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Technological properties of indigenous wine yeast strains isolated from wine production regions of Turkey

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Abstract The purpose of this study was to evaluate the important technological and fermentative properties of wine yeast strains previously isolated from different wine producing regions of Turkey. The determination of the following important properties was made: growth at high temperatures; fermentative capability in the presence of high sugar concentration; fermentation rate; hydrogen sulfide production; killer activity; resistance to high ethanol and sulfur dioxide; foam production; and enzymatic profiles. Ten local wine yeast strains belonging to *Saccharomyces,* and one commercial active dry yeast as a reference strain were evaluated. Fermentation characteristics were evaluated in terms of kinetic parameters, including ethanol yield ($Y_{P/S}$), biomass yield ($Y_{X/S}$), theoretical ethanol yield (%), specific ethanol production rate (q_p; g/gh), specific glucose uptake rate (q_s; g/gh), and the substrate conversion (%). All tested strains were able to grow at 37 °C and to start fermentation at 30° Brix, and were resistant to high concentrations of sulfur dioxide. 60 % of the strains were weak H_2S producers, while the others produced high levels. Foam production was high, and no strains had killer activity. Six of the tested strains had the ability to grow and ferment at concentrations of 14 % ethanol. Except for one strain, all fermented most of the media sugars at a high rate, producing 11.0–12.4 % (v/v) ethanol. Although all but one strain had suitable characteristics for wine production, they possessed poor activities of glycosidase, esterase and proteinase enzymes of oenological interest. Nine of the ten local yeast strains were selected for their good oenological properties and their suitability as a wine starter culture.

Keywords Indigenous wine yeast · *Saccharomyces* · Technological property · Enzymatic profile

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Introduction

In spontaneous wine fermentation, the conversion of grape must into wine is done by yeast strains naturally present on grape surfaces and in winery equipment. Today, commercial wine yeasts generally belong to *Saccharomyces cerevisiae*, and are used as starters to ensure rapid and complete must fermentation, and to achieve a high degree of reproducibility in the quality

of wines (Orlić et al. 2005). However, local, indigenous selected strains of *S. cerevisiae* are preferred, since these strains are well adapted to the environmental conditions of the wine production region, and can easily dominate fermentation. Moreover, the use of local wine yeasts allows production of wines with typical sensory characteristics of each wine area (Regodon et al. 1997; De Cos et al. 1998; Orlić et al. 2005; Nikolaou et al. 2006; Lopes et al. 2007).

The selection of wine yeasts is performed by determining their important technological properties, which can vary among strains. Important characteristics of wine strains include the following: low production of volatile acidity, tolerance to high ethanol concentration, production of ethanol according to sugar content in the must, complete fermentation of sugar in the must, high fermentation rate, ability to grow at high temperatures and high sugar concentrations, tolerance to sulfur dioxide, low production of SO_2 and H_2S, easy settling after fermentation, low foam production, possession of a killer or neutral phenotype, low acetaldehyde production, adequate glycerol production, and a limited production of higher alcohols (Esteve-Zarsozo et al. 2000; Nikolaou et al. 2006). The enzymatic profile of yeasts is also one of the important technological properties that can enhance the varietal character of the wine (Iranzo et al. 1998).

The yeast strains used in this study were selected based on very successful experiences of regional wine producers over many years. These strains have high fermentative capability. The main objective of this study was to evaluate some important technological properties of these indigenous wine yeast strains previously isolated from different wine production areas of Turkey and identified by biochemical testing. Kinetic parameters of alcoholic fermentation conducted with indigenous yeasts were calculated in order to comprehensively determine the fermentative power of strains. In this way, it would be possible to select the most adequate strain for use as starter culture in the wine industry.

Materials and methods

Yeast strains

Ten indigenous *Saccharomyces* yeast strains isolated from wine regions in Turkey were used in this study (Table 1). These isolates had previously been identified by classical biochemical testing based on carbohydrate fermentation patterns, and maintained in the Culture Collection of the Food Engineering Department of Ankara University. A commercial *S. cerevisiae* starter strain Fermicru AR2 *Saccharomyces cerevisiae* No. LO122 was used as a reference standard, and kindly provided by Kavaklıdere Winery, Turkey's leading wine producer. For experiments determining the killer phenotype, three reference and control strains were used: *S. cerevisiae* NCYC 232 (killer strain, K_1), *S. cerevisiae* NCYC 738 (killer strain, K_2), and *S. cerevisiae* NCYC 1006 (sensitive

Table 1 Yeast cultures examined in the study

Culture no.	Yeast species	Culture code in the collection	Region and origin
1	*Saccharomyces cerevisiae* Kalecik II	Y2	Ankara, Akman (1939)
2	*Saccharomyces cerevisiae* T1	Y3	Not known
3	*Saccharomyces cerevisiae* Narince 3	Y7	Amasya and Tokat, Pamir (1974)
4	*Saccharomyces cerevisiae* CA	Y10	Not known
5	*Saccharomyces cerevisiae* Kalecik I	Y15	Ankara, Akman (1939)
6	*Saccharomyces cerevisiae* Narince 4	Y17	Amasya and Tokat, Pamir (1974)
7	*Saccharomyces cerevisiae* J33	Y21	Not known
8	*Saccharomyces cerevisiae* CH	Y23	Not known
9	*Saccharomyces cerevisiae* Hasandede	Y25	Ankara, Akman (1939)
10	*Saccharomyces bayanus* 1A (syn. *Saccharomyces uvarum*)	Y47	Nevşehir, Şahin (1978)
Reference	Fermicru AR2 *Saccharomyces cerevisiae* No. LO122	–	DSM Food Specialities, Holland

strain). A *Candida krusei* strain was also used as positive control for the hydrogen sulfide (H_2S) production experiments. This strain was kindly provided by Prof. Dr. Yeşim Özbaş from the Culture Collection of Hacettepe University.

