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Unbalanced wine fermentation by cryotolerant *vs.* non-cryotolerant *Saccharomyces* strains

by

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S u m m a r y : Cryotolerant *Saccharomyces cerevisiae* p.r. *uvarum* and *bayanus* strains (fermentation temperature range 6-30 °C, T_{opt} < 30 °C) differ from ordinary non-cryotolerant strains for their ability to synthesize rather than decompose malic acid, higher glycerol and succinic acid production, lower acetic acid production and lower ethanol yield. Thanks to these traits, the fermentative action of cryotolerant strains modifies wine composition to a much more considerable extent than ordinary non-cryotolerant *Saccharomyces* strains.

K e y w o r d s : Saccharomyces, cryotolerance, wine composition, glycerol, acidic ratio.

Introduction

The ability to produce, anabolically or catabolically, different amounts of secondary fermentation compounds is generally a stable and hereditary trait in *Saccharomyces cerevisiae* strains. This fact has been demonstrated for the production of acetic acid (Giudici and Zambonelli 1992), higher alcohols (Giudici *et al.* 1990, 1993), glycerol (Eustace and Thornton 1987) and for action on malic acid generally decomposed with varying intensity or synthesized (Dakin 1924; Fatichenti *et al.* 1984; Schwartz and Radler 1988; Farris *et al.* 1989).

At more interesting enological levels, these traits are rarely found together in a single *S. cerevisiae* p.r. *cerevisiae* strain. However, the combination of very low acetic acid production and a capability of synthesizing malic acid with lower ethanol yield is found in cryotolerant *Saccharomyces* strains (Castellari *et al.* 1992a, b). Cryotolerant or cold-resistant strains are those that ferment well at between 6 and 30 °C (rather than between 12 and 36 °C) with an optimum < 30 °C. As Walsh and Martin (1977) showed, these cryotolerant *S. cerevisiae* strains belong more frequently to the p.r. *uvarum* group A (group B = non-cryotolerant), but also to p.r. *bayanus* (Castellari *et al.* 1992a).

This study sets out to determine differences in the composition of wine produced with normal *S. cerevisiae* p.r. *cerevisiae* or with cryotolerant p.r. *uvarum* and *bayanus* strains. The two kinds of strain were compared in a biometric study of their action on malic acid and on the amount of acetic acid, succinic acid and glycerol produced during must fermentation.

Materials and methods

O r g a n i s m s : We used 53 strains of cryotolerant *Saccharomyces* strains isolated from must after low temperature (2-4 °C) enrichment and 53 non-cryotolerant *Saccharomyces* strains randomly selected from our collection. The cryotolerant strains were *S. cerevisiae* p.r. *uvarum* group A (47 strains) or p.r. *bayanus* (6 strains) and have been described in a previous article (Castellari *et al.* 1992a). The non-cryotolerant strains were p. r. *cerevisiae* (47 strains) or group B p.r. *uvarum* (6 strains).

F e r m e n t a t i o n t e s t s : The fermentation tests were carried out in triplicate with the procedure followed by Eustace and Thornton (1987). A single crushing of *Vitis vinifera* cultivar Trebbiano grape juice was used throughout the biometric study. Grape juice (4 ml) was inoculated with 1 ml of a 24 h culture which had been incubated in liquid YPD medium (0.5% yeast extract, 0.5 % peptone, 2 % dextrose) at 25 °C. After 24 h incubation, 95 ml of sterilized grape juice was inoculated with the 5-ml culture and incubated semianaerobically (fermentation traps without shaking) at 25 °C. Fermentations were usually complete in 10-12 d. Total sugar depletion was assayed with a clinitest kit (Miles) and samples were taken and analyzed for malic, acetic and succinic acid, glycerol and ethanol.

Other fermentation tests were made with musts from different *Vitis vinifera* cultivars. These tests with a limited number of strains were made in 5 l containers of must and repeated three times.

A n a l y s i s : Malic acid, acetic acid, succinic acid, glycerol, ethanol and fermentable sugar were determined enzymatically (Bergmeyer *et al.* 1970) with specific Boehringer kits.

