Influence of temperature and pH on *Saccharomyces bayanus* var. *uvarum* growth; impact of a wine yeast interspecific hybridization on these parameters

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Abstract

The species *Saccharomyces bayanus* var. *uvarum* possesses interesting enological characteristics but produces high concentration of volatile fermentative compounds not desirable in Sauvignon blanc wines. Interspecific hybrids between *Saccharomyces cerevisiae* and *S. bayanus* var. *uvarum* were made in order to join the main parental advantages. Two hybrids were selected on the basis of their fermentation characteristics and their karyotypes, i.e. they have a different mitochondrial DNA. In order to produce these hybrids as active dry yeast to be used as starter in winemaking, their optimal environmental conditions for growth, i.e. temperature and pH, were determined as the objective of our work. Using a two-level factorial design it was found that the two parental strains have different optimal temperature but for the two strains, pH does not have a significant influence on growth. The influence of temperature on biomass productivity for hybrid strains were strictly identical, so we suppose that the main genes coding for temperature sensitivity were not contained in mitochondrial DNA, but in nuclear DNA. Moreover the reactions of hybrid strains to the temperature variations were similar to the one of *S. bayanus* var. *uvarum*. This latter strain could have a majority of genes responsible of temperature sensitivity dominant in comparison with those of the strain *S. cerevisiae*.

Keywords: *Saccharomyces bayanus* var. *uvarum*; *Saccharomyces cerevisiae*; Temperature; Growth; Interspecific hybrid; Wine yeast

1. Introduction

Saccharomyces bayanus var. *uvarum* is considered as a cryotolerant yeast in winemaking (Kishimoto and Goto, 1995; Naumov, 1996; Rainieri et al., 1998). Cryotolerant strains possess a number of advantages compared with non-cryotolerant strains. They are used as starters in winemaking from low-acid musts since they synthesize malic acid and succinic acid (Rainieri et al., 1998), inhibit malolactic fermentation (Caridi and Corte, 1997) and produce more glycerol than *Saccharomyces cerevisiae* and less acetic acid and

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ethanol (Bertolini et al., 1996; Castellari et al., 1994). The use of these yeasts makes it possible to decrease the quantity of sulphites added to stabilize wines (Caridi and Corte, 1997), a considerable advantage for sulphite-sensitive consumers. *S. bayanus* var. *uvarum* strains were also described by different authors as producing high concentration of volatile fermentative compounds such as β-phenylethanol and its acetate. Masneuf et al. (1996) showed that these strains are present in the spontaneous fermentation of various white variety musts from the Val de Loire area. Those compounds are not suitable in Sauvignon blanc wines because they can mask the characteristic aroma of this variety. Thus, the use of a pure culture of *S. bayanus* var. *uvarum* for Sauvignon blanc winemaking is unsuitable. However, because they possess interesting enological characteristics, these strains were retained for an interspecific hybridization with a strain of the species *S. cerevisiae*, the best wine yeast. The hybrids *S. bayanus* var. *uvarum* × *S. cerevisiae* obtained were satisfactory with intermediary enological characteristics to those parental strains. Their use for winemaking needs their industrial production as active dry yeast.

The main aim of this work was to determine the environmental conditions, i.e. temperature and pH, for an optimal production of these hybrid strains. However, before characterizing hybrid strains, we took interest in the study of the influence of environmental conditions on the parental strains growth. In a first step, we determined the optimal conditions (temperature and pH) for parental strains growth by using a two-level factorial design. Differences of results between the two parental strains were discussed. In a second step these results were compared with those obtained for the two hybrid strains.

2. Materials and methods

2.1. Yeast strain

All strains (Table 1) are maintained in the collection of the Faculté d'Oenologie de Bordeaux. *S. cerevisiae* 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 must (Murat et al., 2001). *S. bayanus* var. *uvarum* P3 was isolated from spontaneous fermentation of Sauvignon blanc must in Val de Loire and Bordeaux (Masneuf et al., 1998). The two hybrid strains were obtained in the laboratory by crossing spores of monosporic clones from homothallic yeasts P3 and VL3c (Masneuf et al., 2002). The hybrid strain H9 has a mitochondrial DNA predominantly *S. bayanus* var. *uvarum* while the hybrid strain 14a has a mitochondrial DNA predominantly *S. cerevisiae*.