Technological properties of yeast strains

Growth at high temperature

The ability to grow at high temperature was determined by inoculating a loopful of 24-h yeast cultures in YPG Broth and incubating at 37° and 42 °C for 5 days (Nikolaou et al. 2006).

Fermentative capability at high sugar concentration

Certain modifications (Iranzo et al. 1998) to the method suggested by Parish and Carroll (1987) were implemented. Briefly, a loopful of 24-h yeast cultures were inoculated into YPG Broth adjusted to Brix 30° and included the use of implemented Durham tubes. After a three-day incubation period at 30 °C, the tests were considered as positive if the yeast strains produced gas in two-thirds of the Durham tubes.

Fermentation rate

Fermentations were carried out in duplicate at 25 °C in 500 mL brown-colored fermentation flasks containing 200 mL YPG Broth with 20 % (w/v) glucose adjusted to pH 3.5. The YPG Broth was inoculated with a certain volume of 24-h yeast cultures at a concentration of 10^6 cells/mL. The flasks were plugged with glass fermentation traps containing sterile distilled water to allow only CO_2 to emerge from the system. The fermentation was monitored by measuring the weight loss every day until the end of fermentation during which the weight loss due to CO_2 production became constant. The fermentation rate was expressed as "g CO_2/Lh" based on the weight loss within the first and third days of fermentation (Iranzo et al. 1998; Pérez-Coello et al. 1999).

H_2S production

The H_2S production of the yeast strains was evaluated on the basis of their colony color on Bismuth Sulfite Glucose Glycine Yeast (BIGGY) Agar (Sipiczki et al. 2001; Vicente et al. 2006). *Candida krusei* strains form silvery brown-black colonies on BIGGY Agar plates, as specified in the manufacturers' instructions (Anonymous 2014). Therefore, a *Candida krusei* strain was used as positive control. Briefly, yeast strains were inoculated on BIGGY Agar and incubated at 30 °C for 3 days. Brown and black colonies were considered as H_2S producers. The color of colonies was graded according to the following scale; 1: white, 2: cream, 3: light brown, 4: brown, 5: dark brown, and 6: black.

Killer activity

The killer activity and sensitivity of the strains to K_1, K_2 killer toxins were determined using the agar diffusion technique on YPG-MB Agar (10 g/L yeast extract, 20 g/L glucose, 10 g/L peptone, 20 g/L agar, 0.03 g/L methylene blue) buffered at pH 4.5 with 0.1 M citrate–phosphate as described by Özçelik et al. (1996). *S. cerevisiae* NCYC 232 (killer strain, K_1), *S. cerevisiae* NCYC 738 (killer strain, K_2) and *S. cerevisiae* NCYC 1006 (sensitive strain) were used as reference yeast strains.

Tolerance of ethanol and SO_2

Ethanol and SO_2 tolerance of yeast strains were determined according to Parish and Carroll (1987). YNB (Yeast Nitrogen Base, pH 3.3) containing 0, 8, 10, 12, 14 % (v/v) ethanol and 1 % (w/v) glucose were inoculated with a certain volume of 24-h yeast cultures at a concentration of 10^6 cells/mL. After inoculation, the media was covered with Vaseline and left for incubation at 30 °C for 3 days. Cloudiness in the tubes was accepted as positive yeast growth, whereas the movement of Vaseline towards the surface was accepted as positive fermentation ability. The same procedure was applied to the SO_2 tolerance analysis using 0, 50, 100, 150, 200 mg/L concentrations of SO_2.

Foam production

Foam production was examined according to Regodon et al. (1997). Yeast strains (10^6 cells/mL, grown in YPG Broth at 28 °C for 24 h) were inoculated into (16×160 mm^2) test tubes containing 10 mL sterile must and incubated at 25 °C. The foam height was

measured every day throughout the fermentation. The yeasts were classified into three groups according to the maximum foam height reached: F0: producing below 2 mm foam; F1: producing 2–4 mm foam; and F2: producing above 4 mm foam.

Enzymatic profile

API ZYM (bioMérieux) test kits were used to determine the enzymatic profile of the yeast strains.

Analysis performed during fermentation experiments

Sugar

The initial and final glucose concentrations were enzymatically determined using Boehringer, R-Biopharm commercial kits (Cat. No. 0 716 251).

