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preliminary communication

Oenological Properties of *Saccharomyces bayanus* and *Saccharomyces cerevisiae* Interspecific Hybrids

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Summary

Interspecific hybrids between Saccharomyces bayanus and Saccharomyces cerevisiae are sterile. The sterility prevents genetic improvement but the hybrids themselves are interesting from an oenological point of view. They are more vigorous and competitive than the parental strains over a wide temperature range. The minor compounds of fermentation are always produced in medium quantities, while the parents are highly differentiated in this regard: this is true of glycerol, succinic acid, acetic acid and higher alcohols. They display an intermediate action on malic acid which is degraded by Sacch. cerevisiae and synthesised by Sacch. bayanus.

Keywords: Saccharomyces, interspecific hybrids, oenological properties, temperature response

Introduction

Sacch. bayanus and *Sacch. cerevisiae* have different temperature profiles and possess different properties which can be important from an oenological point of view. *Sacch. cerevisiae* strains have an optimal temperature of growth (t_{opt}) greater than 30 °C and a maximum temperature of growth (t_{max}) greater than 37 °C and ferment well between 12 and 36 °C. *Sacch. bayanus* strains have a t_{opt} lower than 30 °C and a t_{max} lower than 37 °C. They ferment well between 6 and 30 °C and are also known as cryotolerant strains *(1)*.

Sacch. cerevisiae is the yeast producing the cleanest alcoholic fermentation with maximum ethanol yield. *Sacch. bayanus* on the other hand offers distinctly unbalanced fermentations: it produces high amounts of glycerol and succinic acid, very low amounts of acetic acid (less than 0.1 g/L) it synthesises malic acid and produces very high amounts of higher alcohols *(2–5)*.

Cryotolerant *Sacch. bayanus* strains and non-cryotolerant *Sacch. cerevisiae* strains produce sterile interspecific hybrids *(6,7)*. This paper reports on numerous studies determining the properties of the hybrids and deciding if they are of oenological interest.

Materials and Methods

Organisms

The research was carried out with cryotolerant *Sacch. bayanus* strains 7877, 11204 and 12233 and the mesophilic *Sacch. cerevisiae* strains 6167, 7070, 9109, 11052, 11883. All the strains belonged to the collection of the DIPROVAL - University of Bologna. They were homotallic and selfdiploidizing, producing diploid single spore cultures.

Hybridization

Single spore cultures with the same characteristics of the correspondent natural strain were employed for the hybridization. The hybrids were obtained by conjugation of the aforementioned spores following the methods of Winge and Laustsen *(8)*. Asci dissection, spore separation, crosses and tetrad analysis were performed with a de Fonbrune pneumatic micromanipulator in an oil chamber.

Temperature profile

The optimal temperature of growth was determined using a temperature gradient incubator constructed according to the basic design of Packer *et al.* *(9)*. This method allows the determination of the temperature of maximum cellular multiplication to a limited extent for the first 12–18 hours. The cell growth was expressed as absorbance at 450 nm 18 hours after inoculation.

Fermentation tests

Fermentation tests were carried out in sterilised grape juice of several *Vitis vinifera* cultivars from different zones using the procedure of Eustace and Thornton

(10). Fermentation progress was tested by determining weight loss caused by CO_2 release. The wines obtained were stabilised for 2 days at 4 °C, filtered and analysed.

Wine analysis

The common analysis of pH, total acidity, volatile acidity, ethanol and sugar were carried out on the grape juices and on the wines using standard methods *(11)*. Glycerol, succinic acid, acetic acid and malic acid were determined enzymatically with specific kits following the procedures specified by the manufacturer (Boehringer Mannheim Germany) Phenylethanol was detected by HPLC according to the method of Williams *et al. (12)* modified by Bertolini *et al. (5)*.

Statistical analysis

Differences in fermentation products were tested by one-way analysis of variance (Duncan's test) using Statistical Analysis System Software (SAS Institute Inc. Cary NC).

Results

Hybrid characteristics

Hybrids between cryotolerant *Sacch. bayanus* strains and mesophilic *Sacch. cerevisiae* strains are sterile. They sporulate well and produce numerous asci whose spores do not germinate. Their sterility confirms the fact that the parental strains belong to different species. Since the normal reproduction of wine yeast strains is asexual budding, and not sexual reproduction, these sterile hybrids can be used perfectly for wine fermentation.

The hybrids have an optimal growth temperature between the t_{opt} of the parental strains (27 to 33 °C) (Fig. 1). They grow and ferment well both at low (6 °C) and high (36 °C) temperatures. They are more vigorous and competitive than the parents over a wide range of temperatures, including the temperatures of oenological interest (20–30 °C).

The hybrids produce glycerol, succinic acid, acetic acid and phenylethyl alcohol midway between the parent production levels. They can synthesise malic acid but at levels distinctly lower than the cryotolerant parental strains. Table 1 compares hybrid and parent production of fermentation compounds.

