HYBRIDS SACCHAROMYCES CEREVISIAE X *SACCHAROMYCES BAYANUS* VAR. *UVARUM* HAVING A HIGH LIBERATING ABILITY OF SOME SULFUR VARIETAL AROMAS OF *VITIS VINIFERA* SAUVIGNON BLANC WINES

DES HYBRIDES SACCHAROMYCES *CEREVISIAE X SACCHAROMYCES BAYANUS* VAR. *UVARUM* PRÉSENTANT UNE FORTE APTITUDE À RÉVÈLER DES COMPOSÉS SOUFRÉS CARACTÉRISTIQUES DE L'ARÔME VARIÉTAL DES VINS BLANCS DE SAUVIGNON

Isabelle MASNEUF[1], Marie-Laure MURAT[2], G.I. NAUMOV[3], T. TOMINAGA[2] and D. DUBOURDIEU[2]

1 : ENITA de Bordeaux, 1 cours du Général de Gaulle, BP 201, 33175 Gradignan cedex, France
2 : Faculté d'Œnologie, Université Victor Segalen Bordeaux 2, 351 cours de la Libération, 33400 Talence, France.
3 : State Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia

Summary : We measured ability of some indigenous *Saccharomyces bayanus* var. uvarum wine yeasts to release volatile thiols from their S-cysteine conjugate precursors, odorous compounds responsible for the characteristic aroma of Sauvignon blanc wines. We also made interspecific hybrids between *Saccharomyces cerevisiae* and *Saccharomyces bayanus* var. *uvarum* strains and verified their hybrid origin with karyotypes and *MET2* PCR-RFLP analysis. As compared to the parents, some hybrids could release high amounts of volatile thiols from the S-cysteine conjugate precursor without producing excessive amounts of β-phenylethyl alcohol and its acetate. One hybrid was retained for industrial production under a dry form and successfully compared with *Saccharomyces cerevisiae* strains in experimental tests in different cellars.

Résumé : L'aptitude de certaines souches de l'espèce *Saccharomyces bayanus* var. *uvarum* à libérer, au cours de la fermentation alcoolique, les thiols volatils de leurs précurseurs cystéinylés est étudiée dans ce travail. Les deux souches de levures testées présentent une forte aptitude à révéler les composés majeurs de l'arôme variétal des vins blancs de Sauvignon blanc. En revanche, elles produisent des teneurs importantes en phényl-éthanol et son acétate, composés qui au-delà d'une certaine concentration, banalisent l'arôme de certains cépages. Des hybrides interspécifiques entre deux souches de l'espèce *Saccharomyces cerevisiae* et *Saccharomyces bayanus* var. *uvarum* sont alors construits au laboratoire. La nature hybride des souches obtenues est vérifiée par l'analyse des caryotypes en électrophorèse en champs pulsés et par l'analyse PCR-RFLP du gène *MET2*. En milieu modèle au laboratoire, les souches hybrides libèrent de fortes teneurs en thiols volatils, en comparaison avec leur parent *Saccharomyces cerevisiae* sans produire des quantités excessives de phényl-éthanol et de son acétate. Une souche hybride, retenue pour la production industrielle, est testée avec succès lors d'expérimentations menées en grand volume dans différentes caves sur des moûts de Sauvignon. Ce travail constitue un exemple d'utilisation de souches de levures de l'espèce *Saccharomyces bayanus* var. *uvarum* dans un programme d'amélioration de levure industrielle de l'espèce *Saccharomyces cerevisiae*, selon un critère original jamais étudié jusqu'à présent, la libération des thiols volatils à partir de leurs précurseurs S-conjugués à la cystéine.