Ethanol

The initial and final ethanol content was analyzed through with a direct injection of gas chromatography (Shimadzu GC-14B, Kyoto, Japan) equipped with a flame ionization detector (FID). Prior to injection, the samples were filtered through a 0.45 μm PVDF (polyvinylidene fluoride) filter (Millipore, Bedford, MA, USA). One microliter of samples was injected into the GC system with a glass column with 1/8″ inner diameter and 2 m length filled with Chromosorb 101 (80/100 mesh). The column oven, injection and detector temperatures were maintained at 160, 220 and 250 °C, respectively. Nitrogen was used as carrier gas, and the fuel gas was a mixture of hydrogen and air.

Volatile acidity

The volatile acidity in the final samples of fermentation was determined by steam distillation, followed by titration with 0.1 N NaOH, and was expressed as acetic acid (g/L) (Anonymous 2013a).

Biomass values

For dry cell weight determination, 5 mL culture samples taken at the beginning and end of each fermentation were centrifuged at 5,000 rpm for 10 min, washed twice, and re-suspended in cold, sterile distilled water. These cell suspensions were placed on pre-weighed dishes and dried at 105 °C for constant weight. The biomass values were obtained by subtracting the initial yeast culture dry weight from the final one (Lopes et al. 2007).

Results and discussion

Technological properties of *Saccharomyces* strains

Ten indigenous yeast strains and commercial yeast were evaluated for their suitability as wine starters by using assays to assess fermentative capacity, and adaption to stressful conditions. Wine yeast strains were selected for certain desirable characteristics: growth at 37 and 42 °C, ability to start fermentation in the presence of high sugar concentration (Brix 30°), high fermentation rate, low production of H_2S, presence of killer activity, resistance to high ethanol and sulfur dioxide, and low foam production.

Growth characteristics of the yeast strains at 37 as well as 42 °C were carried out because of the possibility of temperature increases occurring during wine fermentation. The 42 °C growth criterion in the wine starter selection is important for warm wine production regions such as Greece, Spain (Regodon et al. 1997; Nikolaou et al. 2006). All strains were able to grow at 37 °C, whereas no growth was observed at 42 °C. Similar results were obtained by other groups (Regodon et al. 1997; Nikolaou et al. 2006, 2007). Regodon et al. (1997) reported that all selected strains of *S. cerevisiae* they used grew at 37 °C, but that 75 % of the strains also grew at 42 °C. Nikolaou et al. (2006) reported that all six strains of *S. cerevisiae* they selected from among 110 yeast isolates grew at 37 °C, and four of these also grew at 42 °C. Nikolaou et al. (2007) reported that all tested 27 *S. cerevisiae* isolates from naturally fermented Greek wines grew at 37 °C, and from these two-thirds grew at 42 °C also. It is known that *S. bayanus* can not grow at 37 °C (Kurtzman and Fell 1998). Contrary to expectations, it was found that *S. bayanus* 1A was able to grow at 37 °C. This could be due to the misidentification of this isolate in previous years. As the genus *Saccharomyces* contains closely related species, it is difficult to discriminate these species by conventional biochemical tests (Blättel et al. 2013).

Resistance to high sugar concentrations is a key property for the fermentation of musts having high sugar contents in hot regions (Iranzo et al. 1998; Ortiz et al. 2013). When the sugar concentration is very high in the grape and must, yeast growth can be inhibited due to increases in osmotic pressure (Hutkins 2006). High initial sugar content in the grape must can affect growth by extending the lag phase, decreasing the growth rate, and reducing the biomass and ethanol tolerance in the final stages of fermentation (Charoenchai et al. 1998). All strains were able to start fermentation at 30° Brix. Parish and Carroll (1987) reported that all *Saccharomyces* strains they examined were able to initiate fermentation at 30° Brix. Iranzo et al. (1998) found that all 74 *Saccharomyces* strains they screened grew in the must at 30°Brix. Charoenchai et al. (1998) showed that an increase in sugar concentration in the must from 200 to 300 g/L resulted in a decreased growth rate for some yeasts, and a decreased final cell biomass for all yeasts. They suggested that these results were related to a higher need for energy to maintain their viability under high sugar level conditions.

One of the most important properties used for the selection of the wine starter is the fermentation rate (Reed and Nagodawithana 1988). This trait is particularly important in warm winemaking regions due to the high sugar content of the must that may give rise to stuck or sluggish fermentations as a result of the inhibition of yeast metabolism (Ortiz et al. 2013). It is desirable for wine yeast to complete the fermentation with a given time, and that it possess a capability of fermenting all of the fermentable sugars present in the must (Esteve-Zarsozo et al. 2000; Ullivarri et al. 2011). With the exception of *S. cerevisiae* J33, we found that all tested strains fermented the medium vigorously at a rate of over 1.0 g CO_2/Lh (Table 2). Although *S. cerevisiae* J33 had the lowest fermentation rate among the tested strains, it showed a higher fermentation rate than the value (0.25 g CO_2/Lh) considered suitable for wine yeast by Iranzo et al. (1998). Fermentation rates of yeasts in this study were higher than those in other studies by Ciani and Maccarelli (1998) and Orlić et al. (2005) (0.60–0.72 g CO_2/Lh, 0.83 g CO_2/Lh, respectively). However, rates were similar to a study conducted by Budroni et al. (2006) (0.96–1.13 g CO_2/Lh).