S t a t i s t i c a l a n a l y s i s : Differences between cryotolerant and non-cryotolerant strains for malic acid, acetic acid and glycerol production were tested by one way analysis of variance.

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Results

Biometric study

Malic acid: The amount of malic acid in wine at the end of the fermentation showed that cryotolerant strains are quite different from non-cryotolerant ones. Both p.r. *uvarum* and p.r. *bayanus* cryotolerant strains usually cause an increase in malic acid and the amount is yeast strain-dependent. From an initial level of 1.95 g/l, the max. increase noted was +0.86 and +1.04 g/l in strains 11719 and 12233, respectively. Only 4 strains gave a decrease in malic acid with a max. value of -0.15 g/l (strain 12244). Aside from the extremes, all the other strains showed an average increase of +0.41 g/l (see Fig. 1).

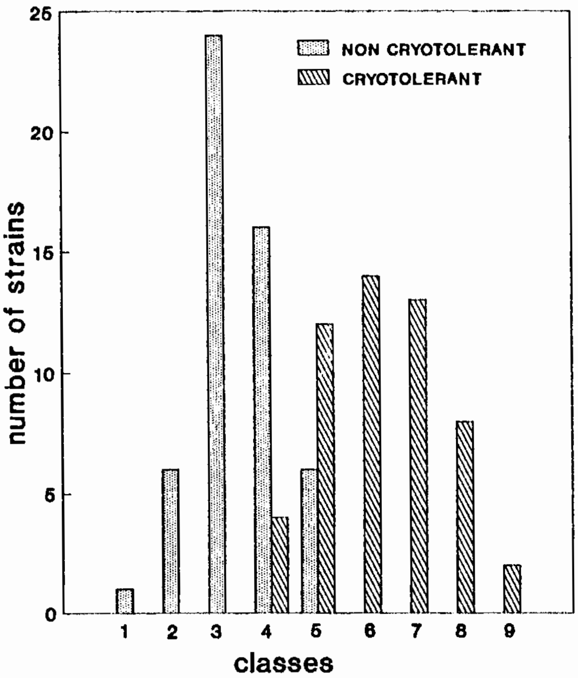

Fig. 1. Frequency histogram for the action on malic acid by cryotolerant and non-cryotolerant *Saccharomyces* strains. Level classes (g/l): 1= from 1.21 to 1.40; 2= from 1.41 to 1.60; 3= from 1.61 to 1.80; 4= from 1.81 to 2.00; 5= from 2.01 to 2.20; 6= from 2.21 to 2.40; 7= from 2.41 to 2.60; 8= from 2.61 to 2.80; 9= from 2.81 to 3.00. — Malic acid in must: 1.95 g/l.

The non-cryotolerant strains, independent of their p.r., also change the amount of malic acid, but this change was a decrease with only a few instances of a slight increase. Once again the variations are entirely yeast strain-dependent. Fig. 1 illustrates the situation between the two extremes of -0.63 and +0.12 g/l. The non-cryotolerant p.r. *uvarum* group B behaved in the same fashion as the p.r. *cerevisiae* strains with -0.59 and +0.02 g/l at the extremes.

Acetic acid: The acetic acid values at the end of fermentation show that cryotolerant strains are considerably different from the non-cryotolerant ones. Cryotolerant strains produce low amounts of acetic acid, usually < 100 mg/l (mean value 85.90 mg/l). Acetic acid production is yeast strain-dependent and, as shown in Fig. 2, can be divided into well distributed level classes.

The non-cryotolerant *S. cerevisiae* p.r. *cerevisiae* and group B p.r. *uvarum* strains produce higher amounts of acetic acid. Depending on the strain, acetic acid production goes from a low of 96.6 mg/l to a high of 880 mg/l as shown in the bar graph of Fig. 2.