Keywords: interspecific hybrid, *Saccharomyces bayanus* var. *uvarum*, volatile thiol, cysteinylated precursor

Mots clefs : hybride interspecifique, *Saccharomyces bayanus* var. *uvarum*, thiol volatile, précurseur S-conjugué à la cystéine

*Correspondance : I-masneuf@enitab.fr

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INTRODUCTION

Selection of yeasts for winemaking is often made from indigenous yeast flora of spontaneous must fermentations. Two yeast species belonging to the genus *Saccharomyces sensu stricto* are involved in alcoholic fermentation : *S. cerevisiae* and *S. bayanus* var. *uvarum* (NAUMOV, 2000; NGUYEN and GAILLARDIN, 1997; RAINIERI *et al.*, 1999). The latter yeast was identified in natural fermentations of grapes originated from cool wine producing areas, e.g. Val de Loire (France) (MASNEUF *et al.*, 1996; NAUMOV *et al.*, 2000). Most industrial dried yeasts are *S. cerevisiae*. Recently, an industrial strain, initially classified as *S. uvarum*, was identified as an hybrid between *S. cerevisiae* and *S. bayanus* (MASNEUF *et al.*, 1998).

During the wine fermentation, yeasts are transforming sugars into ethanol and producing or releasing volatile compounds that affect the wine's flavor and aroma. For example, the compounds responsible for the characteristic aroma of Sauvignon blanc wines are volatile thiols (DARRIET *et al.*, 1995 ; TOMINAGA *et al.*, 1996, 1997 and 1998a). The 4-mercapto-4-methylpentan-2-one (4MMP) is responsible for the «box tree» and «broom aromas» while the 3-mercaptohexan-1-ol (3MH) evokes the «passion fruit» odour. These volatile thiols also contribute to the aroma of wines made from other grape varieties e.g. Muscat, Riesling, Colombard, etc. (TOMINAGA *et al.*, 2000). The sulphur compounds exist in the must in a non-volatile form as an S-cysteine conjugate (TOMINAGA *et al.*,1998a). The volatile thiols are released during the alcoholic fermentation of the must due to the transformation of the corresponding S-cysteine conjugate by the yeast. The final concentration of these thiols varies and depends on the yeast strain used (DARRIET *et al.*,1991 ; MURAT *et al.*, 2001). However, the mechanism by which the yeast transforms the cysteinylated precursor into aroma remained unknown. In our previous studies, we have shown that *S. bayanus* var. *uvarum* strains are present in the spontaneous fermentation of various Sauvignon blanc musts from the Val de Loire area (MASNEUF *et al.*, 1996 ; NAUMOV *et al.*, 2000) but their role in the Sauvignon blanc winemaking process has not been investigated before. Wine yeast *S. bayanus* var. *uvarum* strains were described by different authors to produce high concentration of volatile fermentative compounds such as β-phenylethyl alcohol and its acetate. Those compounds are not desirable in Sauvignon blanc wines because they can mask the characteristic aroma of this variety. Thus, we considered that using a pure culture of *S. bayanus* var. *uvarum* for Sauvignon blanc winemaking is undesirable. Interspecific hybrids *S. cerevisiae* x *S. bayanus* var. *uvarum* were shown to produce intermediate amounts of β-phenylethyl alcohol and its acetate (KISHIMOTO, 1994 ; MASNEUF *et al.*, 1998, SHINIHARA *et al.*, 1994 ; ZAMBONELLI *et al.*, 1997).

Our objectives in this study were to determine the ability of *S. bayanus* var. *uvarum* strains to liberate volatile thiols from their S-cysteine conjugates and to construct *S. cerevisiae* x *S. bayanus* hybrids for Sauvignon blanc winemaking. We attempt to receive a strain with a high ability to liberate 4MMP and 3MH from their cysteinylated precursor without producing exaggerated amount of β-phenylethyl alcohol and its acetate.

MATERIALS AND METHODS

I- YEAST STRAINS AND MEDIA

All strains (table I) are maintained in the collection of the Faculté d'Œnologie de Bordeaux. *S. cerevisiae* strain VL3c was selected for its good ability to liberate the volatile thiols from the corresponding S-cysteine conjugates among 90 different strains isolated from natural fermentations of Sauvignon blanc. *S. bayanus* var. *uvarum* strains were isolated from spon-

Table I - Yeast strains used in this study.

