UDC 663.12:663.251
ISSN 1330-9862

professional paper

Oenological Properties of Cryotolerant and Thermotolerant *Saccharomyces* Strains

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Received: September 15, 1997.
Accepted: November 11, 1998

Summary

Strains belonging to Saccharomyces sensu stricto are mesophilic yeasts but, in some cases, their temperature performance is peculiar. Some strains, known as cryotolerant, ferment well at low temperatures (6 °C), while other, known as thermotolerant, can ferment at temperatures up to 42 °C. When these two types of Saccharomyces yeasts are used in vinification they produce wines of different composition, especially regarding the fermentation byproducts. Cryotolerant strains produce very low amounts of acetic acid, large amounts of glycerol and succinic acid, and synthetise malic acid rather than degrading it. Thermotolerant strains produce large amounts of glycerol and perform a very intense malo-alcoholic fermentation converting up to 50% of malic acid into ethanol.

Keywords: Saccharomyces, cryotolerance, thermotolerance, wine composition

Introduction

The *Saccharomyces* sensu stricto group is made of typical mesophilic yeasts, nevertheless some strains and some species, still being mesophilic, exhibit a peculiar temperature profile.

Strains with optimal growth temperature (t_{opt}) lower than 30 °C are known as cryotolerant. They do not grow at temperatures above 37 °C and can ferment well at 6 °C. These yeasts belong to the *Saccharomyces bayanus* and *Saccharomyces pastorianus* species sensu Vaughan-Martini and Martini *(1)*. According to more recent studies, the *Saccharomyces bayanus* species is not homogeneous and can be divided into two sub-groups called *bayanus* and *uvarum (2)*.

Strains with t_{opt} higher than 30 °C and growing at temperatures above 37 °C are called mesophilic. They belong to the *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* species *(1)*. Only some *Saccharomyces cerevisiae* can grow well at high temperatures, up to 42 °C, and are known as thermotolerant *(3–6)*.

The present work is the synthesis of several different researches *(6–9)* of these different types of strains and sets out to determine their specific oenological properties regarding fermentation temperature and wine composition.

Material and Methods

Organisms

The work was carried out with 53 cryotolerant strains of *Saccharomyces bayanus* sub-group *uvarum*, 53 mesophilic strains of *Saccharomyces cerevisiae* and 37 thermotolerant *Saccharomyces cerevisiae* strains. Cryotolerant and thermotolerant strains were isolated from must and other media such as milk, whey and other beverages, using the enrichment technique *(6,10)*. The mesophilic strains were randomly selected from our collection.

Fermentation tests

Fermentation tests were carried out in sterilised grape juice of *Vitis vinifera* from different cultivars. Samples of 0.1 or 1.0 L of sterilised grape juice were inoculated 24 h with a 5% culture, in the same must, and incubated anaerobically without shaking. Fermentation progress

was tested determining the weight loss caused by CO_2 release. The wines obtained were stabilised for two days at 4 °C, filtered and analysed. Tests were carried out in triplicate at seven different temperaures: 6, 12, 18, 24, 30, 36 and 42 °C.

Temperature

The optimal temperature of growth was determined in YPG (yeast extract 2%, peptone 2%, glucose 4%) in a temperature gradient incubator constructed according to Packer *et al.* (*11*). Using this method the optimal temperature corresponds to the most intense cellular multiplication 18 h after inoculation.

Wine analysis

Glycerol, succinic acid, acetic acid and malic acid were determined enzymatically using specific kits and following the procedures specified by the manufacturer (Boehringer, Mannheim, Germany). The concentration of glycerol, succinic acid and acetic acid was expressed as mg or g per 100 mL of ethanol produced. The concentration of malic acid was expressed in mg/L of wine. The common analysis of pH, total acidity, volatile acidity, ethanol and sugar were carried out on musts and wines with standard methods.

Statistical analysis

Differences in fermentation products between the strains studied were tested by one-way analysis of variance (Sheffe's test) using Statistical Analysis System Software (SAS Institute Inc. Cary NC, USA).

Results

Temperature performance

Cryotolerant strains exhibited a t_{opt} at approximately 28 °C. Thermotolerant and mesophilic strains had the same t_{opt} of 31 to 33 °C, but thermotolerant strains demonstrated the ability to grow well at higher temperature (Fig. 1).

The fermentation performance of the three types of strain is quite different depending upon the temperature. At 6 °C only cryotolerant strains can complete musts fermentation, even in 60 days (Fig. 2). At 18 to 30 °C all strains complete fermentation, but the more vigorous are the mesophilic strains (Fig. 3). At 36 °C thermotolerant strains are the more vigorous, cryotolerant strains are nearly inhibited and mesophilic strains can complete the fermentation process very slowly and only during long time (Fig. 4).