A high level of volatile acidity can result in a vinegary taint; a very undesirable characteristic for wines (Esteve-Zarsozo et al. 2000; Sipiczki et al. 2001). Wines having elevated concentrations of acetic acid (more than 0.8 g/L) are regarded as defective (Swiegers et al. 2005; Lopes et al. 2007). The values of volatile acidity were between 0.52 g/L (*S. cerevisiae* CA) and 1.14 g/L (*S. cerevisiae* J33) (Table 2). The culture numbers 7, 9 and 10 surpassed the value of 0.8 g/L acetic acid. There are many indications that *S. cerevisiae* J33 is not a convenient strain for wine production: a low fermentation rate, low ethanol production, a low rate of sugar consumption, and a relatively high volatile acid formation. The *S. bayanus* 1A strain is also a high volatile acidity producer. Similarly, Şahin (1982) showed that the highest volatile acidity was produced by *S. bayanus* among the tested yeast strains. However, many authors reported that *S. bayanus* produced lower quantities of acetic acid than *S. cerevisiae* (Castellari et al. 1994; Antonelli et al. 1999; Masneuf-Pomarède et al. 2010). This discrepancy could be due to the misidentification of this isolate in previous years or the strain dependence of acetic acid production.

As an overall consideration of volatile acidity values, its production by all strains exceeded 0.5 g/L acetic acid. This may have been due to the low volumes used in the fermentation experiments. It should be noted that others have reported that the formation of varying levels of volatile acidity in *Saccharomyces* appear to differ by strain: 0.1-0.9 g/L (Giudici and Zambonelli 1992), 0.21–0.66 g/L (Shinohara et al. 1994), 0.46–0.80 g/L (Regodon et al. 1997), 0.22–1.2 g/L (Orlić et al. 2005), 0.31–0.48 g/L (Nikolaou et al. 2006), 0.56–0.69 g/L (Ooi et al. 2008).

H_2S production during wine fermentation is an undesirable property due to its unpleasant rotten odor (Sipiczki et al. 2001; Nowak et al. 2004). H_2S can arise from the reduction of sulfur-containing amino acids by sulfite reductase activity in the yeasts during fermentation (Budroni et al. 2006; Ullivarri et al. 2011). The yeast strains forming cream- and light brown-colored colonies were considered to be weak H_2S producers, while ones forming brown and dark brown colonies were considered strong H_2S producers. 60 % of the strains (*S. cerevisiae* Kalecik I, *S. cerevisiae* Narince 3, *S. cerevisiae* Narince 4, *S. cerevisiae* J33, *S. cerevisiae* CH, *S. bayanus* 1A) were weak H_2S producers, whereas the remaining strains produced high levels of H_2S. *S. bayanus* 1A was found to

Table 2 The technological properties of yeast strains

Culture no.	Yeast strains	Growth at high temperature 37 °C/ 42 °C	Fermentation at high sugar concentration (Brix 30)[a]	Fermentation rate (g CO_2/Lh)	Volatile acidity (g/L)	H_2S production	Killer activity[b]	Resistance to killer toxin[c] K1/K2	Resistance to ethanol (% v/v)	Resistance to SO_2 (mg/L)	Foam production[d]
1	*S.cerevisiae* Kalecik II	+/−	+	1.48 ± 0.08	0.62 ± 0.065	3:light brown	–	−/−	14	200	F2
2	*S.cerevisiae* T1	+/−	+	1.52 ± 0.03	0.58 ± 0.041	4:brown	–	−/−	14	200	F2
3	*S. cerevisiae* Narince 3	+/−	+	1.55 ± 0.08	0.53 ± 0.018	3:light brown	–	−/−	12	200	F2
4	*S.cerevisiae* CA	+/−	+	1.42 ± 0.14	0.52 ± 0.078	4:brown	–	−/−	12	200	F2
5	*S.cerevisiae* Kalecik I	+/−	+	1.56 ± 0.06	0.56 ± 0.060	4:brown	–	−/−	14	200	F2
6	*S.cerevisiae* Narince 4	+/−	+	1.34 ± 0.02	0.80 ± 0.066	3:light brown	–	−/−	14	200	F2
7	*S.cerevisiae* J33	+/−	+	0.59 ± 0.06	1.14 ± 0.011	3:light brown	–	−/−	8	200	F1
8	*S.cerevisiae* CH	+/−	+	1.09 ± 0.01	0.72 ± 0.027	3:light brown	–	−/−	14	200	F2
9	*S.cerevisiae* Hasandede	+/−	+	1.25 ± 0.09	0.86 ± 0.058	4:brown	–	−/−	14	200	F2
10	*S.bayanus* 1A	+/−	+	1.06 ± 0.03	1.10 ± 0.044	2:cream	–	−/−	12	200	F2
Reference	Fermicru AR2 *S. cerevisiae* No. LO122	+/−	+	1.30 ± 0.04	0.70 ± 0.085	3:light brown	+	−/+	10	200	F2

[a] + positive growth, − negative growth

[b] + killer phenotype, − no killer phenotype

[c] + resistant to K1/K2 toxin, − sensitive to K1/K2 toxin

[d] *F1* producing 2-4 mm foam, *F2* producing above 4 mm foam

produce the lowest levels of H_2S based on its cream-colored appearance on BIGGY Agar. This finding complies with results from Esteve-Zarsozo et al. (2000), who showed no tested *S. bayanus* strains that produced H_2S. Conversely, Sipiczki et al. (2001) reported that *S. bayanus* was strong H_2S producer.

