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Monoterpene alcohols release and bioconversion by *Saccharomyces* species and hybrids

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ABSTRACT

Terpene profile of Muscat wines fermented by *Saccharomyces* species and hybrid yeasts was investigated. The amount of geraniol decreased in most wines with respect to the initial must except for *Saccharomyces bayanus* wines. On the other hand, alpha-terpineol amount was higher in wines fermented by *Saccharomyces cerevisiae* and hybrid yeasts. The amount of linalool was similar in all wines and comparable to the amount in the initial must. Lower levels of beta-D-glucosidase activity were found in the hybrid yeasts with respect to *S. cerevisiae*. Moreover, no relationship between beta-D-glucosidase activity and terpenes profile in Muscat wines fermented with *Saccharomyces* species and hybrids was found. Growth of yeasts on minimum medium supplemented with geraniol showed bioconversion of geraniol into linalool and alpha-terpineol. Percentages of geraniol uptake and bioconversion were different between *Saccharomyces* species and hybrids. Strains within *S. bayanus*, *Saccharomyces kudriavzevii* and hybrids showed higher geraniol uptake than *S. cerevisiae*, whereas the percentage of produced linalool and alpha-terpineol was higher in *S. cerevisiae* and hybrid yeasts than in *S. bayanus* and *S. kudriavzevii*. The relationship between geraniol uptake and adaptation of *Saccharomyces* species to grow at low temperature is discussed.

1. Introduction

The yeasts responsible for alcoholic fermentation had been traditionally identified as strains pertaining to the species *Saccharomyces cerevisiae* and *Saccharomyces bayanus* var. *uvarum* (Demuyter et al., 2004; Naumov et al., 2000; Nguyen and Gaillardin, 2005; Pretorius, 2000). Recent studies based on genetic characterization of wine yeast strains have revealed the presence of hybrids *S. cerevisiae* × *S. bayanus* and *S. cerevisiae* × *Saccharomyces kudriavzevii* conducting wine fermentations in Central Europe (González et al., 2006; Masneuf et al., 1998; Nguyen et al., 2000). Moreover, several *Saccharomyces* hybrids are presently being commercialized for performing fermentations at low temperature and enhancing of varietal aroma in wines (Lallemand, Montreal, Canada).

Varietal aromas in wines are attributed to grape-derived flavour-active precursor compounds. These compounds are in free form in the "aromatic" grapes, although most of them are present as non-volatile and odourless glycoconjugates (Baumes, 2009; Loscos et al., 2007; van Rensburg and Pretorius, 2000; Ugliano and Henschke, 2009). Among the most important key odorants in the so-called "aromatic" grape varieties (e.g., Muscat) are monoterpenes such as linalool, geraniol, nerol, citronellol and α-terpineol. (Gunata et al., 1985; Loscos et al., 2007; Maicas and Mateo, 2005; Strauss et al., 1986; Ugliano and Henschke, 2009).

During must fermentation the grape glycosidic precursors are hydrolysed by action of glycosidases and the aromatic volatile compounds released into the wine. Among the most important glycosidases are β-glucosidases, α-L-arabinofuranosidases, α-L-rhamnosidases and β-D-xylosidases (Maicas and Mateo, 2005; van Rensburg and Pretorius, 2000; Sarry and Gunata, 2004). Characterization of wine yeasts with regard to glycosidase activities and release of varietal aromas has been done by different research groups on *S. cerevisiae* and non-*Saccharomyces* (Charoenchai et al., 1997; Esteve-Zarzoso et al., 1998; Fernández et al., 2000; Fleet, 2008; McMahon et al., 1999; Strauss et al., 2001; Ugliano et al., 2006; Zoecklein et al., 1997). Unfortunately, there are very few studies concerning the production of glycosidic enzymes and increase of terpene metabolites by different species within the genus *Saccharomyces*. Mateo and Di Stefano (1997) analysed the β-D-glucosidase activity of three *S. cerevisiae*, one *S. bayanus* and one hybrid *S. bayanus* × *S. cerevisiae* strain, and Ugliano et al. (2006) studied the β-D-glucosidase, α-L-rhamnosidase and α-L-arabinosidase activity and the volatile compounds released by two *S. cerevisiae* and one *S. bayanus* strains.