Cryotolerant strains ferment well in a temperature range of 6 to 30 °C, mesophilic strains in a range of 12 to 36 °C and thermotolerant strains in a range of 18 to 42 °C. The temperature profiles of the three types of strains are summarized in Fig. 5.

Minor compounds of fermentation

The three types of strains studied were shown to possess different fermentation profiles, especially regarding the production of the minor fermentation compounds.

Glycerol. The cryotolerant and thermotolerant strains produced larger amounts of glycerol than the mesophilic strains. The average amount of this compound in wine was 7.90, 8.32 and 5.10 g/100 mL of ethanol for cryotolerant, thermotolerant and mesophilic strains, re-

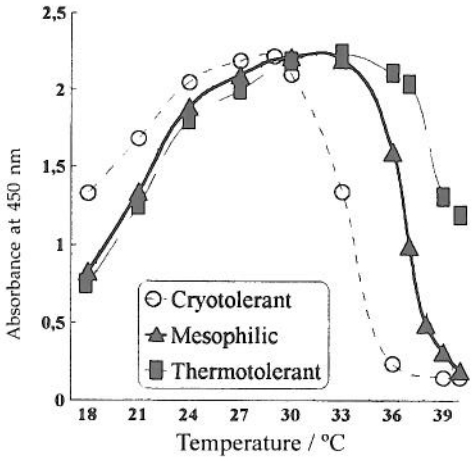

Fig. 1. Optimum temperature determined as intensity of cellular multiplication after 18 h

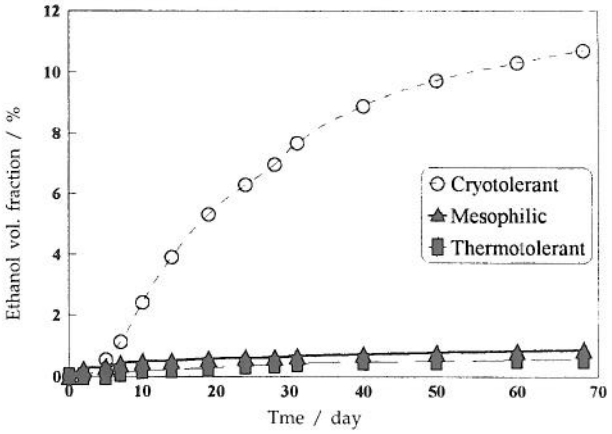

Fig. 2. Fermentation curves at 6 °C for representative strains. Only the cryotolerant strains can complete must fermentation

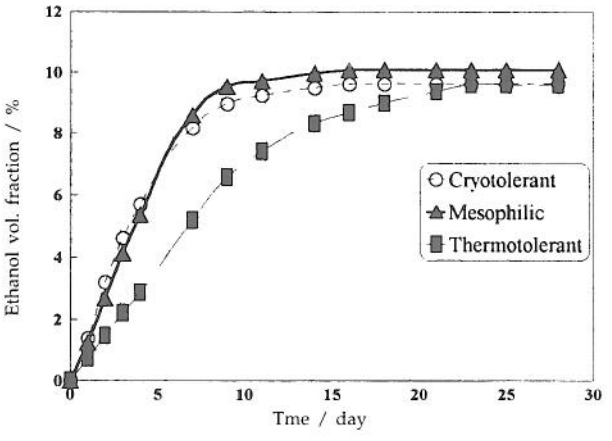

Fig. 3. Fermentation curves at 24 °C for representative strains. The more vigorous are the mesophilic strains

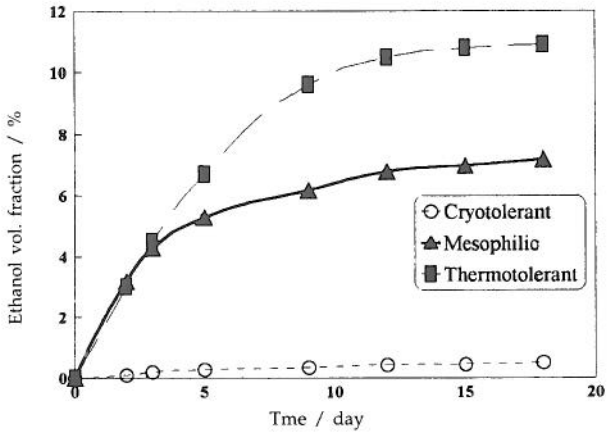

Fig. 4. Fermentation curves at 36 °C for representative strains. The thermotolerant strains are the more vigorous

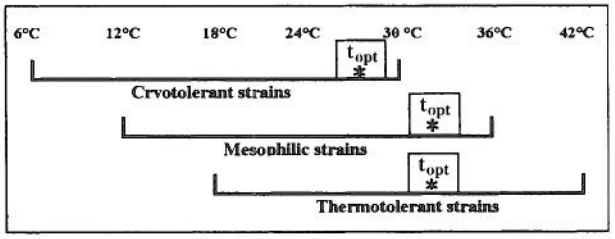

Fig. 5. General behaviour of the three types of strains with regard to temperature