All tested strains in this study produced H_2S to some degree. A survey of the literature shows varying levels of H_2S produced in *Saccharomyces*. Orlić et al. (2005) and Budroni et al. (2006) reported that *S. cerevisiae* strains produced low levels of H_2S, Lopes et al. (2007) reported moderate levels, and Iranzo et al. (1998) and Sipiczki et al. (2001) found high levels. In contrast, Pérez-Coello et al. (1999) reported that most of the strains they tested did not produce H_2S.

In respect to killer activity, none of the indigenous strains showed killer phenotype or resistance to K1/K2 toxin, whereas the reference strain had killer activity and resistance to K2 toxin. Resistance to K2 killer toxin is important in wine fermentation, since it is active at the pH level typical of wine products (Lopes et al. 2007).

Wine yeast starter cultures are required not only to resist high concentrations of ethanol, but also to be able to ferment favorably in the presence of 12 % (v/v) ethanol (Nikolaou et al. 2006). Six of the tested strains (no. 1, 2, 5, 6, 8, 9) had the ability to grow and ferment at concentrations of 14 % ethanol in this study. The majority of *Saccharomyces* strains had both growth and fermentation capabilities at 10 % (v/v) ethanol in most other studies pertinent to ours (Iranzo et al. 1998; Nikolaou et al. 2006; Lopes et al. 2007).

All yeast strains resisted the maximum SO_2 concentration tested (200 mg/L). Parish and Carroll (1987) analyzed the SO_2 tolerance of four *S. cerevisiae* strains and found that two of them tolerated 300 mg/L SO_2, and one tolerated 200 mg/L SO_2. The other yeast strains were sensitive to SO_2 levels of 25 mg/L. Iranzo et al. (1998) reported that their *Saccharomyces* strains were resistant to 250 mg/L SO_2, while Regodon et al. (1997) indicated a resistance to 300 mg/L SO_2.

Foam production during fermentation is not desired since it decreases the available capacity of the fermentation vessel and encourages contamination. However, almost all strains, including the reference strain, produced high foam (F2). Only *S. cerevisiae* J33 strain produced moderate foam (F1), which was probably due to the slow growth characteristic of this strain. Low foam production of *Saccharomyces* was reported by Regodon et al. (1997). Nikolaou et al. (2006) assayed selected *S. cerevisiae* strains, showing foaming to 1.5–3.5 mm heights.

API-ZYM results revealed that local yeast strains showed similar characteristics with reference to the commercial strain in terms of enzymatic profiles (Table 3). Leucine arylamidase and acid phosphatase activities were found in all strains. In addition, *S. cerevisiae* Kalecik II, *S. cerevisiae* T1, *S. cerevisiae* CA, *S. cerevisiae* Kalecik I, *S. cerevisiae* Narince 4, *S. cerevisiae* CH, *S. cerevisiae* Hasandede and the reference strain possessed naphthol-AS-BI-phosphohydrolase activity at different levels. The reference strain exhibited cystine arylamidase activity, which was also detected in local test strains at low levels.

Glycosidases are responsible for the hydrolysis of glycosidic monoterpenes, converting them into a free odiferous form which contributes to the aromatic character of wine (Nikolaou et al. 2006). Among the glycosidases assayed (α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucoaminidase, α-mannosidase, α-fucosidase), only a slight α-glucosidase activity was observed in *S. cerevisiae* T1. These results reflect the poor level of glycosidase activity of *Saccharomyces*, and were in agreement with the reports of others (Iranzo et al. 1998; Orlić et al. 2005; Nikolaou et al. 2006). Yeast esterase activity also plays an important role in varietal and fermentative flavor of wine. Yeast strains examined in this study showed weak esterase and esterase/lipase activities.

One of the other enzyme groups important in wine production is the proteinases. Since proteins in the must are not heat stable, and thus, a haziness from denaturation can result within white wines after bottling and during storage. Therefore, enzymatic hydrolysis of these haze-forming proteins is important for wine stabilization and clarification (Moreno-Arribas and Polo 2005). High leucine arylamidase and low cystine arylamidase activity was observed in all strains in this study. Iranzo et al. (1998) reported that the strains they studied did not possess endopeptidases (trypsin, α-chymotrypsin) or exopeptidases (arginine, proline, leucine-glycine, pyroglutamic acid, glycine, histidine, and glutamil-glutamic acid arylamidase). These strains only possessed low levels of leucine, valine, cystine, phenylalanine, tyrosine, alanine and serine arylamidase activity. Overall, tested indigenous strains possessed poor enzymatic profiles in terms of