Souches de levures utilisées.

Strains designation*	Origin or source	Species
VKM Y-502	VKM	*S. cerevisiae*
VKM Y- 1146	VKM	*S. bayanus* var. *uvarum*
EG8	INRA Colmar	*S. cerevisiae*
VL3c	Faculté d'Œnologie de Bordeaux	*S. cerevisiae*
VL1	Faculté d'Œnologie de Bordeaux	*S. cerevisiae*
P3	Faculté d'Œnologie de Bordeaux	*S. bayanus* var. *uvarum*
TBC 28	Faculté d'Œnologie de Bordeaux	*S. bayanus* var. *uvarum*

*VKM Y-502=CBS 5287, VKM Y^{-1}146=CBS 8687 - EG8=CLIB 2030, VL3c=CLIB 2016, VL1=CLIB 2015

VKM = All-Russian Collection of Microorganisms, Moscow, Russia. CBS = Centraalbureau voor Schimmelcultures, Delft, Holland. CLIB = Collection de Levures d'Intérêt Biotechnologique, Thiverval-Grignon, France.

taneous fermentation of Sauvignon blanc must in Val de Loire and Bordeaux (MASNEUF *et al*., 1996 ; NAUMOV *et al*., 2000). Yeasts were grown on complete YPD medium (1 % yeast extract, 1 % peptone, 2 % glucose, 2 % agar) at 28 °C; sporulation was induced by incubation of cells at 25 °C for two days on acetate medium (1 % CH_3COONa, 0.5 % KCl, 2 % agar).

II- HYBRIDISATION

The two strains were sporulated and individual ascospores were isolated with a micromanipulator (Singer MSM Manual) and tested for sporulation. Ascus walls were destroyed with crude stomach enzyme complex prepared from snail *Helix pomatia*. Hybrids of homothallic yeasts were obtained by crossing spores of monosporic clones using a micromanipulation method of Winge (NAUMOV *et al*., 1986). Each hybrid originated from a single zygote.

III- MOLECULAR GENETIC ANALYSIS OF THE HYBRIDS

1- *MET2* PCR-RFLP

PCR amplification was conducted with yeast cells after growth on solid YPD medium until stationary phase (MASNEUF and DUBOURDIEU, 1994). Amplification reactions were performed with a Perkin-Elmer (Emeryville, California, USA) DNA thermal *Thermocycler* 480 by using primers specific for *MET2* amplification (HANSEN and KIELLAND-BRADT, 1994). PCR products were precipitated, and aliquots were digested with *Eco*RI or *Pst*I. Restriction fragments were analysed by electrophoresis on a 1.8 % agarose gel. A Boehringer Mannheim (Meylan, France) DNA molecular weight marker VIII was used as standard.

2- CHEF (Contour clamped homogeneous electric field) Gel electrophoresis

Chromosomal DNA was prepared in agarose plugs (FREZIER and DUBOURDIEU, 1992). A CHEF DRII apparatus (Bio-Rad, Richmond, Californie, USA) was used to separate chromosomal DNAs. Electrophoresis buffer (0.5 X TBE) was circulated around the gel and cooled to 14 °C. Electrophoresis was carried out at 200 V for 15 h with a switching time of 60 s and then for 9 h with a switching time of 90 s. A standard set of *S. cerevisiae* YNN 295 chromosomes was obtained commercially (Bio-Rad) and used for comparison.