Table 1
Yeast strains used in this study

Strains designation	Source	Species
VL3c	Faculté d'Oenologie de Bordeaux	*S. cerevisiae*
P3	Faculté d'Oenologie de Bordeaux	*S. bayanus* var. *uvarum*
H9	Faculté d'Oenologie de Bordeaux	Hybrid
14a	Faculté d'Oenologie de Bordeaux	Hybrid

2.2. Culture media

A synthetic medium contained [g l^{-1}]: glucose monohydrated, 50; ammonium sulphate, 15; KH_2PO_4, 7; $CaCl_2 \cdot 2H_2O$, 2.5; NaCl, 0.5; $MgCl_2 \cdot 6H_2O$, 6 and yeast extract (Oxoid) 1. Ammonium sulphate and the other components of the medium were separately heat-sterilized (121 °C, 20 min) to avoid the Maillard reaction, then mixed and complemented with 10 ml l^{-1} of a sterile vitamin and mineral solution. The vitamin solution had the following composition [mg l^{-1}]: biotin, 3; calcium pantothenate, 40; inositol, 250; pyridoxine HCl, 50; thiamine HCl, 100; and the mineral solution contained [mg l^{-1}]: FeSO4 · $7H_2O$, 556; $ZnSO_4 \cdot 7H_2O$, 576; $CuSO_4 \cdot 5H_2O$, 14; $Na_2MoO_4 \cdot 2H_2O$, 50; $CoCl_2 \cdot 6H_2O$, 50; $MnSO_4 \cdot H_2O$, O, 338; H_2SO_4, 10 drops.

2.3. Culture conditions

Precultures were grown in shake-flask on synthetic medium at 28 °C, 250 rpm. These cultures were used to inoculate batch cultures which were run in 2-l bioreactor (Sétric Set 3M, Toulouse, France) stirred at 200 rpm. Temperature and pH were, respectively, controlled from

21.5 to 38 °C and from 3.3 to 5.5 depending on the experiment and the strain used. The pH control was managed by adding 1 M sodium hydroxide solution.

2.4. Experimental design

2.4.1. Factors and experimental domain

Two factors were studied: pH and temperature which were seen as key parameters (Prescott et al., 1995). For each factor, three equidistant levels were chosen. The coded values were: −1, 0 and +1.

2.4.2. Responses

Response to variations in pH and temperature was quantified by biomass productivity (Z1, g l^{-1} h^{-1}) and maximal specific growth rate (Z2, h^{-1}). The biomass productivity was defined as the difference between maximal and initial biomass concentration divided by the time needed for obtaining maximal concentration.

2.4.3. Model

A priori a first-order model was proposed to fit the responses Z1 and Z2 as a function of the different factors in coded units. This model took into account the main effect of each factor (a_1 and a_2) and was written as:

$$Z = A + a_1 \cdot \mathrm{pH_C} + a_2 \cdot T_C$$

where $\mathrm{pH_c}$ and T_c were the coded values of each variable.

2.4.4. Experimental design

A two-level factorial design was chosen to analyze simultaneously the effect of two growth parameters, pH and temperature, to optimize cell production (Schimmerling et al., 1998). The experimental design limited the number of experiments. In this case, it needed the realization of 7 experiments defined by coded values of the two parameters: 3 center points of coded values (0, 0) allowing the estimation of experimental variance and 4 factorial points. The calculation of the experimental conditions was made with a step of 0.71 for pH and 3.55 for temperature, according to the following equations:

$$\mathrm{pH} = \mathrm{pH_0} + 0.71\mathrm{pH_C}$$

$$T = T_0 + 3.55T_C$$

where $\mathrm{pH_0}$ and T_0 are the experimental values assigned to center points and $\mathrm{pH_C}$ and T_C represent the coded value of each parameter. Table 2 shows the experimental design allowing the measurement of Z1 and Z2.

Regression coefficients were estimated by the standard least-squares method, with a confidence level higher than 95%. The coefficient of multiple correlations R^2 indicates the quality of the multivariable linear regression. The Durbin-Watson's value (D_w) is a test for first-order serial correlation in the residuals of a time series regression. A value superior to 1.4 for the Durbin-Watson's statistic indicates that there is no serial correlation. Then, the Fisher's value allows determining the independence of the experimental variables. At last, the Student's test is used to check the statistic meaning of each independent variable.

2.5. Biomass evaluation

Two methods were used to estimate the biomass growth in the cultures: optical density and dry weight.