Table 3 Enzymatic profiles of yeast strains by API-ZYM test kits

Enzyme assayed	Yeast species										
	S. cerevisiae Kalecik II	*S. cerevisiae* T1	*S. cerevisiae* Narince 3	*S. cerevisiae* CA	*S. cerevisiae* Kalecik I	*S. cerevisiae* Narince 4	*S. cerevisiae* J33	*S. cerevisiae* CH	*S. cerevisiae* Hasandede	*S. bayanus* 1A	Reference
Control	0	0	0	0	0	0	0	0	0	0	0
Alkaline phosphatase	0	0	0	0	0	0	0	0	0	0	0
Esterase (C 4)	1	2	1	1	1	2	2	1	1	1	2
Esterase Lipase (C 8)	1	1	2	1	1	1	1	1	1	1	2
Lipase (C 14)	0	1	0	1	0	0	0	0	0	0	0
Leucine arylamidase	5	5	5	5	5	5	5	5	5	5	5
Valine arylamidase	0	1	1	1	0	1	0	0	0	0	2
Cystine arylamidase	1	2	1	2	1	1	1	1	1	1	3
Trypsin	0	1	0	1	0	0	0	0	0	0	0
α-chymotrypsin	0	0	0	1	0	0	0	0	0	0	0
Acid phosphatase	5	5	5	5	5	5	3	5	5	5	5
Naphthol-AS-BI-phosphohydrolase	4	3	2	4	4	3	1	3	4	1	5
α-galactosidase	0	0	0	0	0	0	0	0	0	0	0
ß-galactosidase	0	0	0	0	0	0	0	0	0	0	0
ß-glucuronidase	0	0	0	0	0	0	0	0	0	0	0
α-glucosidase	0	1	0	0	0	0	0	0	0	0	0
ß-glucosidase	0	0	0	0	0	0	0	0	0	0	0
N-acetyl-ß-glucosaminidase	0	0	0	0	0	0	0	0	0	0	0
α-mannosidase	0	0	0	0	0	0	0	0	0	0	0
α-fucosidase	0	0	0	0	0	0	0	0	0	0	0

0 negative activity; *1–4* intermediate activity; *5* maximum activity

glycosidases, esterases and proteinases of oenological interest. Therefore, it seems that the use of these strains will probably not enhance the varietal character of the wine.

On the basis of these results, all but one (*S. cerevisiae* J33) of the yeast strains had suitable characteristics for wine production, and could be recommended as commercial wine starters. However, because of their low levels of enzymatic activities, they lacked the suitable properties required. Consequently, nine yeast strains examined in this study were selected for their valuable oenological properties; showing their suitability as a wine starter culture.

Selection of ideal strains for wine production

In order to select the most appropriate strains for wine production, the schematic exclusion test of Pérez-Coello et al. (1999) was applied (Fig. 1). In this context, the yeast strains were first grouped based on their fermentation rate. Since all strains had fermentation rates higher than 0.2 g CO_2/Lh, the strains producing undesirably high H_2S (no. 2, 4, 5, 9) were then discarded. Of the remaining six strains, no.7 had residual sugar over than 4 g/L and was thus rejected as suitable for wine production. Thus, five of all tested strains (no. 1, 3, 6, 8, 10) were selected for the desirable properties of having a high fermentation rate, low H_2S production, low residual sugar concentration, high tolerance to SO_2 and ethanol, and the ability to begin fermenting at 30° Brix. However, none of these strains showed killer or neutral activity. With respect to enzymatic activities, which play an important role in wine aroma, the five selected strains showed quite limited profiles. Those strains eliminated (no. 2, 4, 5, 9) due to their high production of H_2S, could also be evaluated for use in wineries because of other appropriate oenological properties. These strains had respective fermentation rates of 1.52, 1.42, 1.56, 1.25 g CO_2/Lh; respective ethanol tolerance of 14, 12, 14, 14 % (v/v); respective ethanol production of 11.5, 11.6, 12.1, 12.4 % (v/v); and respective volatile acidity of 0.58, 0.52, 0.56, 0.86 g/L. These strains also had ability to grow at 37 °C and to start fermentation at 30° Brix; they were resistant to 200 mg/L SO_2. With the exception of strain *S. cerevisiae* J33, all tested strains showed at least similar if not better properties to the reference commercial strain, and were therefore suitable for use as starter culture in wine production. Nurgel et al. (2002a, 2002b) have reported that the use in wine fermentation of indigenous selected strains from the Emir and Kalecik karası grape varieties of Turkey increases the fermentation rate compared to commercial yeast strains.

Results of fermentation experiments

Fermentations having residual sugar levels lower than 0.4 % are considered to be complete fermentations. Wines with high sugar concentrations after fermentation are more susceptible to microbial spoilage, and should not be bottled without their microbial stability being confirmed (Bisson 1999). In general, residual sugar levels should be less than 4 g/L at the end of fermentation (Regodon et al. 1997). Table 4 lists all the strains that exhausted nearly all glucose in the medium at the end of fermentation. This list excludes *S. cerevisiae* J33 which at the end of fermentation had a residual sugar concentration of 65.9 g/L. The strain *Saccharomyces bayanus* 1A also had a partially higher residual sugar (0.53 g/L) than other strains tested. Our findings agree with Esteve-Zarsozo et al. (2000) who found that *S. bayanus* left more residual sugar than *S. cerevisiae* at the end of fermentation.

