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Research Note

Characterisation of wine yeasts isolated at different temperatures using the enrichment technique

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Summary: *Saccharomyces cerevisiae* strains isolated from fermenting grape must incubated at extreme fermentation temperatures (40 and 5 °C) were oenologically characterised. These cultures compared with *S. cerevisiae* wine strains, show a wider optimum temperature for growth and can ferment vigorously in a wider temperature range (27 to 35 °C).

K e y w o r d s : *Saccharomyces cerevisiae*, extreme temperatures, wine.

Introduction: Selection of wine yeasts carried out by isolating cultures from must or wine under ordinary fermentation conditions favours the isolation of the common strains of *Saccharomyces cerevisiae*. Strains with particular traits can be obtained more easily by means of enrichment, *i.e.*, under fermentation conditions which do not favour normal strains. For example, using low-temperature must incubation, it was possible to isolate a large number of cryotolerant *S. uvarum* strains, usually overcome by the more common and mesophilic *S. cerevisiae* (Castellari *et al.* 1992; Massoutier *et al.* 1998). The present research was carried out to find *Saccharomyces* strains with novel oenological traits. *Saccharomyces* strains were isolated from grape juice incubated at low and high temperatures. The newly isolated cultures were identified, oenologically characterised and compared with reference strains.

Material and Methods: Cultures were isolated from musts obtained from 20 vineyards in the Molise region (Southern Italy). Each must was divided into three lots of 5 l each and incubated at 5, 25 and 40 °C. Isolation of cultures was carried out in fully fermenting must. Cultures were maintained on YPG agar (yeast extract, 2 % w/v; peptone, 2 % w/v; glucose, 4 % w/v; agar, 1.5 % w/v). Cultures were selected considering their shape and size on the plates and their appearance under the optical microscope. An average of 18 colonies was isolated from each must. Cultures were identified using the methods described by Kurtzman and Fell (1998). The isolated strains were compared to two reference strains; a *S. cerevisiae* wine strain (Diproval 6167) and a *S. uvarum* strain (Diproval 12233) both isolated from grape must and characterised in previous studies (Castellari *et al.* 1994; Rainieri *et al.* 1999).

Preparation of chromosomal DNA and pulsed-field gel electrophoresis (PFGE) were carried out using the Schwartz and Cantor (1984) technique modified by Giudici *et al.* (1998). Spore germinability was determined using a de Fonbrune micromanipulator and performing the direct breakage of the asci wall. Single spores were then transferred to microdrops of YPD.

Fermentation tests were carried out in triplicate on 500 ml pasteurised (90 °C for 2 min) *Vitis vinifera* cv. Trebbiano must. Must samples were inoculated with a 10 % preculture of each yeast grown in the same substrate for 48 h and incubated at 6, 16, 26 and 36 °C. Fermentation was assessed by determining daily the weight loss caused by CO_2 release. Wine analysis was carried out enzymatically and with standard methods. Differences in fermentation products between strains were tested by one-way analysis of variance (Sheffe's test) using Statistical Analysis System Software (SAS Institute Inc., Cary, NC). The optimum temperature for growth was determined using a temperature gradient incubator constructed as described by Packer *et al.* (1973). This method establishes the temperature at which the maximum cell multiplication rate occurs after 15-20 h from inoculation.

Results and Discussion: Altogether, 154 *Saccharomyces cerevisiae* strains were isolated from musts incubated at 5, 25 and 40 °C. Of the *S. cerevisiae* strains isolated at the extreme fermentation temperatures, 16 differed from ordinary *S. cerevisiae* wine strains. These cultures showed a wider optimum temperature for growth than *S. cerevisiae* wine strains. As shown in the Figure, they grew at the same intensity in a temperature range of 27 to 35 °C.