IV- FERMENTATION EXPERIMENTS

In the laboratory, we used a sterile filter sterilized Sauvignon blanc must or model medium supplemented with S-4-(4-methylpentan-2-one)-L-cysteine (420 nmoles l^{-1}) (TOMINAGA *et al*., 1998). The yeast inoculums were obtained from overnight cultures grown on diluted fermentation medium. The quantity of yeast was measured by OD600 in order to inoculate the must or model medium at a level of $3\text{-}4\times10^6$ cells l^{-1}. The fermentation experiments were carried out in 750 ml bottles at temperature 18 °C. The turbidity of the medium was adjusted to 200 NTU (Nephelometric Turbidity Unit) with must Sauvignon blanc solids for grape juice or with cellulose for model medium to improve the fermentation velocity (OLLIVIER *et al*., 1987). In cellar, the same Sauvignon blanc must having a turbidity adjusted at 200 NTU was divided in identical barrels (225 l) and then inoculated with 200 mg l^{-1} of dried yeast.

In cellars experiments, implantation control was performed at mid-fermentation. Sterile samples were taken from must. The implantation of the strains was checked by comparative analysis of the karyotypes of the inoculate yeast strain and the total biomass, using pulsed-field electrophoresis (FREZIER and DUBOURDIEU, 1992). At the end of the alcoholic fermentation, when reducing sugars content dropped below 2 g l^{-1}, the bottles were placed at 10 °C, SO_2 was adjusted to 60 mg l^{-1} and the wines were rapidly analyzed. The volatile thiols released from Sauvignon blanc must or model medium after the fermentation were analysed according to the method of TOMINAGA *et al*. (1998b) with modifications (TOMINAGA *et al*., 2000). Higher alcohols and esters contents was determined by gas chromatography coupled with a flame ionisation detector (FID) (CARBOWAX 20M capillary column type BP20, length 50 metre, internal diameter 0.25 mm, film thickness 0.50 micron; VARIAN 3400 gas chromatograph, Merck D-2500 chromato-integrator). The wines amounts of alcohol and volatile acidity were quantified by the official methods of the European Union (Europeen Union Reglementation, n°2676/90, 1990).

V- STATISTICAL PROCESSING OF THE RESULTS

The 4MMP, 3MH, β-phenylethyl alcohol and its acetate contents of Sauvignon blanc wines after alcoholic fermentation in laboratory were subjected to double-factor analysis of variance (yeast strains, year) without repetition ($\alpha = 0.01$, ANOVA, Statbox Software©). The same double-factor analysis was done on the 4MMP, 3MH, β-phenylethyl alcohol and its acetate contents, ethanol production and volatile acidity amounts of Sauvignon blanc wines (1999) after alcoholic fermentation in cellar (yeast strains, must origin). Where there were statistically differences between the yeast strains, the

significance of the differences was assessed with a Newman-Keuls comparison test (p = 0.05, ANOVA, Statbox Software©, Grimmer Logiciels).

RESULTS AND DISCUSSION

I- SELECTION OF PARENTAL STRAINS

Fermentation experiments were done in the laboratory on Sauvignon blanc musts obtained in 1995, 1996 and 1997. Enological properties of two *S. bayanus* var. *uvarum* strains P3 and TBC28 were compared to those of three industrial *S. cerevisiae* strains VL3c, EG8, VL1, well known for their high ability to liberate the aromatic potential of the Sauvignon blanc must (MURAT *et al.*, 2001). At mid-fermentation, the strain implantation verification was done by comparison of the pure culture strain's karyotype with the sample taken from the experiment.

The average amounts of 4MMP, 3MH, β-phenylethyl alcohol and its acetate in wines fermented in 1995, 1996 and 1997 by each strain are reported in table II. The wines fermented by *S. bayanus* var. *uvarum* strains have the highest average amount of the 4MMP and the 3MH. Moreover, there were significant differences in the 4MMP content of the wines depending on the species used. The *S. bayanus* var. *uvarum* strains analysed produced also high amount of β-phenylethyl alcohol and its acetate comparing to *S. cerevisiae* strains (table II). Similar observations were described earlier (GUIDICI *et al.*, 1995; MASNEUF *et al.*, 1998). Despite the important difference between the β-phenylethyl alcohol and its acetate data of the two *S. bayanus* var. *uvarum*, only P3 is producing significantly higher amount of these both compounds compare to VL3c, EG8 and VL1. This result is explaining by the strong vintage effect (statistically significant at $\alpha < 0.05$). The strains P3 and VL3c were retained for hybrids construction.