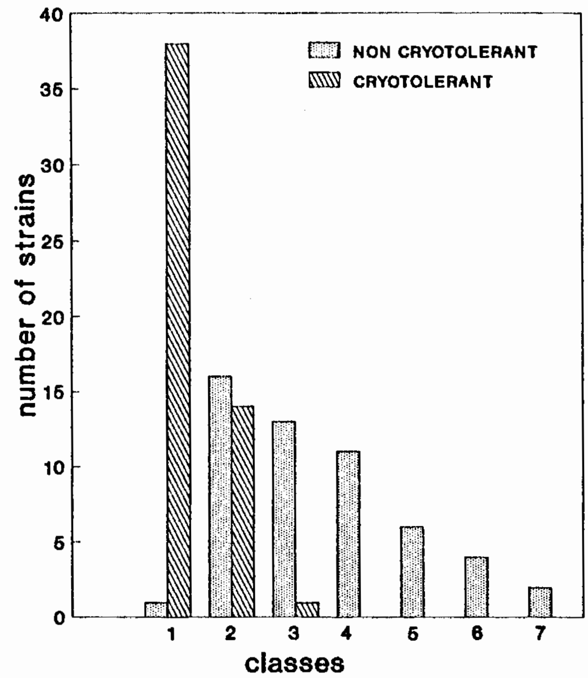

Fig. 2. Frequency histogram for acetic acid production by cryotolerant and non-cryotolerant *Saccharomyces* strains. Level classes (g/l): 1= up to 0.10; 2= from 0.11 to 0.20; 3= from 0.21 to 0.30; 4= from 0.31 to 0.40; 5= from 0.41 to 0.50; 6= from 0.51 to 0.60; 7= more than 0.60.

Glycerol: Cryotolerant strains produce higher amounts of glycerol than non-cryotolerant strains. The average amount of this compound in the wine was 5.765 and 4.916 g/l, respectively.

The trait is very variable with extremes at 4.10 and 7.60 g/l for cryotolerant and 3.52 and 6.32 g/l for non-cryotolerant strains. This trait is also strain-dependent and, as shown of Fig. 3, can be divided into well distributed level classes.

Succinic acid: Production of succinic acid by cryotolerant strains was very high. The amount produced in the wine ranged from 0.75 to 1.36 g/l, and average amount was 1.09 g/l. Non-cryotolerant strains produced much lower quantities of succinic acid: 0.37-0.80 g/l, and average of 0.53 g/l.

Both in cryotolerant and non-cryotolerant strains this trait is strain-dependent and can be divided into well distributed level classes (Fig. 4).

Ethanol: Cryotolerant strains, with the same amount of fermented sugar, produce less ethanol than non-cryotolerant strains. This lower alcoholic fermentation yield is about 3% since the mean values for the two groups of strains are 77.15 and 79.56 g/l, respectively.

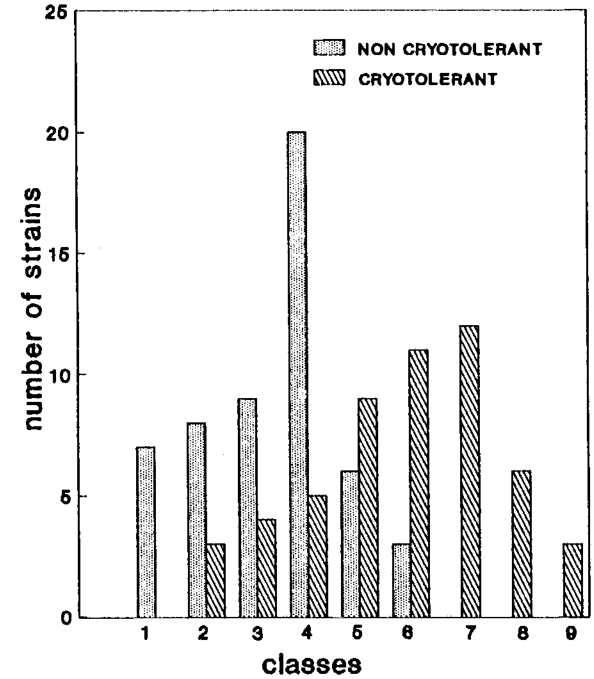

Fig. 3. Frequency histogram for glycerol production by cryotolerant and non-cryotolerant *Saccharomyces* strains. Level classes (g/l): 1= from 3.51 to 4.00; 2= from 4.01 to 4.50; 3= from 4.51 to 5.00; 4= from 5.01 to 5.50; 5= from 5.51 to 6.00; 6= from 6.01 to 6.50; 7= from 6.51 to 7.00; 8= from 7.01 to 7.50; 9= from 7.51 to 8.00.