With the exception of *S. cerevisiae* J33, the yeast strains in these experiements had a high fermentative capability considering their low residual sugar, and compared to results of similar studies. Regodon et al. (1997) reported that residual sugar levels ranged from 1.00 to 4.00 g/L at the end of their fermentation experiments with selected *S. cerevisiae* strains in the 21.5°Brix must. Fermentation experiments by Clemente-Jimenez et al. (2004) showed that *S. cerevisiae* could ferment virtually all initial glucose (199.08 g/L) in the must with a residual glucose concentration of 0.25 g/L. Nikolaou et al. (2006) reported that their selected yeast strains left 0.2–3.9 g/L sugar after fermentation.

The fermentation power is defined as the maximum amount of ethanol (as volume percent [%v/v]) obtainable by a yeast strain in the presence of an excess of sugar (Martini, 2003; Cocolin et al. 2004; Lopes et al. 2007). Table 4 shows that the lowest ethanol-producing strain was *S. cerevisiae* J33, with 7.7 ± 0.8 % (v/v) ethanol. This result is not surprising given that this strain had already showed a low fermentation rate, low ethanol tolerance, and high residual sugar after

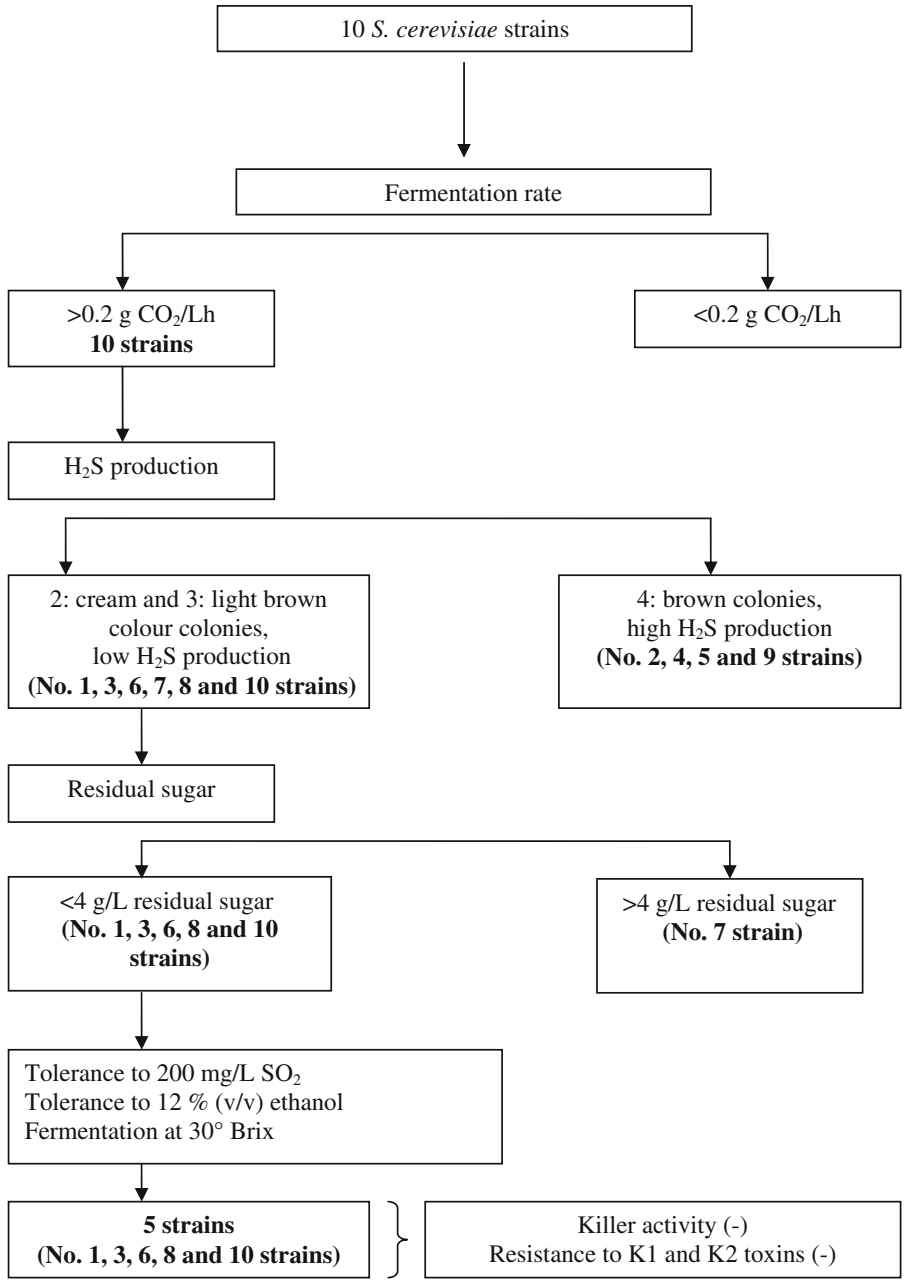


Fig. 1 Exclusion test for selection of the yeast strains with the best technological properties for wine production