Table II - Yeast strains and species effect on 4MMP, 3MH, β-phenylethyl alcohol and β-phenyl-ethyl acetate amounts in Sauvignon blanc wines after alcoholic fermentation (average of three experiments on musts from vintages 1995, 1996 and 1997).

Effet de la souche et de l'espèce de levure sur les teneurs en 4MMP, 3MH, phényl-éthanol et acétate de phényl-éthyle dans des vins de Sauvignon blanc après fermentation alcoolique (moyenne de trois expérimentations réalisées sur des moûts des millésimes 1995, 1996 et 1997).

Strains	*Saccharomyces bayanus* var. *uvarum*		*Saccharomyces cerevisiae*		
	P3	TBC28	VL3c	EG8	VL1
3-mercaptohexan-1-ol (ng l^{-1})	5212 a	4642 a	3507 a	3607 a	3423 a
4-mercapto-4-methylpentan-2-one (ng l^{-1})	47 a(1)	43 a	22 b	19 b	17 b
β-phenylethyl alcohol mg l^{-1}	301 a	187 ab	37 b	37 b	40 b
β–phenylethyl acetate mg l^{-1}	6,3 a	1,9 ab	0,48 b	0,56 b	0,40 b

The values represent the mean of three independent replications. (1)Values followed by different letters are statistically different (Newman-Keuls test)

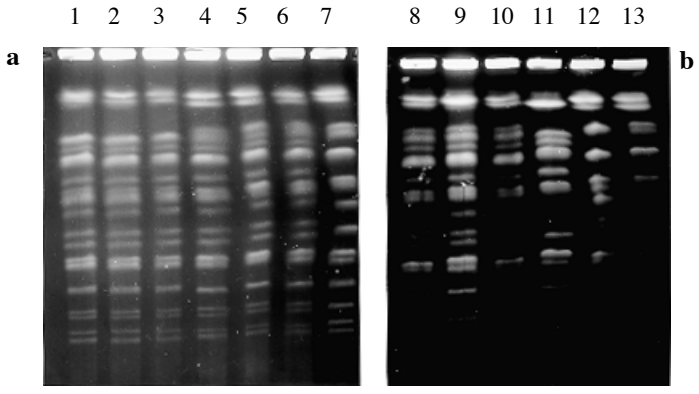

Fig. 1 - A : Electrophoretic karyotyping of hybrids H1, H6, H7, H2, H5, H8 (lane 1 to 6), standard *S. cerevisiae* YNN 295 (lane 7). B : hybrids H3, H4, H9 (lane 8 to 10), *Saccharomyces bayanus* var. *uvarum* P3 (lanes 11), of *Saccharomyces cerevisiae* VL3c (lane 12) and standard *S. cerevisiae* YNN 295 (lane 13).

A : Caryotypes des hybrides H1, H6, H7, H2, H5, H8 (pistes 1 à 6), souche témoin *S. cerevisiae* YNN 295 (piste 7). B : hybrides H3, H4, H9 (pistes 8 à 10), *Saccharomyces bayanus* var. *uvarum* P3 (piste 11), *Saccharomyces cerevisiae* VL3c (piste 12) et souche témoin *S. cerevisiae* YNN 295 (piste 13).