2.5.1. Optical density

Samples were poured into 1-cm optical length spectrometric cuvets (Alliance Concept, Toulouse, France). The optical density of yeast suspensions was measured with a spectrophotometer (Anthelic Advanced; Secoman, Toulouse, France) at 620 nm. A linear regression between optical density and biomass concentration was observed for a measured optical density range of 0.1 to 0.8. For higher values, samples were diluted with distilled water. We defined the optical density as the measured optical density value multiplied by the dilution factor.

Table 2
Experimental design levels of temperature (T) and pH

Run no.	Design matrix		Work matrix	
	T (°C)	pH	T (°C)	pH
1	1	1	28.5	4.7
2	1	−1	28.5	3.3
3	−1	1	21.5	4.7
4	−1	−1	21.5	3.3
5	0	0	25.0	4.0
6	0	0	25.0	4.0
7	0	0	25.0	4.0

Table 3
Correlation between biomass concentration (y) and optical density (x) for the strains used

	Slope	R^2
S. bayanus var. *uvarum* P3	0.2741	0.9911
S. cerevisiae VL3c	0.2631	0.9997
Hybrid H9	0.2323	0.9981
Hybrid 14a	0.2408	0.9921

2.5.2. Dry weight

For measuring the dry weight (biomass concentration), 10 ml of culture samples were centrifuged for 10 min at 545 g. The cells were washed twice with distilled water to eliminate compounds of the culture medium and then dried using a humidity analyzer (model HA60; Precisa, Zurich, Switzerland). The linear regression of biomass concentration (y) versus optical density (x) was given by the equations, according to the strain used (Table 3).

3. Results

The aim of this study was to determine the optimal temperature and pH conditions for the production of hybrids *S. cerevisiae* × *S. bayanus* var. *uvarum*. The first step was to analyze the behaviour of *S. bayanus* var. *uvarum*, being of interest for winemaking, which is not as well known as *S. cerevisiae*.

3.1. Influence of the factors on biomass productivity for the S. bayanus var. uvarum strain

The predictive first-order model for the productivity was:

$$Z1 = 9.6.10^{-2} + 1.7.10^{-2}T_C + 8.3.10^{-4}\mathrm{pH}_C$$

The R^2 coefficient of determination of this fitting model was 0.89 indicating that a high proportion of variability was explained by the model. For the 3 center points, we measured a productivity of 0.090, 0.091 and 0.090 g l^{-1} h^{-1}. Thus, we noted a good reproducibility, that is an important factor for the validation of the first-order model. For the Fisher's test, the variance analysis returned a value of 15.8 which was widely superior to the critical value F_C (variables: 2; degrees of freedom: 4; risk: 0.05)=6.94 (Lacaze et al., 1997). So the established correlation was significant, in other words the relation observed between the dependant variable (productivity) and the independent variables (T and pH) was not due to the hazard. The Durbin-Watson's statistic was 1.6, superior to 1.4, indicating that there was no serial correlation between residues. This result was foreseeable because all the experiments were realized with the same protocol and the same culture medium. So all the parameters were controlled and only the study factors changed.

Fig. 1 represents the productivity of *S. bayanus* var. *uvarum* as a function of temperature and pH. Throughout the experimental design, temperature was the factor which had the main influence on the response. It had a high positive effect, i.e. productivity increased when temperature increased. The pH was less important since if we modified its value with the temperature fixed to its mean level, we could observe a weak variation of productivity (0.0944 and 0.0967 g l^{-1} h^{-1} for pH values of 3 and 5, respectively). These results were confirmed by the Student's test: for the temperature factor, the Student's t value was 5.61 whereas for the pH factor it was 0.27. If the Student's t value was superior to the critical value (=2.77, Lacaze et al., 1997), the considered criterion was a significant variable in the model estimation. So we could conclude that the temperature had a real influence on the productivity of the strain *S. bayanus* var. *uvarum* contrary to the pH, in the studied range.

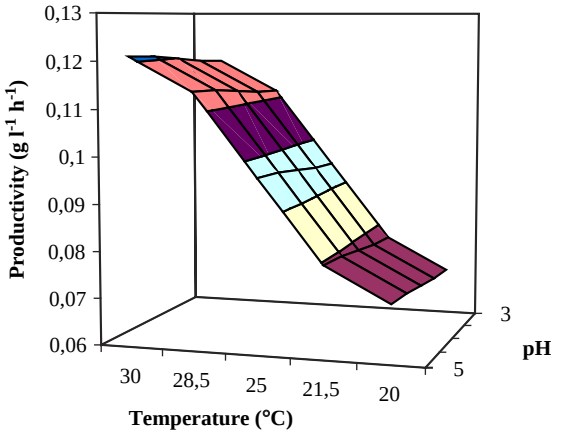

Fig. 1. Response surface of *S. bayanus* var. *uvarum* for the productivity factor (defined as the difference between maximal and initial biomass divided by the time needed to obtain maximal biomass).