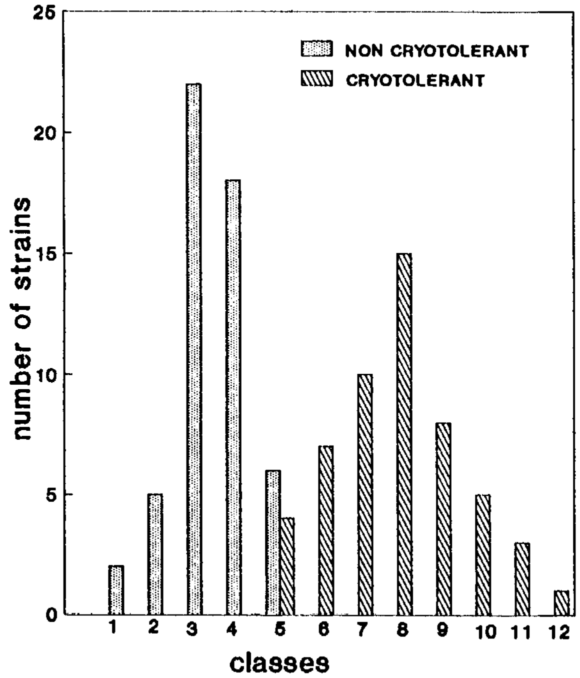

Fig. 4. Frequency histogram for succinic acid production by cryotolerant and non-cryotolerant *Saccharomyces* strains. Level classes (g/l): 1=from 0.31 to 0.40; 2= from 0.41 to 0.50; 3= from 0.51 to 0.60; 4=from 0.61 to 0.70; 5= from 0.71 to 0.80; 6= from 0.81 to 0.90; 7=from 0.91 to 1.00; 8= from 1.01 to 1.10; 9= from 1.11 to 1.20; 10=from 1.21 to 1.30; 11= from 1.31 to 1.40; 12= from 1.41 to 1.50.

Statistical analysis

Significance of the results: The two groups of strains are statistically different (p=0.01) as regards their action on malic acid and production of glycerol, acetic acid and succinic acid (Tab. 1). This conclusion was drawn from direct comparison of the mean values for these three traits for cryotolerant and non-cryotolerant strains. The difference between the groups is not significant for ethanol yield.

The degree of significance for the differences between cryotolerant and non-cryotolerant strains becomes unequivocal if all three traits are considered together. In this overall context, even ethanol yield acquires a precise significance since it allows us to correct the fermentation balance.

Table 1

Differences between cryotolerant and non-cryotolerant *Saccharomyces* strains

Groups	Number of strains	Mean*	Standard deviation	Minimum value	Maximum value
Action on malic acid (g/L)					
cryotolerant	53	2.356	0.2613	1.80	2.99
non cryotolerant	53	1.770	0.1785	1.32	2.17
Glycerol production (g/L)					
cryotolerant	53	5.765	1.046	4.10	7.60
non cryotolerant	53	4.916	0.806	3.42	6.32
Acetic acid production (g/L)					
cryotolerant	53	85.90	35.284	53.30	280
non cryotolerant	53	311.96	164.649	96.60	880
Succinic acid production (g/L)					
cryotolerant	53	1.090	0.25	0.750	1.460
non cryotolerant	53	0.530	0.18	0.370	0.800

*Averages are significantly different at the 0.01 level (Sheffe's test)

Statistical analysis confirmed that in both cryotolerant and non-cryotolerant strains action on malic acid and the capability of producing high or low amounts of glycerol, acetic acid and succinic acid are strain-dependent traits. In all cases the difference was not significant for the strains belonging to adjacent level classes (data not shown); the differences became significant for the majority of strains separated by two level classes (Scheffe's test p=0.05).