II- HYBRIDISATION

Spores of the strains P3 and VL3c were isolated by a micromanipulator (Singer MSM manual). Analysis of 17 tetrads of each strain showed that they were homothallic giving diploid monosporic cultures. The percentage of germination was 53 % for *S. cerevisiae* VL3c and 98 % for *S. bayanus* var. *uvarum* P3. The fermentation rate, the acetic acid production, the revelation of the 4MMP and the production of β-phenylethyl alcohol and its acetate were determined for 10 monosporic clones of each parent in laboratory fermentation experiments using the model medium supplemented with S-4-(4-méthylpentan-2-one)-L-cysteine (420 nmoles l^{-1}). Finally, three monosporic clones of each parent have been chosen on the basis of the previous criteria by comparison with the initial parental strains (data not shown). Hybrids of homothallic yeast were obtained by the «spore-to-spore» mating method using the micromanipulator` (NAUMOV *et al*., 1986). Nine hybrids, H1 to H9, were obtained.

III - GENETIC CHARACTERIZATION OF THE HYBRIDS H1 TO H9

When two yeast cells are crossed, the zygote obtained contains the nuclear material from both parents. The nuclear chromosomes are transmitted equally to the daughter cells. Many authors in previous works have shown that electrophoretic karyotyping and *MET2* gene PCR-RFLP analysis can be used to differentiate *S. cerevisiae* and *S. bayanus* (CARDINALLI and MARTINI, 1994 ; NAUMOV *et al*., 1993 ; KISHIMITO and GOTO, 1995 ; MASNEUF *et al*.,1996). As it was previously described (GUIDICI *et al*., 1998 ; KISHIMOTO, 1994 ; MARINONI *et al*., 1999 ; MASNEUF *et al*., 1998), the karyotypes of the nine hybrids were found to be a combination of the DNA bands of the parental strains. The three parental *S. bayanus* var. *uvarum* monosporic clones used in hybridisation experiments displayed identical karyotypes whereas the karyotypes of the three parental *S. cerevisiae* monosporic clones were found different (data not shown). As consequence, three combination were found among the hybrid strains. The strains H1, H6, H7 exhibited the same karyotypes whereas the strains H2, H5, H8 and H3, H4, H9 displayed different patterns (figure 1). For the PCR-RFLP analysis, an *Eco*RI and a *Pst*I restriction fragment patterns of three bands were obtained for the 9 strains, with the same intensity and the same length as those obtained for *S. cerevisiae* with *Eco*RI and for *S. bayanus* var. uvarum with *Pst*I. The restriction patterns confirmed the hybrid nature of all 9 strains (figure 2).

IV- FERMENTATIVE PROPERTIES OF THE HYBRIDS H1 TO H9

The hybrids and their parents were tested on the model medium for their aptitude to release the 4MMP from S-4-(4-methylpentan-2-one)-L-cysteine and their production of β-phenylethyl alcohol and its acetate. At mid-fermentation, strain implantation was verified by electrophoretic karyotyping. The results of the wine analysis are presented in the table III. According to pre-