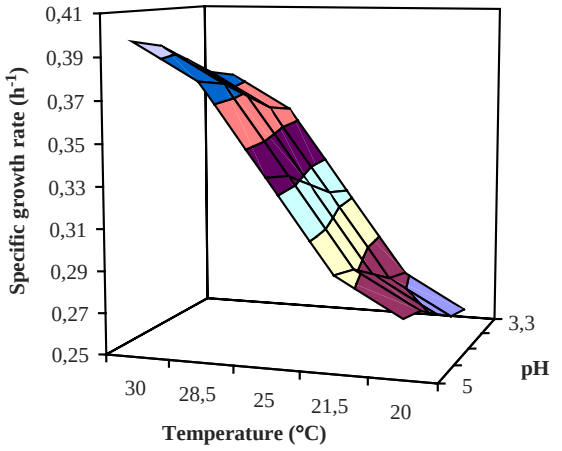

Fig. 2. Response surface of *S. bayanus* var. *uvarum* for the maximal specific growth rate factor.

3.2. Influence of the factors on the maximal specific growth rate for the S. bayanus var. uvarum strain

The predictive first-order model for the maximal specific growth rate was:

$$Z2 = 3.2.10^{-1} + 4.2.10^{-2}T_C + 8.5.10^{-3}pH_C$$

The R^2 coefficient of determination of this fitting model was 0.95 indicating that a high proportion of variability was explained by the model. For the 3 center points, we measured a maximal specific growth rate of 0.334, 0.335 and 0.335 h^{-1}. We noted a good reproducibility. For the Fisher's test, the variance analysis returned a value of 35.32 which was widely superior to the critical value F_C (2; 4; 0.05)=6.94. So the established correlation was significant. The Durbin-Watson's statistic was 1.7, superior to 1.4, indicating that there was no serial correlation between residues.

Fig. 2 shows the maximal specific growth rate of *S. bayanus* var. *uvarum* as a function of temperature and pH. Like previously, throughout the experimental design, temperature was the factor which had the main influence on the response. The pH was less important since if we modified its value with the temperature fixed to its mean level, we could observe a weak variation of maximal specific growth rate (0.3137 and 0.3377 h^{-1} for pH values of 3 and 5, respectively). These results were confirmed by the Student's test: for the temperature factor, the Student's t value was 8.24 whereas for the pH factor it was 1.65. So we could conclude that temperature had a real influence on the maximal specific growth rate of the strain *S. bayanus* var. *uvarum* contrary to pH, in the studied range.

The specific growth rate could be expressed as a function of the temperature by the Arrhenius relation:

$$\log\mu = \log K - \frac{E_a}{2.3 \times R}\left(\frac{1}{T}\right)$$

where E_a: activation energy (cal mol^{-1}); K: attempt frequency (h^{-1}); R: Boltzmann's constant (1.99 cal mol^{-1} K^{-1}); T: temperature (K).

The representation of 'log μ' as a function of '1/T' allows to determine the activation energy needed by the yeast cells for the growth to start. Fig. 3 shows the activation energy of the strain *S. bayanus* var. *uvarum* for different pH values. According to pH values of the culture medium, the strain *S. bayanus* var. *uvarum* exhibited activation energy of 6.3 to 6.8 kcal mol^{-1} for a range of temperature of 20 to 30 °C. The lower the pH the higher the demand of the metabolic reactions of yeast cells for activation energy. However, these activation energy variations as a function of pH were not visible on the productivity or the maximal specific growth rate.

3.3. The optimal temperature for S. bayanus var. uvarum growth

The experimental design showed that pH did not have a significant influence on the *S. bayanus* var. *uvarum* growth. Moreover the response surface did not provide the optimal temperature, but only a line of

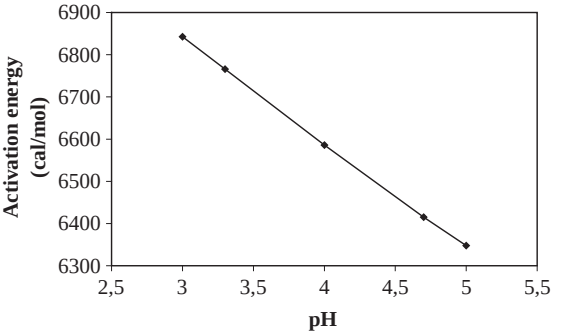

Fig. 3. Influence of pH on the activation energy for *S. bayanus* var. *uvarum* growth (derived from log μ as a function of 1/T).

