UDC 663.14:663.2:663.12
ISSN 1330–9862

original scientific paper

(FTB-1315)

Biodiversity of a Natural Population of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* from Aglianico del Vulture

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Received: March 22, 2004
Accepted: July 6, 2004

Summary

A total of 140 strains of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum*, isolated from grapes and musts in the Basilicata region in Italy, were differentiated on the basis of fermentation behaviour and production of secondary compounds in Aglianico del Vulture must. A significant natural biodiversity of the strains was determined. In particular, within each species, the strains were differentiated for the fermentative activity and for the production of secondary compounds. Great strain variability was found in *Saccharomyces cerevisiae*.

Key words: fermentation, *Hanseniaspora uvarum*, *Saccharomyces cerevisiae*, wine yeast

Introduction

It is demonstrated that differences in wine quality are clearly related to the levels of secondary compounds, which are principally determined by the yeast species involved in the fermentation process (*1*). This has recalled the attention of wine-researchers and wine-makers to the autochthonous strains with the aim of selecting starter cultures that are potentially better adapted to the growth in a specific grape must, also emphasising the sensorial and typical quality of the specific wine. The more frequently encountered yeasts in the early formation phase are non-*Saccharomyces* species, such as the strains of *Hanseniaspora*, *Candida* and *Metschnikowia*, which initiate the fermentation during the first 3–4 days, after which they die off, due to the toxicity of the increasing concentrations of ethanol. At this stage, the more ethanol tolerant wine yeasts, *Saccharomyces cerevisiae* and related species, become predominant and complete the fermentation (*2*,*3*). Indeed, *S. cerevisiae* is quite capable of conducting the entire fermentation by itself, but it is not dominant as a natural contaminant in freshly crushed juice and it must grow competitively with other non-*Saccharomyces* species in the earlier stages of fermentation. It must be stressed that these non-*Saccharomyces* yeast species perform biochemical activities producing some important reactions in the grape must, thus influencing positively or negatively the quality of the wine (*4*,*5*). Of these yeasts, *Hanseniaspora uvarum* is more frequently the principal species and also great strain diversity has been found among the strains of this species, which is of technological interest (*6*). In particular, during the fermentation process, these yeasts, in addition to the production of ethanol, generate many secondary compounds that strongly impact the wine flavour, and are key determinants of wine quality (*7*).

In this work autochthonous yeast strains were isolated from a natural population of Aglianico del Vulture wine and this research was focused on the two species

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most frequently found on grapes and during must fermentation, *S. cerevisiae* and *H. uvarum*. The isolates were characterised for the production of secondary compounds with the aim to individuate natural biodiversity of the strains and to correlate the expression of a specific metabolic yeast behaviour with the isolation environment.

Materials and Methods

Yeast strains

A total of 140 strains, belonging to the collection of the Wine Microbiology Laboratory (Basilicata University), was used. The strains, 70 of *S. cerevisiae* and 70 of *H. uvarum*, previously isolated from different vineyards of Aglianico del Vulture grapes (*8,9*), were maintained at 4 °C on slant of Sabouraud Agar (Oxoid).

Micro-fermentations

To evaluate strain biodiversity for fermentation performance, the strains of the two species were tested by micro-fermentations in grape must. The experiments were carried out in Aglianico del Vulture, which represents an ancient grape variety and is mainly produced in a special geographical location, Venosa, where Orazio Flacco was born. Aglianico is the major wine produced in Basilicata and is a red wine of distinguished characteristics.

Grapes, collected directly in vineyards, were crushed in the laboratory and the must obtained was distributed in 125-mL Erlenmeyer flasks (filled with 100 mL of must), which were autoclaved at 100 °C for 20 min. Each sample was inoculated with 10^4 cells from 24-h pre-cultures in the same must. The grape must surface was covered with a thin layer of sterilised paraffin oil in order to avoid air contact. The samples were incubated at 25 °C and the fermentation was followed by CO_2 evolution, expressed as grams of CO_2 produced from 100 mL of fermenting grape must. The weight loss was monitored every day and the quantity (in g) of CO_2 produced was used to express strain fermentation vigour (FV) and power (FP). When the CO_2 evolution ceased, the fermentation was considered completed, the samples were refrigerated for 1 day at 4 °C, racked and stored at –20 °C until required for analysis. The fermentations were carried out in duplicate.

Analytical determinations

At the end of the fermentation process, the obtained wines were analysed for alcohol and volatile compounds, such as higher alcohols and esters. Analytes were determined following the procedure reported by Romano *et al.* (*10*).

Statistical analysis

The data were statistically analysed by ANOVA, Box-plot and PCA using the software »Statistica for Windows« version 5.0, 97 Edition (Statsoft).

Results and Discussion

Various statistical methods were applied in order to select some strains of each species in function of the different technological behaviour.