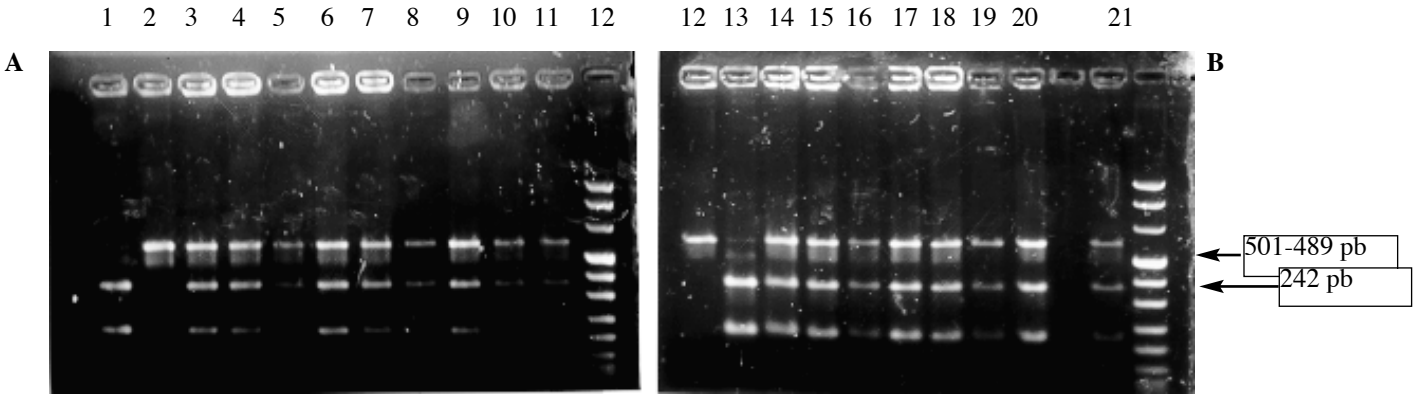

Fig. 2 - RFLP on PCR-amplified *MET2* gene fragment. A : Lane 1 to 11 : restriction analysis with *Eco*RI. B : Lane 12 to 21 : restriction analysis with *Pst*I. Lane 1 and 12: *Saccharomyces cerevisiae* type strain 1171 ; lane 2 and 13 : *Saccharomyces bayanus* VKM Y- 1146 ; lane 3 to 11 and 14 to 21: hybrids H1 to H9 (except H8 for *Pst*I) ; M : molecular weight marker (marker VIII from Boerhinger Mannheim).

Fig. 2 - Electrophorèse en gel d'agarose des fragments amplifiés du gène *MET2* par PCR-RFLP. A : Pistes 1 à 11 : restriction avec *Eco*RI. B : pistes 12 à 21 : restriction avec *Pst*I. Pistes 1 et 12: souche type *Saccharomyces cerevisiae* 1171 ; pistes 2 et 13 : souche type *Saccharomyces bayanus* VKM Y- 1146 ; pistes 3 à 11 et 14 à 21: hybrides H1 à H9 (à l'exception de H8 pour *Pst*I) ; M : marqueur de poids moléculaire (marqueur VIII de Boerhinger Mannheim).

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Table III - Production of 4MMP, β-phenylethyl alcohol and β-phenylethyl acetate by interspecies hybrids and their parents *S. cerevisiae* VL3c and *S. bayanus* var. *uvarum* P3.

Production de 4MMP, phényl-éthanol et acétate de phényl-éthyle par les hybrides interspécifiques et leurs parents *S. cerevisiae* VL3c et *S. bayanus* var. *uvarum* P3.

	VL3c	H1	H2	H3	H4	H5	H6	H7	H8	H9	P3
4-mercapto-4-methylpentan-2-one ng l^{-1}	2034	5596	3593	3148	3652	2448	2188	1948	6048	5606	6384
β-phenylethyl alcohol mg l^{-1}	11	21	15	20	35	12	21	20	10	13	115
β-phenylethyl acetate mg l^{-1}	0.5	1.5	0.8	0.4	1.8	0.7	0.4	0.2	0.1	0.4	1.9

Table IV - Production of 4MMP, 3MH, β-phenylethyl alcohol, β-phenylethyl acetate, ethanol and volatile acidity by the hybrid strain H9 and different *S. cerevisiae* strains (experimental test in cellar, vintage 1999).

Production de 4MMP, 3MH, phényléthanol, acétate de phényl-éthyle, éthanol et acidité volatile par la souche hybride H9 et différentes souches *S. cerevisiae* (expérimentations menées en caves, millésimes 1999).