bigger slope, indicating guidance for this value. So the determination of optimal temperature was continued, but without an experimental design. For all the experiments, the pH was kept constant at 4 whereas the temperature varied in the range 30 to 35 °C. Table 4 shows all the responses for the different temperatures. The optimal temperature for *S. bayanus* var. *uvarum* growth was 29–30 °C. Beyond 30 °C the productivity and the maximal specific growth rate decreased clearly because of a growth stop more precocious than for inferior temperatures.

3.4. The optimal temperature for the S. cerevisiae strain growth. Comparison of the two parental strain growth

A similar two-level factorial design was conducted with the second parental strain VL3c: a *S. cerevisiae* strain. Temperature and pH varied in the range 25 to 35 °C and 3.5 to 5.5, respectively. The predictive first-order models for the productivity and the maximal specific growth rate were:

$$Z1 = 2.2.10^{-1} + 3.9.10^{-2}T_C + 8.7.10^{-3}\mathrm{pH}_C$$

$$Z2 = 6.1.10^{-1} + 7.3.10^{-2}T_C + 2.8.10^{-2}\mathrm{pH}_C$$

The R^2 coefficients of determination of these fitting models were 0.968 and 0.935 indicating that a high proportion of variability was explained by the model. For the 3 center points, we measured a productivity of 0.227, 0.222 and 0.223 g l^{-1} h^{-1} and a maximal specific growth rate of 0.334, 0.335 and 0.335 h^{-1}. We noted a good reproducibility. For the Fisher's test, the variance analysis returned values of 61.12 and 28.7, respectively for productivity and maximal specific growth rate fitting models, which were widely superior to the critical value F_C (2; 4; 0.05)=6.94. So the established correlation was significant. The Durbin-Watson's statistics were 2.02 and 2.3, superior to 1.4, indicating that there was no serial correlation between residues.

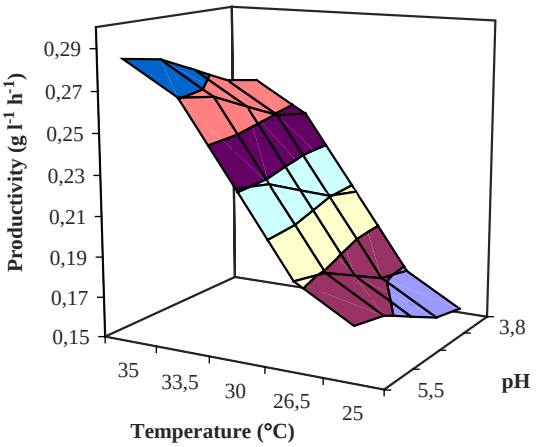

Fig. 4. Response surface of *S. cerevisiae* for the productivity factor (see legend of Fig. 1).

Fig. 4 represents the productivity of *S. cerevisiae* as a function of temperature and pH. Like previously, throughout the experimental design, the temperature was the factor which had the main influence on the response. The pH was less important. These results were confirmed by the Student's test: for the temperature factor, the Student's t value was 10.79 whereas for the pH factor it was 2.42.

The experimental design showed that pH did not have a significant influence on the *S. cerevisiae*

Table 4
Influence of growth temperature on biomass productivity (see legend of Fig. 1) and maximal specific growth rate of *S. bayanus* var. *uvarum* batch culture

Temperature (°C)	Productivity (g l^{-1} h^{-1})	Maximal specific growth rate (h^{-1})
21.5	0.083	0.277
25.0	0.090	0.335
28.5	0.117	0.362
30.0	0.119	0.481
31.5	0.086	0.423
33.0	0.081	0.396
35.0	0.064	0.386

Table 5
Influence of growth temperature on biomass productivity (see legend of Fig. 1) and maximal specific growth rate of *S. cerevisiae* batch culture

Temperature (°C)	Productivity (g l^{-1} h^{-1})	Maximal specific growth rate (h^{-1})
25.0	0.153	0.5065
26.5	0.177	0.5366
30.0	0.219	0.6101
33.5	0.255	0.6836
35.0	0.254	0.7089
36.0	0.235	0.6851
38.0	0.169	0.5624