Fermentation vigour and power

Fermentations were performed in Aglianico del Vulture grape must and strain fermentation performances, such as vigour and fermentation power of the 140 strains (*S. cerevisiae* and *H. uvarum*), are reported in Fig. 1 as average and standard deviation values. The fermentative activity differed significantly among the strains of the two species, confirming the behaviour recognised as typical of the two species. As expected, *S. cerevisiae* strains showed the highest activity with an average CO_2 evolution of 13.61 g/100 mL of grape must, whereas the maximum CO_2 formed was about 18.0 g/100 mL of grape must. *H. uvarum* strains exhibited low and more uniform fermentation vigour and power. Only one strain of *H. uvarum* demonstrated elevated fermentation efficiency for this species (6.99 g/100 mL of grape must). It must be stressed that significant differences in the fermentation activity were recorded among *S. cerevisiae* strains, some of which have high fermentative power (17.94 g/100 mL of grape must) and others low fermentative power (less than 10.75 g/100 mL of grape must).

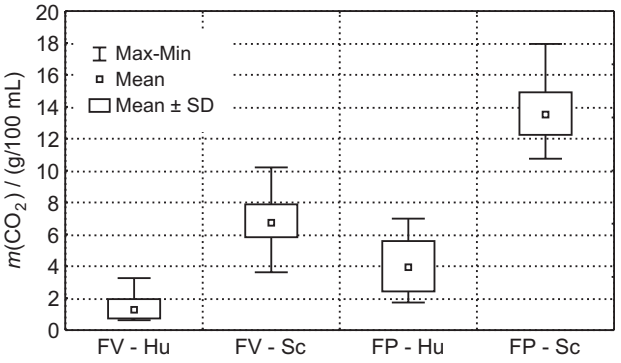

Fig. 1. Fermentation vigour (FV) and power (FP) of *S. cerevisiae* (Sc) and *H. uvarum* (Hu) strains

Production of secondary compounds

The experimental wines, obtained by inoculated fermentation with each strain, were analysed for the content of some by-products. In Fig. 2 the analysis of variance (ANOVA) is reported as F-value of the Fisher test. The comparison between *S. cerevisiae* and *H. uvarum* strains yielded a significant variance (1 ‰) for all the parameters considered with the exception of acetaldehyde and ethyl acetate. In particular, a considerable variability was determined for amyl alcohol, isoamyl alcohol and acetoin.

The data regarding secondary compounds were subjected to PCA, as reported in Fig. 3. PCA explained 63 % of the total variance using the first and second component. The first component discriminated the strains into two main groups (A and B), which represented the

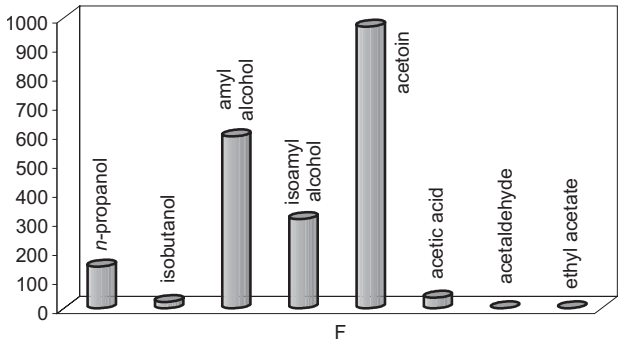

Fig. 2. Variance analysis by Fisher test

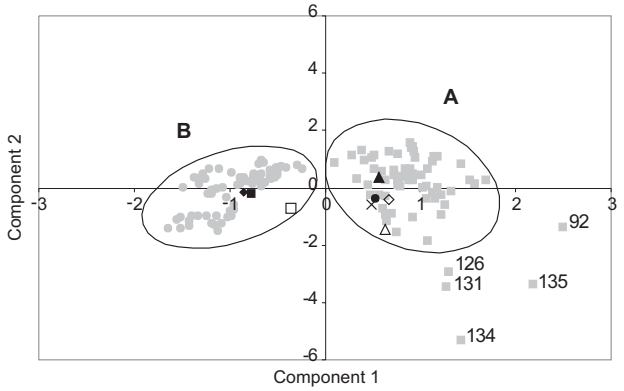

Fig. 3. Discrimination of the experimental wines obtained with strains of *Saccharomyces cerevisiae* and *Hanseniaspora uvarum* by PCA based on secondary compounds: ■ *Saccharomyces cerevisiae*, ● *Hanseniaspora uvarum*, △ Aglianico wine, ▲ *n*-propanol, ◇ isobutanol, ◆ acetoin, × isoamyl alcohol, ■ acetic acid, ● acetaldehyde, • ethyl acetate