Table 1. Production of minor compounds of fermentation for cryotolerant and non-cryotolerant parents and hybrids in grape juice (the results are the average of three indipendent replications)

	Cryotolerant parent	Hybrid	Non-cryotolerant parent
		γ / g L^{-1}	
Glycerol	10.89 A	9.25 B	7.22 C
Succinic acid	1.038 A	0.765 B	0.453 C
Acetic acid	0.080 B	0.150 AB	0.270 A
Total higher alcohols	0.270 A	0.270 A	0.250 A
2-phenylethanol	0.180 A	0.110 B	0.037 C
Malic acid	2.67 A	1.92 B	1.48 C

Values followed by different letters are statistically different (Duncan's test, $P < 0.01$)

Table 2. Characteristics of wines produced by hybrid 12233-3a × 6167-1c (cryo × non-cryotolerant) compared with parents. The data are the average of 9 tests

Composition	Cryotolerant parent	Hybrid	Non-cryotolerant parent
φ(ethanol)/%	10.07 B	10.48 AB	10.84 A
γ(sugar)/g L^{-1}	<1	<1	<1
Total acidity/g L^{-1}*	8.22 A	7.62 B	5.75 C
pH	3.25	3.30	3.36
Volatile acidity /g L^{-1}**	0.140 B	0.23 AB	0.35 A
γ(extract)/g L^{-1}	19.91 A	18.47 B	15.90 C

*As tartaric acid; **as acetic acid.
Values followed by different letters are statistically different (Duncan's test, $P < 0.01$)

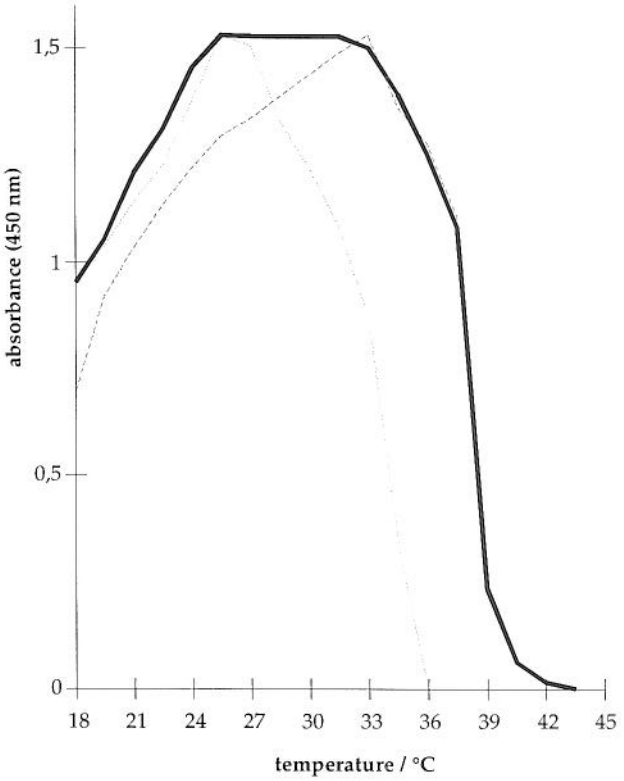

Fig. 1. Optimum temperature determined as intensity of cellular multiplication after 18 hours. Cryotolerant parent 12233-3a (····), non-cryotolerant parent 6167-1c (- - - -), and hybrid (——).

Wine composition

The wines produced from the hybrids and those produced by the parents are quite different in their composition. Depending on their properties, the hybrids produce wines whose analytical parameters are midway between those of the parents. Table 2 details the overall data and fermentation tests results of the hybrid 12233 3a x 6167 1C in 3 musts of different origin. The data are the average of three replications.

Conclusions

Hybrids between cryotolerant and non-cryotolerant *Saccharomyces* strains possess the species characteristics of both the parental strains summed together (*e.g.* the optimal temperature of growth) and an average of the strains characteristics. They are extremely interesting in oenological terms both for their characteristics of competitiveness and stability and for their fermentation products. Their fermentative vigour is greater than that of the parents and they ferment well over a much wider temperature range.

The hybrids produce wines whose acidity, alcoholic degree and other characteristics are intermediate with respect to those of the two parental strains. Also the amount of 2-phenylethyl alcohol produced does not give the wine an unpleasant taste. The hybrids possess the properties of the cryotolerant strains but to a lesser degree, they can be employed when a fairly full-bodied wine is required and when the acid level is to be maintained or slightly increased.

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Enološka svojstva interspecifičnih hibrida *Sacch. bayanus* i *Sacch. cerevisiae*

Sažetak

Sterilni su interspecifični hibridi između Sacch. bayanus i Sacch. cerevisiae. Sterilnost sprječava genetičko poboljšanje, ali su hibridi kao takvi interesantni s enološkoga gledišta. Bili su mnogo aktivniji od roditeljskih sojeva u širokom temperaturnom rasponu. Uvijek su proizvodili manju količinu nusproizvoda fermentacije, dok su se roditeljski sojevi u tom pogledu bitno razlikovali po udjelu proizvedenoga glicerola, jantarne kiseline, octene kiseline i viših alkohola. Hibridi su na jabučnu kiselinu djelovali posredno jer je Sacch. cerevisiae razgrađuje, a Sacch. bayanus sintetizira.