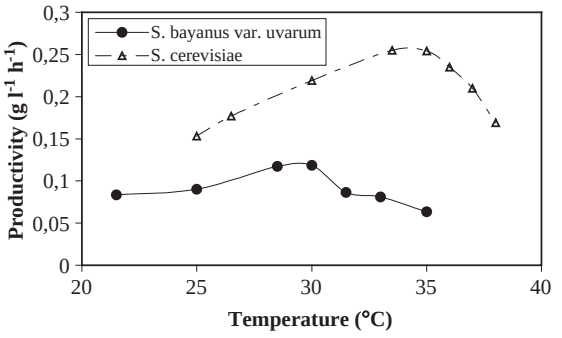

Fig. 5. Influence of temperature on the biomass productivity for *S. bayanus* var. *uvarum* and *S. cerevisiae* growth (see legend of Fig. 1).

growth. Moreover the response surface did not provide the optimal temperature. So the determination of optimal temperature was continued, but without an experimental design. For all experiments, the pH was kept constant at 5 whereas the temperature has varied between 35 and 40 °C. Table 5 shows all the responses for the different temperatures. The optimal temperature for *S. cerevisiae* growth was 33–34 °C.

For comparison, Fig. 5 shows the influence of temperature on the biomass productivity for the two parental strains growth and Fig. 6 represents the growth activation energy of these strains for different pH values.

3.5. Influence of temperature on the interspecific hybrids growth

Crossing of strains *S. bayanus* var. *uvarum* P3 and *S. cerevisiae* VL3c allowed to obtain many hybrids selected on their fermentation characteristics (Massneuf et al., 2002). The influence of temperature on two hybrids growth was studied in the same way that for the parental strains. For all the experiments, the pH was kept constant at 4 whereas the temperature varied in the range 25 to 38 °C in order to surround the optimal temperatures of parental strains.

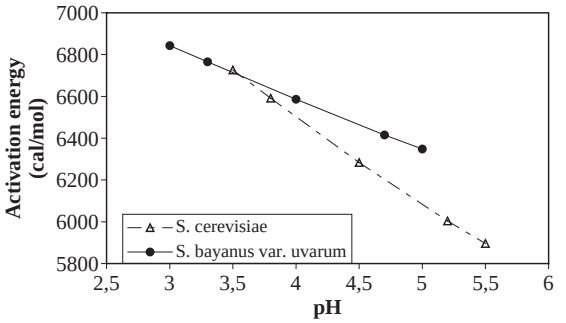

Fig. 6. Influence of pH on the growth activation energy of the two parental strains (derived from log μ as a function of $1/T$).

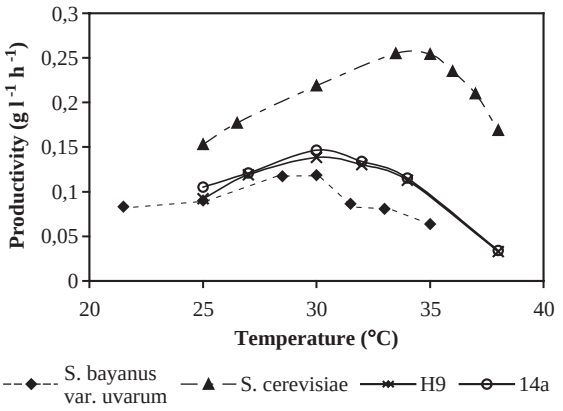

Fig. 7. Influence of temperature on the biomass productivity for hybrid and parental strains (see legend of Fig. 1).

The productivity profiles obtained for the two hybrid strains were strictly identical (Fig. 7). The hybrid strains had their optimum growth at 30 °C, like the parental strain *S. bayanus* var. *uvarum*.

4. Discussion

Several criteria, such as process characteristics, composition of growth medium and environmental conditions are important when producing yeast strains for winemaking. In this study, the environmental conditions, i.e. temperature and pH, for an optimal production of hybrids *S. cerevisiae* × *S. bayanus* var. *uvarum* as active dry yeast were studied. In a first part the influence of temperature and pH on growth of parental strains was studied by using a two-level factorial design. In a second part these results were compared with those obtained for two hybrid strains.

Temperature was the factor which had the main influence on the yeast growth. For the two parental strains, we observed a same temperature profile: below the optimal temperature, the responses, i.e. productivity and maximal specific growth rate, increase progressively whereas after the optimal temperature the fall of responses is fast. Prescott et al.