ng l^{-1}	Bordeaux 1	Bordeaux 2	Pessac -Léognan	Graves	Average
4-mercapto-4-methylpentan-2-one					
H9	23	16	17	22	19,5 a(1)
VL3c	12	12	12	10	11,5 b
EG8	8	9	15	8	10 b
VL1	6	2	7	6	5,25 c
3-mercaptohexan-1-ol					
H9	1715	3463	587	780	1636
VL3c	2161	3261	413	991	1706
EG8	2894	4581	460	1135	2267
VL1	2077	2227	305	1457	1516
PE (ng.l^{-1})					
H9	47	46	92	25	52,5 a
VL3c	14	19	40	10	20,75 b
EG8	13	19	42	13	21,75 b
VL1	18	12	38	10	19,5 b
APE (ng.l^{-1})					
H9	1,60	2,00	2,25	1,00	1,71 a
VL3c	0,37	0,40	0,74	0,32	0,46 b
EG8	0,40	0,75	1,08	0,34	0,64 b
VL1	0,64	0,42	0,92	0,50	0,55 b
Éthanol production (% by vol)					
H9	11,20	11,10	12,40	12,55	11,81
VL3c	11,45	11,10	12,80	12,60	11,99
EG8	11,65	11,40	12,55	12,50	12,02
VL1	11,65	10,85	12,65	12,70	11,96
V. A. g l-1 H_2SO_4					
H9	0,15	0,17	0,15	0,20	0,17 b
VL3c	0,22	0,16	0,20	0,21	0,20 b
EG8	0,37	0,26	0,35	0,33	0,33 a
VL1	0,18	0,14	0,17	0,21	0,17 b

(1)Values followed by different letters are statistically different (Newman-Keuls test)

vious reports (KISHIMOTO, 1994 ; SHINOHARA *et al*., 1994), the quantity of β-phenylethyl alcohol and β-phenylethyl acetate produced by the hybrids obtained in this study are intermediate compared to their *S. cerevisiae* and *S. bayanus* var. *uvarum* parents, but closer to the amount produced by their *S. cerevisiae* parent. The amounts of 4MMP in wines fermented by the hybrids depended on the crossing combinations ; however they were twice or three times higher comparing to the quantity of 4MMP liberating by the parent *S. cerevisiae* for the hybrids H1, H8 and H9. The best fermentative properties, such as low production of volatile acidity and complete alcoholic fermentation, were obtained for H9 (data not shown). This hybrid was retained for industrial production under a dry form and was compared with *S. cerevisiae* strains in 1999 experimental tests in cellar from different Bordeaux vineyards. At mid-fermentation, the strain implantation was verified by electrophoretic karyotyping. The amounts of 4MMP, 3MH, β-phenylethyl alcohol and its acetate, ethanol and volatile acidity obtained for each strain are reported in the table IV. The quantity of 4MMP for H9 is statistically different from the quantity of 4MMP produced by VL3c and EG8, yeast strains which were previously described with a high aptitude to release 4MMP from its precursor (MURAT *et al*., 2001). Those results are in accordance with the data obtained in model medium with the S-4-(4-methylpentan-2-one)-L-cysteine precursor (table III). However, there was no significant variation in the 3MH contents of wines fermented with the different yeast strains. This fact can be explained by the strong effect of the must origin on the amount of 3MH in the wines which mask the strains effect. The increase in the concentration of the 4MMP may have a real olfactory impact in the wine considering the very low perception thresholds of the 4MMP (0.8 ng l^{-1} in model solution, (DARRIET *et al*., 1995)). The quantity of β-phenylethyl alcohol and its acetate in the wines fermented by the hybrid is significantly higher comparing with the amounts obtained with *S. cerevisiae* strains but below their threshold in model medium, 200 mg l^{-1} and 3 mg l^{-1} respectively (SHINOHARA, 1984). No statistical differences were found concerning the ethanol production by the yeast strains whereas the volatile acidity amount of the wines fermented by EG8 was significantly higher than the other strains.

In this study, we clearly showed, for the first time, that *S. bayanus* var. *uvarum* strains and hybrids *S. cerevisiae* x *bayanus* var. *uvarum* present a high ability to release the volatile thiols from their natural precursors. Irrespective to the origin of the must, one strain H9 was able to produce high amount of 4MMP without producing excess amount of the β-phenylethyl alcohol and its acetate. Our work represents a new and original example of the use of the yeast *S. bayanus* var. *uvarum* as a new genetic resource in breeding programs. The yeast S-cysteine conjugate released mechanisms should be elucidated further as well as the high liberating ability of some *S. bayanus* var. *uvarum* strains.

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