two yeast species. Thus, all the strains of *S. cerevisiae* were in the group A and all the strains of *H. uvarum* in the group B. The variables that mainly contributed to characterise the apiculate strains were acetoin, acetic acid and ethyl acetate, while isobutanol, acetaldehyde and *n*-propanol determined the grouping of *S. cerevisiae* strains. Conversely, the second component differentiated the strains within each group, showing the strain variability of each species. It must be noted that *H. uvarum* strains (group B) exhibited a more uniform behaviour than *S. cerevisiae* strains (group A), which showed a significant variability. In particular five strains of *S. cerevisiae* were located very far from the others, characterised by specific and different profiles. In particular, the strain no. 92 was correlated with the lowest production level of acetic acid and high production of isobutanol. The strains nos. 126 and 131 were related to high levels of acetaldehyde, acetic acid and isoamyl alcohol production. The strain no. 134 was characterised by high levels of isobutanol, acetic acid and isoamyl alcohol production, whereas the strain no. 135 was correlated with low production of acetic acid and high production of isoamyl alcohol. The production of the secondary compounds is reported in Table 1, as determined by PCA.

Conclusions

Today, when there is a sound scientific and technical understanding of the role of yeasts in the fermentation process and when winemakers have greater confidence in their ability to manage this operation, there is an increasing interest to add further value to their wines by enhancing quality through natural fermentations.

In this context, the determination of a considerable strain biodiversity allowed the classification of the strains, of each of the species considered, in phenotypic groups, differentiated for opposite behaviour for each parameter. The first results in the work, performed on a great number of strains of both species, allowed to individuate the dominant phenotype, which can represent the typical yeast adapted to »Aglianico del Vulture« habitat and can consequently be potentially more suitable to conduct the fermentation. Among *H. uvarum*, a certain strain biodiversity was found, but however, the statistical analysis grouped the apiculate strains in a unique phenotype, group B, which represents the dominant metabolic phenotype of *H. uvarum*, characterised by high

Table 1. Levels of secondary compounds (mg/L) produced in Aglianico del Vulture by strains of the two yeast species

		PR	ISB	ACE	ISM	ACT	ADE	ETAC
H. uvarum	Group B							
Mean		15.2	24.3	41.2	135.9	1000.4	22.0	25.1
S.D.		4.6	5.7	13.7	28.7	220.3	4.8	21.4
CV/%		30.4	23.3	33.2	21.1	22.0	21.9	85.3
S. cerevisiae	Group A							
Mean		70.8	50.7	12.0	225.0	460.0	46.4	13.5
S.D.		60.1	28.7	6.8	131.5	206.5	28.5	12.7
CV/%		84.8	56.7	56.7	58.4	44.9	61.6	93.7
Different strains								
Sc 92		64.0	186.9	8.3	220.4	395.6	54.9	4.0
Sc 126		14.5	48.9	2.9	766.7	889.9	44.3	26.4
Sc 131		18.4	57.2	17.9	141.0	1024.0	229.9	53.6
Sc 134		22.0	129.8	7.6	518.4	1106.8	98.5	90.4
Sc 135		9.2	125.4	12.4	748.5	493.6	37.8	16.8

PR = *n*-propanol; ISB = isobutanol; ACE = acetoin; ISM = isoamyl alcohol; ACT = acetic acid; ADE = acetaldehyde; ETAC = ethyl acetate

production of acetic acid, ethyl acetate and acetoin, and low production of isobutanol, *n*-propanol and acetaldehyde. Conversely, among *S. cerevisiae*, a more significant strain biodiversity was discovered and, together with the dominant metabolic phenotype (group A), some strains exhibited a specific and unique behaviour. The group A of *S. cerevisiae* is characterised by high production of *n*--propanol isobutanol, isoamyl alcohol and acetaldehyde and low production of acetoin, acetic acid and ethyl acetate.

Taking into account that the use of starter cultures results in a more predictable control of fermentation and quality of wine, our results emphasise that the selection of yeast strains for winemaking should be addressed to the choice of strains conferring a specific typicality to the wine, respecting the individual characteristics of the vine variety.

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Biološka raznolikost kvasaca *Saccharomyces cerevisae* i *Hanseniaspora uvarum* s područja Aglianico del Vulture

Sažetak

Iz grožđa i mošta s područja Basilicata u Italiji izolirano je ukupno 140 sojeva *Saccharomyces cerevisae* i *Hanseniaspora uvarum*. Razvrstani su prema značajkama koje su pokazivali pri vrenju i u proizvodnji sekundarnih spojeva u moštu s područja Aglianico. Utvrđena je znatna prirodna raznolikost pojedinih sojeva. Unutar svake vrste sojevi su se razlikovali prema fermentativnoj aktivnosti i proizvodnji sekundarnih metabolita. U *Saccharomyces cerevisae* nađena je velika raznolikost pojedinih sojeva.