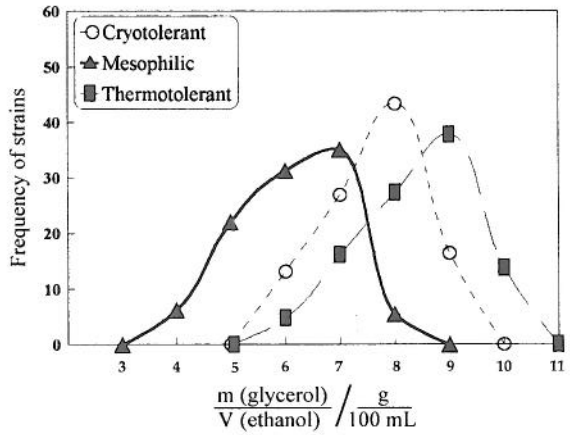

Fig. 6. Frequency curves for glycerol production by cryotolerant, mesophilic and thermotolerant strains

spectively. The trait could be divided into well distributed level classes (Fig. 6).

Succinic acid. Cryotolerant strains produced more succinic acid than thermotolerant and mesophilic strains. The average concentration of succinic acid was 1.10, 0.80 and 0.65 g/100 mL of ethanol for cryotolerant, thermotolerant and mesophilic strains, respectively, and could be divided into level classess (Fig. 7).

Acetic acid. Cryotolerant strains produced very low amounts of acetic acid, less than 100 mg/100 mL of ethanol. Mesophilic strains produced between 100 and 800 mg with an average of approximately 300 mg/100 mL

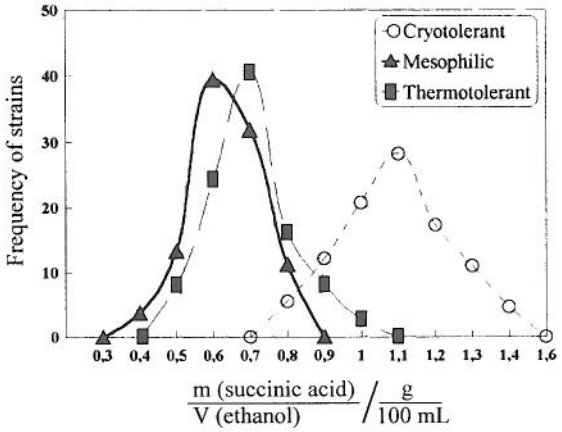

Fig. 7. Frequency curves for succinic acid production by cryotolerant, mesophilic and thermotolerant strains

of ethanol. The acetic acid production of thermotolerant strains was equally large (from 200 to 900 mg/100 mL ethanol) though the average at approximatively 400 mg was higher than in mesophilic strains (Fig. 8).

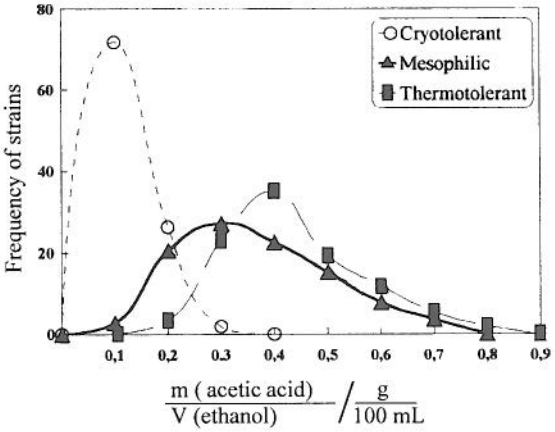

Fig. 8. Frequency curves for acetic acid production by cryotolerant, mesophilic and thermotolerant strains

Action on malic acid. During alcoholic fermentation this acid is normally degraded by the *Saccharomyces* through malo-alcoholic fermentation. Average degradation of malic acid by mesophilic strains was generally between 10 and 30% (Fig. 9). Thermotolerant strains degrade much more malic acid than the mesophilic and some strains achieve a 50% and more over the level necessary to halve the original amount. Cryotolerant strains performance is different since, rather than degrading malic acid, they synthesise it and increase its final concentration. The intensity of malic acid synthesis is strain-characteristic and some strains can even double its concentration.