Wines produced under laboratory conditions with natural *Saccharomyces* hybrids have been found more aromatic than those produced by *S. cerevisiae* (Gangl et al., 2009; González et al., 2007). Moreover, a slight colour loss in *S. kudriavzevii* wines attributable to anthocyanin-β-

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D-glucosidase (Manzanares et al., 2000; van Rensburg and Pretorius, 2000) was indicative of potential enzymatic activity related to terpene release. Consequently, as the new *Saccharomyces* hybrids start playing a role in wine fermentation it is interesting to analyze the glycosidases spectrum and terpenes profile displayed by these hybrid yeasts in comparison with their parental species.

Grape aroma compounds are potential subjects to biochemical transformation by yeast enzymatic activity (Ugliano and Henschke, 2009). According to Darriet (1992) change in terpene content along alcoholic fermentation is the result of the joint action of several phenomena including mutual conversions, formation of terpene oxides, sweeping by CO_2 released during alcoholic fermentation, enzymatic and chemical hydrolysis of glycosidic bounds and adsorption on yeast cell walls.

Nevertheless, the differences in terpenes profile during fermentations performed by different yeasts cannot be fully accounted for, unless a direct effect of the physiological activity of the yeasts is considered (Zea et al., 1995). Limited studies concerning biotransformation of terpenes by *S. cerevisiae* have shown reduction of geraniol to citronellol, translocation of geraniol to linalool, isomerization of nerol to geraniol and cyclicizations of linalool to α-terpineol (Gramatica et al., 1982; King and Dickinson, 2000; Zea et al., 1995; Zoecklein et al., 1997).

The aim of the present study was to characterize *Saccharomyces* species and hybrids attending to their glycosidase activities, terpene release profiles and changes in terpene composition derived from yeast metabolism.

2. Materials and methods

2.1. Yeast strains

22 yeast strains belonging to the species *S. cerevisiae*, *S. kudriavzevii*, *S. bayanus* and hybrids between them were tested. Strains denomination and origin are listed in Table 1.

Yeast cultures used in all assays were grown overnight on GPY liquid medium (0.5% yeast extract, 0.5% peptone and 4% glucose; pH 6.5).

2.2. Plate assays of glycosidase activities

Plate assays of glycosidase (β-D-glucosidase, β-D-xylosidase, α-L-arabinofuranosidase and α-L-rhamnosidase) activities were done following the methodology of Manzanares et al. (1999) and Strauss et al. (2001).

2.3. β-D-Glucosidase activity, cellular location and enzymatic assays

Cells of *Saccharomyces* hybrids and parental species growing on 5 mL Muscat must were used to test the production of glycosidic enzymes. Must was sterilized by adding 1 mg/L of dimethyl-dicarbonate (Fluka, Switzerland) and allowed to settle overnight. Sterile must was inoculated with 10^6 cells and incubated at 16 °C for 72 h. Experiments were carried out in duplicate.

Enzyme activities were investigated in the culture supernatants (extracellular activity), cell extracts (intracellular activity) and in the whole cells (cell wall bound activity) as described by Manzanares et al. (1999). Activity was measured using ρ-nitrophenyl-β-D-glucopiranoside (pNPG) as substrate following the protocol of Manzanares et al. (1999). Release of ρ-nitrophenol was measured at 405 nm using a spectrophotometer (Ultrospec 2100pro, G.E. Healthcare). Enzymatic measurements were done in duplicate and the results were expressed as milliunits of enzyme activity per milligram of cells (mU/mg cells) (Table 1). One unit of enzyme activity (U) was defined as the amount of enzyme that released 1 μmol of ρ-nitrophenol per hour at 40 °C in McIlvaine buffer at pH 5.

2.4. Microfermentations

Microfermentations were carried out in Muscat must (reducing sugars 250 g/L and pH 3.2). Must was sterilized by adding 1 mg/L of dimethyl-dicarbonate (Fluka, Switzerland) and allowed to settle overnight. The sterilized must was distributed in 50 mL flasks, inoculated with 10^6 cells and incubated at 16 °C. Fermentations were carried out in duplicate. Must fermentation was completed

Table 1
List of strains of *Saccharomyces* species and hybrids used in this study, reference numbers in culture collections, commercial names and isolation sources. Terpene amounts (μg/mL) were detected by GC at the end of Muscat must fermentation. Enzymatic activities (mU/mg cells) were detected in cell wall of whole cells.