(1995) reported a same profile for microorganisms, i.e. an optimal temperature nearer to maximum than minimum growth temperature. Indeed enzymes are very sensitive to temperature variations. Pelmont (1995) observed a characteristic break in the Arrhenius graph applied to enzymatic activity, indicating a variation of activation energy after a definite temperature. For low temperatures the enzymatic kinetics suffer of inactivation; the result is a slow advance of growth before optimal temperature. Moreover, their activities and so the one of global metabolism are favoured by higher temperatures, but beyond a definite temperature, the least supplementary degree damages irremediably the cellular membranes and denatures the enzymes. These changes generate a fast fall of cellular development.

Globally, the parental strains reacted in a same way to temperature (Fig. 5). However a more detailed observation of the sensitivity of each strain to low temperature allowed to see real differences. The productivity of the strain *S. bayanus* var. *uvarum* decreased by 25% between 30 and 25 °C then becomes stable for inferior temperatures whereas productivity of the strain *S. cerevisiae* falls continually (loss of 13% and 40%, respectively, between 35 and 30 °C and between 30 and 25 °C). The strain *S. bayanus* var. *uvarum* is less sensitive to temperature falls than the strain *S. cerevisiae*, this is in agreement with the classification of this species as a cryophilic yeast by Naumov (1996).

Some differences of the yeast strains sensitivities to pH appear using the Arrhenius relation (Fig. 6). According to pH values of the culture medium, the activation energy of the strains *S. cerevisiae* and *S. bayanus* var. *uvarum* varied in the range 5.9 to 6.8 kcal mol^{-1} for a range of temperature 20 to 35 °C. These scales are in agreement with the values generally found in the literature for yeasts: activation energy equals 6 to 8 kcal mol^{-1} if the system is limited by a diffusion process, and activation energy is superior to 12 kcal mol^{-1} in the case of a limitation by the biological reaction (Topiwala and Sinclair, 1971). Globally, for a definite pH value, the strain *S. cerevisiae* needs less energy than the strain *S. bayanus* var. *uvarum* to activate its own development, so its growth was faster and its maximal specific growth rate was more important: in the optimal growth conditions (*T* and pH), *S. cerevisiae* grew at 0.68 h^{-1} whereas *S. bayanus* var. *uvarum* grew at 0.48 h^{-1}. However, a decrease in the pH value generates an increase of the activation energy more important for the strain *S. cerevisiae* than for the strain *S. bayanus* var. *uvarum* (415 cal mol^{-1}/unit of pH for *S. cerevisiae* but only 247 cal mol^{-1}/unit of pH for *S. bayanus* var. *uvarum*). In other words, the choice of the pH value for the yeast growth is more determining for the strain *S. cerevisiae* than for the strain *S. bayanus* var. *uvarum*. *S. bayanus* var. *uvarum* is less sensitive to acidification of the culture medium.

Hybrids of homothallic yeasts *S. cerevisiae* and *S. bayanus* var. *uvarum* were obtained by crossing spores of monosporic clones. Two hybrids were selected on the basis of their fermentation characteristics and their karyotypes: H9 had a mitochondrial DNA predominantly *S. bayanus* var. *uvarum* whereas 14a had a mitochondrial DNA predominantly *S. cerevisiae*. The aim of this crossing was to join together the best parental characteristics in a unique strain. In spite of their different mitochondrial DNA and of the different optimal temperatures for the parental strains growth, the productivity profiles of the two hybrid strains as a function of temperature were strictly identical. These results allowed to suppose that the main genes coding for temperature were not contained in mitochondrial DNA, but in nuclear DNA. Moreover the hybrid strains had their optimum temperature growth at 30 °C like *S. bayanus* var. *uvarum* parental strain. However when the temperature of the culture medium was superior to optimal temperature, the productivity decreases rapidly, like for the *S. cerevisiae* parental strain. These results were in accordance with those described in literature (Kishimoto, 1994; Zambonelli et al., 1997; Rainieri et al., 1999) for high temperatures: better performance of hybrid strains than *S. bayanus* var. *uvarum* parental strain for temperatures superior to 28 °C.

The hybrid strains reacted differently to the temperature variations than the parental strains, but globally, their reactions were similar to the one of *S. bayanus* var. *uvarum*. Hybrids have inherited of all nuclear chromosomes of the two parental strains. The genes responsible for the temperature sensitivity are certainly numerous considering that all the enzymatic reactions are thermosensitives. Because the hybrid strains behaviour was not intermediate to those of the parental strains, it is allowed to suppose that a

majority of these genes were dominant in the strain *S. bayanus* var. *uvarum* in comparison with those of the strain *S. cerevisiae*. This can explain a behaviour of the two hybrid strains close to the parental strain *S. bayanus* var. *uvarum* one, but not totally identical. The use of microarrays during batch cultures would be a good way to verify this hypothesis and to understand the response mechanics of these yeast strains to temperature variations.