Correlation between traits: Interesting correlations between effect on malic acid and glycerol and acetic acid production levels in cryotolerant strains can be noted. Cryotolerant strains that produce larger amounts of acetic acid also cause a reduction in malic acid. When we calculated regression in these two compounds within the group of cryotolerant strains, we found a highly significant inverse correlation between the two activities. It also exists in the non-cryotolerant group but the significance is lower.

There is a direct correlation in cryotolerant strains between high malic acid synthesis and the production of high quantities of glycerol. Here, in fact, regression gave this correlation high significance but it could not be shown for non-cryotolerant strains.

In summary, high malic acid synthesizing cryotolerant strains also produce the highest amounts of glycerol and succinic acid and the lowest amounts of acetic acid.

Comparison between the most representative strains

Six cryotolerant strains selected from the highest producers of the three compounds considered (malic acid, glyc-

Table 2

Must fermentation balance of two cultivars by 4 non-cryotolerant *S. cerevisiae* p.r. cerevisiae (c) and 6 cryotolerant *S. cerevisiae* p.r. *uvarum* (u) strains

	cv Sangiovese*					cv Trebbiano**				
Strain	malic acid g/L	acetic acid g/L	glycerol g/L	succinic acid g/L	ethanol in degree	malic acid g/L	acetic acid g/L	glycerol g/L	succinic acid g/L	ethanol in degree
6555 c	2.19	0.665	8.35	0.870	10.65	0.95	0.635	6.08	0.710	10.40
9109 c	2.32	0.110	6.86	0.720	10.97	0.97	0.095	5.05	0.660	10.57
11886 c	2.48	0.120	4.44	0.520	10.87	1.51	0.160	4.22	0.410	10.26
12344 c	1.96	0.170	5.30	0.570	11.01	0.99	0.220	4.57	0.480	10.42
7877 u	3.42	0.070	9.01	1.090	10.44	2.32	0.040	8.72	0.980	9.86
11241u	3.33	0.070	9.44	1.290	10.57	2.18	0.035	7.55	1.150	10.28
11719u	3.88	0.075	9.41	1.120	10.49	2.46	0.045	6.22	1.020	10.22
11822u	3.39	0.075	8.87	0.990	10.38	2.64	0.040	7.58	0.880	10.34
12233u	3.88	0.030	9.12	1.180	10.28	3.27	0.040	7.10	1.040	10.41
12284u	3.52	0.095	7.94	0.910	10.31	2.43	0.065	6.97	0.870	10.37

* Sugar: 17.91 %; malic acid: 2.45 g/l. ** Sugar: 17.60 %; malic acid: 1.40 g/l.

erol and succinic acid) were compared in two different grape musts among themselves and with four non-cryotolerant strains. These latter were selected for high acetic acid and glycerol production (strain 6555), low acetic acid production (strain 9109) and for the highest (11886) and lowest (12344) malic acid production.

The results confirmed the fact that cryotolerant strains synthesize malic acid rather than decomposing it, produce more glycerol and succinic acid and less acetic acid than non-cryotolerant strains and produce less ethanol. As a result of this action, the wine produced by the two types of strains has a very different composition (see Tab. 2). The data from these tests also confirm the fact that single cryotolerant strains differ among themselves and possess the traits discussed here at different intensities. Medium (must) nature can influence the intensity but not the ratios in which the traits appear in the different strains.

Conclusion

Cryotolerant *Saccharomyces* strains possess an entirely peculiar prerogative that differentiates them from other yeasts in the same genus – i.e., malic acid synthesis during grape must fermentation, higher glycerol and succinic acid and very low acetic acid production. These activities, within a given population, have the typical quantitative trait distribution. Cryotolerant strains produce less ethanol and this brings the alcoholic fermentation balance into equilibrium.

This cryotolerant strains behaviour opens interesting perspectives in the enological field. Their use as starters to guide must fermentation would allow wine composition to be corrected biologically without having to employ chemicals. This type of adjustment is usually required in must lacking acidity, where high fixed acidity is needed or when high glycerol content is called for.

Our results are also interesting from a taxonomic point of view. The differences found refer to alcohol fermentation balance and confirm the hypothesis of Walsh and Martin (1977) and Naumov (1989) where cryotolerance is a species-dependent trait that should be taken into account in the classification of the *Saccharomyces* genus.

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