubourdieu, D., 2002, "Hybrids *Saccharomyces cerevisiae* x *Saccharomyces bayanus* var. *uvarum* having a high liberating ability of some sulfur varietal aromas of *Vitis Vinifera* Sauvignon blanc wines" J. Int. Sci. Vigne Vin., 36, pp. 205-212.

[13] Sato, M., Kishimoto, M., Watari, J. and Takashio, M., 2002, "Breeding of Brewer'sYeast by Hybridisation between a Top-Fermenting yeast *Saccharomyces cerevisiae* and a Cryophilic Yeast *Saccharomyces bayanus*" J. Biosci. Bioeng., 93, pp. 509-511.

[14] Naumov, G.I., Kondrat'eva, V.I. and Naumova, E.S., 1986, "Methods for hybridization of homothallic yeast diplonts and haplonts" Biotekhnologiya, 6, pp. 33-36.

[15] Querol, A., Barrio, E. and Ramón, D., 1992, "A comparative study of different methods of yeast strain characterization" System. Appl. Microbiol., 15, pp. 439-446.

[16] Pulvirenti, A., Solieri, L., Gullo, M., De Vero, L. and Giudici, P., 2004, "Occurrence and dominance of yeast species in sourdough." Lett. Appl. Microbiol., 38, pp. 113-117.

[17] Ivorra, C., Pérez-Ortín, J.E. and Del Olmo, M., 1999, "An inverse correlation between stress resistence and stuck fermentations in wine yeasts. A molecular study" Biotechnol. Bioeng., 64, pp. 698-708.

[18] Alberola, T.M., García-Martínez, J., Antúnez, O., Viladevall, L., Barceló, A., Ariño, J. and Pérez-Ortín, J.E., 2004, "A new set of DNA macrochips for the yeast *Saccharomyces cerevisiae*: features and uses" Int Microbiol (in press).

[19] Pulvirenti, A., Caggia, C., Restuccia, C., Giudici, P. and Zambonelli, C., 2000, "Inheritance of mitochondrial DNA in interspecific *Saccharomyces* hybrids" Ann. Microbiol., 50, pp. 61-64.

[20] Pulvirenti, A., Zambonelli, C., Todaro, A. and Giudici, P., 2002, "Interspecific hybridization by digest tract of invertebrates as a source of environmental biodiversity within the *Saccharomyces cerevisiae*" Ann. Microbiol., 52, pp. 61-64.

[21] Goffeau, A., Barrell, B.G., Bussey, H., Davis, R.W., Dujon, B., Feldmann, H., Galibert, F., Hoeisel, J.D., Jacq, C., Johnston, M., Louis, E.J., Mewes, H.W., Murakami, Y., Philippsen, P., Tettelin, H., Oliver, S.G.**,** 1996, "Life with 6000 genes" Science, 274, pp. 546-567.

[22] Hauser, N.C., Fellenberg, K., Gil, R., Bastuck, S., Hoheisel, J.D. and Pérez-Ortín, J.E., 2001, "Whole genome analysis of a wine yeast strain" Comp. Funct. Genom., 2, pp. 69-79.

[23] Cavalieri, D., Towsend, J.P. and Hartl, D.L., 2000, "Manifold anomalies in gene expression in a vineyard isolate of *Saccharomyces cerevisiae* revealed by DNA microarray analysis" PNAS, 97, pp. 12369-12374.

[24] Bon, E., Neuvéglise, C., Casaregola, S., Artiguenave, F., Wincker, P., Aigle, M. and Durrens, P., 2000, "Genomic exploration of the *Hemiascomycetous* yeasts: 5. *Saccharomyces bayanus* var. *uvarum*" FEBS Letters, 487, pp. 37-41.