Wine composition

Thanks to the different production levels for the different minor fermentation compounds, the wines produced with these yeasts will be considerably different. Wine from mesophilic strains have a nicely balanced

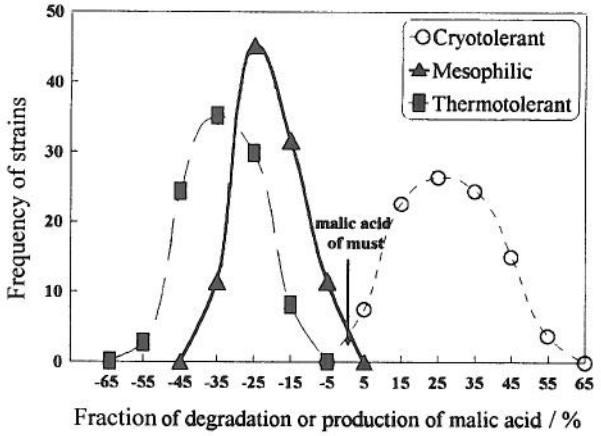

Fig. 9. Frequency curves for the action on malic acid by cryotolerant, mesophilic and thermotolerant strains. Values are expressed as the fraction in % of decrease or increase in malic acid compared with starting content

Table 1. Wine mean data obtained from 10 different musts fermented by more significant cryotolerant, mesophilic and thermotolerant strains

	Cryotolerant strains	Mesophilic strains	Thermotolerant strains
Vol. fraction of alcohol / %	9.88	10.24	9.35
Vol. fraction of theoretical calc. alcohol / %	10.01	10.33	10.10
Concentracion of reducing sugars / g L^{-1}	2.18	1.50	9.25
pH	3.08	3.17	3.26
Total acidity conc. / g L^{-1} *	11.30	7.80	7.15
Volatile acidity conc. / g L^{-1} **	0.08	0.26	0.45

* as tartaric acid, ** as acetic acid

composition while those from cryotolerant strains have a very high fixed acid content due to the synthesis of malic and succinic acid and, hence, a pH lower by several tenths of unit. Ethanol fraction was 0.3 to 0.5% lower because of the higher production of glycerol, malic and succinic acids. Thermotolerant strains, on the other hand, caused an increase in glycerol together with a 20–50% reduction in malic acid with the consequent increase in pH due to the lower acid content. Results of several vinifications carried out with the most representative strains of the three types are shown in Table 1.

Conclusions

Mesophilic strains, namely the typical and representative *Saccharomyces cerevisiae* strains, give the most vigorous and complete musts fermentation, at the standard condition for vinification at temperature of oenological interest. Their alcoholic fermentation is very clean, producing a very low amount of the minor compounds of fermentation. For these reasons they are considered the most suitable in oenological applications.

Cryotolerant strains give an unbalanced alcoholic fermentation compared to mesophilic strains and can be considered as acidifier. The acidifying activity depends upon the high concentration of succinic acid produced and the ability to synthesise malic acid. They are less vigorous at ordinary vinification temperatures and show a high vigour only at low temperatures (6 to 12 °C). During the spontaneous must fermentations they play a marginal role and were found to be dominating the fermentation process only in the case of cold stored musts (at 2 to 4 °C). They can be used as starter for ordinary fermentations when musts lack fixed acidity.

Also, thermotolerant strains give unbalanced alcoholic fermentation compared to mesophilic strains, but in this case they can be considered as desacidifyier. They can provoke intense malo-alcoholic fermentation with a degradation of malic acid up to 50% of the starting concentration. They are not vigorous at temperatures of oenological interest and exhibit a good vigour only above 30 °C. Thermotolerant strains can play an important role in natural must fermentation only when the uncontrolled temperature rises high enough to inhibit the mesophilic strains. Due to their desacidifying activity and high glycerol production they can be employed as starter in the production of red wines.

Cryotolerant and thermotolerant strains possess interesting oenological properties. They can be used in the production of some wines but they can also be used as parental strains in the production of sterile interspecific or fertile intraspecific hybrids with innovative and need-specific combination of properties.

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Enološka svojstva kriotolerantnih i termotolerantnih sojeva *Saccharomyces*

Sažetak

Saccharomyces sensu stricto je soj koji pripada u mezofilne kvasce, a u nekim se slučajevima neobično ponaša pri različitim temperaturama. Neki sojevi, poznati kao kriotolerantni, fermentiraju dobro pri niskim temperaturama (6 °C), dok drugi poznati kao termotolerantni, provode vrenje pri temperaturama do 42 °C. Ako se ta dva tipa kvasca koriste pri vinifikaciji, dobivamo vina različita sastava, koja sadržavaju različite fermentacijske nusproizvode. Kriotolerantni sojevi proizvode vrlo malu količinu octene kiseline, veliku količinu glicerola i jantarne kiseline, a sintetiziraju jabučnu kiselinu umjesto da ju razgrade. Termotolerantni sojevi proizvode veliku količinu glicerola, imaju vrlo intenzivnu jabučno-alkoholnu fermentaciju, pri čemu se čak do 50% jabučne kiseline prevodi u etanol.