Int Microbiol (2003) 6: 207–209
DOI 10.1007/s10123-003-0135-y

REVIEW ARTICLE

Alessandro Martini

Biotechnology of natural and winery-associated strains of *Saccharomyces cerevisiae*

Received: 20 January 2003 / Accepted: 5 June 2003 / Published online: 29 July 2003

Abstract A new body of evidence challenges the original consolidated theory of Pasteur on the natural (vineyard) origin of wine strains of *Saccharomyces cerevisiae* and instead indicates a local, winery-restricted life cycle. The findings open novel biotechnological perspectives for obtaining autochthonous selected starters for the wine industry. A local, individual, and specific fermenting yeast flora, mass selected year after year through many generations of *S. cerevisiae* in grape must, is present on the surfaces of every winery. These yeast strains are endowed with exceptional enological properties and capable of producing an assortment of volatile compounds apparently contributing to the specific bouquet of locally produced wines.

Keywords *Saccharomyces cerevisiae* · Yeast technology · Wine making · Winery autochthonous starters

Introduction

The recent discovery that an overabundance of living cells of *Saccharomyces cerevisiae* is present on all of the surfaces of every winery [6, 8] is providing wine technologists with a large reservoir of strain diversity as a new source of locally selected starters for wine-making. Apparently, *S. cerevisiae* populations endowed with enological properties wholly comparable to those of commercial starters and autochthonous starters of wineries [4, 5] may prevent excessive standardization engendered by the presence of only two to three active dry commercial starters in the international market. Through comparison of strains of *S. cerevisiae* isolated from nature and wineries, it is possible to prove that, year after year and through many duplications in local grape musts, the enological properties of cells colonizing winery surfaces improve.

Wine technologists assign the properties required for the definition of a strain of the species *S. cerevisiae* as a "selected starter for wine making" to two categories [9]: (1) *primary*, defined as those strictly associated with, e.g., the formation of ethyl alcohol by fermentation, and (2) *secondary*, defined as those related to the production of compounds that affect other parameters, such as the body of a wine, the higher alcohols complex (bouquet), and the appearance of undesirable off-flavors. Conventionally, the primary conditions are:(1) High *fermentation vigor*(FV), intended as the uppermost concentration of ethanol obtainable by fermentation from an excess of sugar. Grape musts often contain more than 25% (w/v) sugar, corresponding to an FV of 15% (v/v) ethanol; selected starters in the brewing industry rarely exceed 10% FV. (2) High *fermentation purity*, (FP), expressed as of the ratio between volatile acidity (as g acetic acid/l) and ethanol (% volume) produced at the end of the fermentation process. High values of the ratio denote the ability of a wine starter to form few undesirable by-products in the course of fermentation. Wines cannot be commercialized if volatile acidity exceeds one tenth of the ethanol content. (3) High *fermentation rate* (FR) is the measure of the ability of a starter to bring the fermentative process to a fast completion. It is normally represented as grams of CO_2 developed in 24 h, calculated as the average of a 3-day measurement period. A yeast starter for wine making should form more than 1° (v/v) ethanol.

Secondary conditions affect another level of the quality of a wine, termed the organoleptic specificity (bouquet), which is often more difficult to represent in analytical terms because it results from the interaction of a series of by-products of primary fermentation. Even though the compositional variability of musts (i.e., the

A. Martini
Dipartimento di Biologia vegetale
& Biotecnologie agroambientali,
Industrial Yeasts Collection DBVPG,
Borgo 20 Giugno 74, 06121 Perugia, Italy
E-mail: martinal@unipg.it
Tel.: +39-0755856479
Fax: +39-0755856470

Table 1 Average values of primary enological characters in natural and winery resident strains of *Saccharomyces cerevisiae*

	286 cultures from the Industrial Yeasts Collection DBVPG isolated from environments not associated with the production of wine	531 strains isolated from various surfaces of 26 wineries from three Italian DOC areas: Verdicchio, Sagrantino, Prosecco
Fermentation vigor as maximum ethanol produced	11.1 ± 0.09% (v/v)	15.2 ± 0.08% (v/v)
Fermentation purity as acetic acid (g/l)/fermentation vigor	0.064	0.105
Fermentation rate as grams of CO_2 produced in 24 h	0.42 ± 0.01 g	0.95 ± 0.01 g

precursors of bouquet molecules variably distributed within grape varieties) is considered as the main source of organoleptic specificity, there is today a propensity to reevaluate the role of yeast metabolism (strain-related by-products of fermentation) in the formation of bouquet.

Analytical conditions

Enological performance, as defined by the above-described parameters, of over 500 pure cultures of *S. cerevisiae*, isolated from 250 samples collected from walls, ceilings, floors, vats, and equipment of 26 different wineries, was compared with that of more than 250 strains of the same species isolated from natural sources not associated with wine production. All yeast cultures came from the Industrial Yeasts Collection DBVPG [7; and http//:www.agr.unipg.it].

Microfermentations were carried out under static conditions at 20 °C in 500-ml Erlenmeyer flasks containing 300 ml steam-sterilized grape must of the Pinot Grigio cultivar (reducing sugar 200 g/l, pH = 3.1) and inoculated to reach a concentration of 1×10^6 cells/ml. Samples were frozen for further analyses at −20 °C after filtration.