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References

Bertolini, L., Zambonelli, C., Giudici, P., Castellari, L., 1996. Higher alcohol production by cryotolerant *Saccharomyces strains*. Am. J. Enol. Vitic. 47, 343–345.

Caridi, A., Corte, V., 1997. Inhibition of malolactic fermentation by cryotolerant yeasts. Biotechnol. Lett. 19, 723–726.

Castellari, L., Ferruzi, M., Magrini, A., Giudici, P., Passarelli, P., Zambonelli, C., 1994. Unbalanced wine fermentation by cryotolerant vs. non-cryotolerant *Saccharomyces strains*. Vitis 33, 49–52.

Kishimoto, M., 1994. Fermentation characteristics of hybrids between the cryophilic wine yeast *Saccharomyces bayanus* and the mesophilic wine yeast *Saccharomyces cerevisiae*. J. Ferment. Bioeng. 77, 432–435.

Kishimoto, M., Goto, S., 1995. Growth temperatures and electrophoretic karyotyping as tools for practical discrimination of *Saccharomyces bayanus* and *Saccharomyces cerevisiae*. J. Gen. Appl. Microbiol. 41, 239–247.

Lacaze, B., Mailhes, C., Maubourguet, M., Tourneret, J.Y., 1997. Probabilités et statistiques appliquées—Résumé de cours et illustrations. In: Cépaduès, B. (Ed.), Collection Polytech de l'INP de Toulouse. Cépaduès, Toulouse (355 pp.).

Masneuf, I., Aigle, M., Dubourdieu, D., 1996. Development of a polymerase chain reaction/restriction fragment length polymorphism method for *Saccharomyces cerevisiae* and *Saccharomyces bayanus* identification in enology. FEMS Microbiol. Lett. 138, 239–244.

Masneuf, I., Hansen, J., Groth, C., Piskur, J., Dubourdieu, D., 1998. New hybrids between *Saccharomyces* sensu stricto yeast species found among wine and cider production strains. Appl. Environ. Microbiol. 64, 3887–3892.

Masneuf, I., Murat, M.L., Naumov, G.I., Tominaga, T., Dubourdieu, D., 2002. Hybrids *Saccharomyces cerevisiae* × *Saccharomyces bayanus* var. *varum* having a high liberating ability of some sulfur varietal aroms of *Vitis Vinifera Sauvignon* blanc wines. J. Int. Sci. Vigne Vin. 36, 205–212.

Murat, M.L., Masneuf, I., Darriet, P., Lavigne, V., Tominaga, T., Dubourdieu, D., 2001. Effect of the *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wines. Am. J. Enol. Vitic. 52, 136–139.

Naumov, G.I., 1996. Genetic identification of biological species in the *Saccharomyces* sensu stricto complex. J. Ind. Microbiol. 17, 295–302.

Pelmont, J., 1995. Enzymes: Catalyseurs du Monde Vivant (Nouvelle Édition). Presses Universitaires de Grenoble, Grenoble (1039 pp.).

Prescott, M., Harley, J.P., Klein, D., 1995. Microbiologie. De Boeck université, Bruxelles (1014 pp.).

Rainieri, S., Zambonelli, C., Tini, V., Castellari, L., Giudici, P., 1998. The enological traits of thermotolerant *Saccharomyces strains*. Am. J. Enol. Vitic. 49, 319–324.

Rainieri, S., Zambonelli, C., Tini, V., Castellari, L., Giudici, P., 1999. Oenological properties of an interspecific *Saccharomyces hybrid*. S. Afr. J. Enol. Vitic. 20, 47–52.

Schimmerling, P., Sisson, J.C., Zaïdi, A., 1998. Pratique des plans d'expériences. Tec et Doc Lavoisier, Paris (526 pp.).

Topiwala, H., Sinclair, C.G., 1971. Temperature relationship in continuous culture. Biotechnol. Bioeng. 13, 795–813.

Zambonelli, C., Passarelli, P., Rainieri, S., Bertolini, L., 1997. Technological properties and temperature response of interspecific *Saccharomyces hybrids*. J. Sci. Food Agric. 74, 7–12.