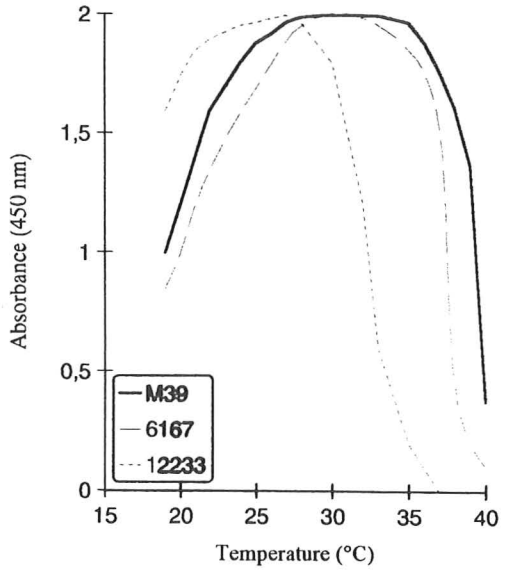

Figure: Thermal profile of one of the selected *Saccharomyces cerevisiae* selected strains (M39), compared with reference strains *S. uvarum* 12233 and *S. cerevisiae* 6167.

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Table

Composition of the must and wines produced by strains isolated at extreme fermentation temperatures (A, B) in comparison to reference strains *S. uvarum* 12233 and *S. cerevisiae* 6167

	Must	12233	6167	A (range)	B M 39
Sugar (g/l)	173	2.58	3.40	1.97 - 3.53	3.50
pH	3.50	3.28	3.42	3.34 - 3.42	3.44
Total acidity (g/l)	6.20	9.70	7.30	7.10 - 8.20	7.20
Fermentation compounds:					
Ethanol (vol%)	9.60		10.10	9.90 - 10.20	10.20
Glycerol (g/l)	8.40	a	4.86 b	4.20 - 5.35 b-c	4.20 c
Succinic acid (g/l)	0.97	a	0.65 b	0.50 - 0.69 b-c	0.50 c
Acetic acid (g/l)	0.07	a	0.24 c	0.17 - 0.54 b-d	0.25 c
Malic acid (g/l) 2.68	3.52	a	1.59 b	1.59 - 2.26 b-c	1.60 b

Numbers with different letters differ at $p < 0.01$ level (Sheffe's test)

The 16 *S. cerevisiae* strains characterised in this study fermented vigorously in grape must at both 6 and 36 oc. At 6 oc they showed an improved fermentation rate compared to the *S. cerevisiae* reference strain 6167 and fermented almost as vigorously as the *S. uvarum* strain 12233. At 36 oc they fermented vigorously and without leaving residual sugars, whereas the reference *S. cerevisiae* wine strain 6167 started the fermentation process but could not exhaust the sugar of the must. The *S. uvarum* reference strain 12233 could not start the fermentation process. At 16 and 26 oc the 16 *S. cerevisiae* strains fermented with the same vigour as both reference strains.

The wines obtained using the 16 *S. cerevisiae* strains as starter cultures did not differ from those obtained using the reference *S. cerevisiae* wine strain 6167. The minor compounds of fermentation (*i.e*. acetic acid, glycerol, malic acid and succinic acid) were produced at levels oscillating around those of the *S. cerevisiae* wine reference strain. The composition of the wines obtained using the *S. uvarum* reference strain differed notably from that of wines obtained using *S. cerevisiae* strains. The Table shows the composition of wines obtained at 26 oc.

The 16 *S. cerevisiae* strains isolated at the extreme fermentation temperatures sporulated well and formed numerous 4-spored asci. The spores demonstrated a high rate of germinability and single spore cultures were obtained from all the strains. The single spore cultures showed the same morphological and physiological traits of the respective parental strains.

The outstanding traits of the *S. cerevisiae* strains described in this study are their wide optimum temperature for growth and their ability to ferment vigorously in a wide temperature range. These characteristics have been described for interspecific hybrids obtained by crossing *S. cerevisiae* and *S. uvarum* strains (ZAMBONELLI *et al*. 1997). Even though the existence of natural hybrids has been confirmed (MASNEUF *et al*. 1998) this does not appear to be true for the *S. cerevisiae* strains described in the present paper. These strains, in fact, have the electrophoretic karyotype of *S. cerevisiae*, are fertile and their single spore cultures have the same characteristics as their parental strains.

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