Fermenting vigor and fermentation rate were tested according to the method described in [13]. Ethanol was measured by gas-liquid chromatography (GLC) [1, 2]; acetic acid was determined enzymatically (kit no. 148261, Boehringer, Mannheim, Germany). Secondary enological properties (acetaldehyde, ethyl acetate, acetoin and higher alcohols) were detected by GLC [3]. Principal components analysis of chemiometric data from microfermentations was carried out using the statistical package Statistica, ver. 3.0 (Statsoft Italia, Padova).

Expression of primary characters in natural and winery-colonizing strains of *S. cerevisiae*

The three main primary properties were analyzed in microfermentations carried out using 531 yeast cultures isolated from 26 wineries, and 286 strains of natural origin as starters. Results are presented in Table 1.

The primary properties of the yeasts colonizing winery surfaces were unequivocally and consistently superior to those of other *S. cerevisiae* strains only occasionally present in natural sites. It appears that the cultures of *S. cerevisiae* that initiated the first grape-must fermentation, when the particular winery first came into activity, underwent radical modifications of their enotechnogical characters over time. Furthermore, the data clearly indicate that a genetic selection occurred in each winery, leading, vintage after vintage, to a progressive adaptation of resident *S. cerevisiae* populations to the nutritionally extreme conditions of grape must. A selective pressure may have been operating that limited environmental factors such as ethanol concentration in favor of high FV strains; or sugar concentration in favor of strains capable of fermenting in adverse osmotic conditions; or high concentrations of SO_2 in favor of strains resistant to its action. A winery-restricted annual cycle of *S. cerevisiae* [11], implying a recurring flow of cells from surfaces to freshly pressed musts and a return at vintage to winery surfaces after a series of about ten generations (multiplied by the number of vats and the total volume of must fermented), may actually have provided enough variability for a selective optimization of resident strains.

Specific changes of secondary enological properties of *S. cerevisiae* in the winery environment

Another noteworthy observation derives from comparison of the chemical analyses of wines obtained with the best performing cultures isolated from each of the following origins: Verdicchio dei Colli di Jesi, Sagrantino di Montefalco, and Prosecco di Valdobbiadene. Bench-scale vinifications were carried out on the same sterile grape must (Pinot grigio) using 18 starters. Chemiometric data, derived from 14 different bouquet-affecting compounds analyzed in the 18 obtained wines, were elaborated by principal components analysis. The first and second principal components were identified, and these accounted for 54% of the variance.

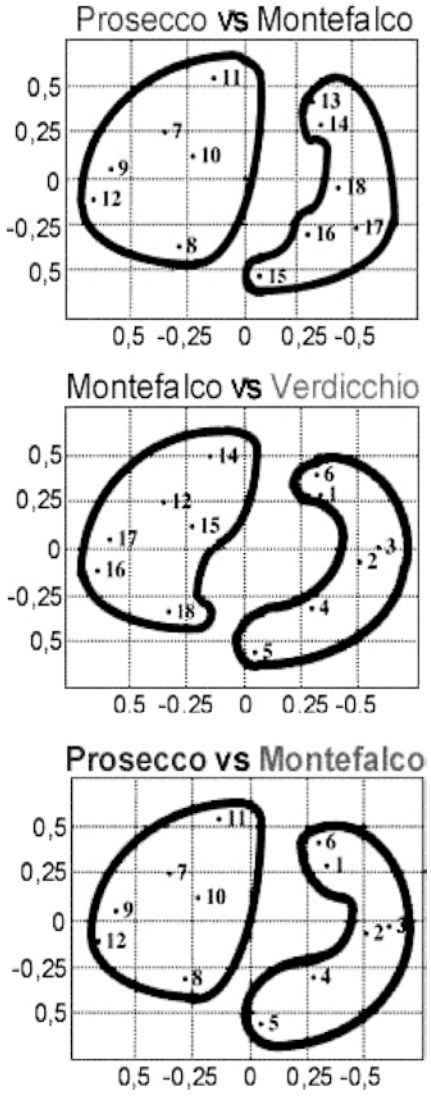

Fig. 1 Projection of points of the two eigenvectors with the highest variance

The scattergram of the two principal components (Fig. 1) was effective in grouping the 18 strains into clearly separated clusters. Apparently, locally isolated starters yielded wines characterized by a highly domain-specific chemical composition. Since the same grape must was used throughout the test, the compositional variability of wines must be ascribed to differences in the secondary metabolism of local yeasts. In other words, it appears highly probable that a selective pressure, contributing to the formation of those organoleptic characters that "fingerprint" the local wine, operates also for secondary compounds.

Concluding remarks

In any winery, there is a local, resident *S. cerevisiae* population consisting of strains technologically optimized for wine making and adapted to produce a set of compounds possibly involved in the formation of the local, individual bouquet. The logical and enological consequence of this observation is that, by means of a simple and inexpensive isolation from internal walls, any winery may obtain its own superselected starter with personalized bouquet characters. The scope of the evidence presented here is to make wine makers throughout the world aware of a new, easily accomplished, inexpensive, and certainly beneficial advance in the biotechnology of wine making.

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