Species	Strain	Isolation source	Terpenes in wine		Enzymatic activity	
			Geraniol	Linalool	α-Terpineol	β-D-Glucosidase
S. cerevisiae	Control[a]		0.15 ± 0.06	0.60 ± 0.02	2.87 ± 0.08	1.90 ± 0.35
	Lalvin T73	Wine, Spain	0.18 ± 0.00	0.58 ± 0.10	2.22 ± 0.43	0.99 ± 0.15
	Fermiblanc Arom	Wine, France	0.18 ± 0.06	0.48 ± 0.04	1.55 ± 0.19	1.02 ± 0.23
	Fermicru Primeur	Wine, France	0.16 ± 0.01	0.47 ± 0.02	1.09 ± 0.01	0.88 ± 0.32
	UCLM S-377	Wine, Spain	0.14 ± 0.03	0.64 ± 0.04	0.92 ± 0.09	1.24 ± 0.12
S. bayanus	CECT 12627	Wine, Spain	0.55 ± 0.03	0.39 ± 0.11	0.23 ± 0.03	0.72 ± 0.04
	CECT 12629	Grape must, Spain	0.55 ± 0.00	0.39 ± 0.00	0.21 ± 0.01	0.61 ± 0.03
	CECT 12638	Grape must, Spain	0.91 ± 0.05	0.53 ± 0.06	0.29 ± 0.07	0.51 ± 0.08
	CECT 12669	Grape, Spain	0.52 ± 0.07	0.38 ± 0.07	0.25 ± 0.03	0.60 ± 0.12
	CECT 12930	Wine, Spain	1.08 ± 0.02	0.48 ± 0.06	0.38 ± 0.06	0.20 ± 0.07
S. kudriavzevii	IFO 1802	Decayed leaf, Japan	0.05 ± 0.01	0.50 ± 0.09	0.13 ± 0.04	0.29 ± 0.08
S.c. × S.b.	Lalvin S6U	Wine, Switzerland	0.05 ± 0.02	0.42 ± 0.05	0.80 ± 0.01	1.08 ± 0.20
	CECT 11000	Beer, Belgium	nd	0.40 ± 0.03	1.77 ± 0.36	0.66 ± 0.16
	CECT 11037	Beer, UK	0.07 ± 0.01	0.41 ± 0.04	1.73 ± 0.25	0.30 ± 0.06
	CECT 1885	Wine, Spain	nd	0.49 ± 0.05	1.18 ± 0.80	0.26 ± 0.09
S.c. x S.k.	Lalvin W27	Wine, Switzerland	0.10 ± 0.02	0.52 ± 0.02	1.69 ± 0.17	0.26 ± 0.09
	Lalvin W46	Wine, Switzerland	0.11 ± 0.02	0.50 ± 0.11	2.07 ± 0.34	0.26 ± 0.08
	SPG 16-91	Wine, Switzerland	nd	0.38 ± 0.08	2.66 ± 0.24	0.22 ± 0.06
	SPG 441	Wine, Switzerland	0.09 ± 0.02	0.44 ± 0.01	2.06 ± 0.08	0.24 ± 0.06
	CECT 1388	Beer, UK	nd	0.46 ± 0.04	1.62 ± 0.26	0.32 ± 0.06
	CECT 1990	Beer, UK	0.05 ± 0.02	0.48 ± 0.15	2.00 ± 0.34	0.29 ± 0.08
S.c. × S.b. × S.k.	CBS 2834	Wine, Switzerland	0.03 ± 0.01	0.41 ± 0.03	1.89 ± 0.47	0.30 ± 0.09
	CID 1	Cider, France	0.32 ± 0.09	0.42 ± 0.03	0.75 ± 0.17	0.33 ± 0.11

nd: not detected.

[a] *S. cerevisiae* recombinant strain YCB3$_5$ (Sánchez-Torres et al., 1998) was used as a control for β-D-glucosidase production.