fermentation, as reported in our results. The other strains produced much higher levels of ethanol, in the range of 11.0–12.4 % (v/v). *S. cerevisiae* Narince 4 and *S. cerevisiae* Hasandede had the highest yield of ethanol (12.4 %). Regodon et al. (1997) indicated that wine yeasts should produce ethanol higher than 8 %. According to the International Organization of Vine and Wine, wines are required to have at least an 8.5 % (v/v) alcohol content (Anonymous 2013b). Tested strains are also suitable for wine production based on this criterion. Orlić et al. (2005) found that 17.4 % of the tested strains produced more than 10 % (v/v) ethanol and the highest ethanol concentration was 11.3 % (v/v). A study by Regodon et al. (1997) showed

Table 4 Kinetic parameters of alcoholic fermentation with yeast strains

Culture no.	Yeast strains	Residual sugar concentration (g/L)	Ethanol % (v/v)	$Y_{X/S}$[a]	$Y_{P/S}$[b]	Theoretical ethanol yield (%)[c]	q_S[d] (g/gh)	q_P[e] (g/gh)	Substrate conversion (%)[f]
1	*S. cerevisiae* Kalecik II	0.01 ± 0.004	12.2 ± 0.1	0.025	0.49	95.8	0.21	0.10	100.0
2	*S. cerevisiae* T1	0.01 ± 0.004	11.5 ± 0.9	0.020	0.46	91.0	0.25	0.12	100.0
3	*S. cerevisiae* Narince 3	0.04 ± 0.003	11.4 ± 0.3	0.020	0.45	88.4	0.26	0.12	100.0
4	*S. cerevisiae* CA	0.04 ± 0.017	11.6 ± 0.7	0.017	0.44	85.9	0.31	0.13	100.0
5	*S.cerevisiae* Kalecik I	0.02 ± 0.005	12.1 ± 0.2	0.015	0.45	87.1	0.34	0.15	100.0
6	*S. cerevisiae* Narince 4	0.07 ± 0.040	12.4 ± 0.3	0.014	0.47	92.8	0.37	0.18	100.0
7	*S.cerevisiae* J33	65.91 ± 5.337	7.7 ± 0.8	0.015	0.46	89.3	0.34	0.16	66.5
8	*S.cerevisiae* Champ. Haut.	0.11 ± 0.016	11.9 ± 0.3	0.016	0.45	88.7	0.33	0.15	99.9
9	*S. cerevisiae* Hasandede	0.07 ± 0.042	12.4 ± 0.3	0.015	0.46	90.4	0.34	0.16	100.0
10	*S. bayanus* 1A	0.53 ± 0.329	11.0 ± 0.1	0.014	0.41	80.0	0.37	0.15	99.7
Reference	Fermicru AR2 *S. cerevisiae* No. LO122	0.09 ± 0.058	11.3 ± 0.2	0.019	0.42	82.7	0.27	0.12	100.0

Fermentation conditions: fermentation medium = YPG Broth with 20 % (w/v) glucose, pH 3.5; volume of fermentation medium = 200 mL; temperature = 25 °C; yeast inoculum concentration = 10^6 cells/mL; duration of fermentation = 8 days

Values express the mean ± standard deviation of duplicate measurements obtained in two independent experiments

[a] $Y_{X/S}$ biomass yield coefficient, ratio of substrate conversion to cells

[b] $Y_{P/S}$ ethanol yield coefficient, ratio of substrate conversion to ethanol

[c] Theoretical ethanol yield (%): $(Y_{P/S}/0.511) \times 100$

[d] q_S g substrate/g biomassxh

[e] q_P g ethanol/g biomassxh

[f] Substrate conversion (%): $(S_0 - S)/S_0 \times 100$

that selected yeast strains produced 8.0-8.5 % (v/v) ethanol in fermentation experiments of 21.5° Brix must.

The alcoholic fermentation of the indigenous strains were also assessed with the following kinetic parameters: ethanol yield ($Y_{P/S}$), biomass yield ($Y_{X/S}$), theoretical ethanol yield (%), specific ethanol production rate (q_p, g/gh), specific glucose uptake rate (q_s, g/gh), and substrate conversion (%). The calculated kinetic values are given in Table 4. The yield of ethanol from glucose ($Y_{P/S}$) obtained at the end of fermentation varied between 0.41 and 0.49 and 80.0–95.8 % of the theoretical ethanol yield. The specific ethanol production of the strains ranged from 0.10 to 0.18 g/gh, and specific glucose uptake rate ranged from and 0.21–0.37 g/gh. The *S. cerevisiae* J33 strain, which our results had already shown was inadequate for wine production with its low fermentative power, presented high kinetic values on the basis of ethanol yield ($Y_{P/S}$), theoretical ethanol yield (%), specific ethanol production rate (q_p), specific glucose uptake rate (q_s). These calculations were based on consumed sugar concentrations. All other strains utilized the totality of sugars in the media, with substrate conversion rates of 99.7–100 %. In contrast the substrate conversion rate was 66.5 % for the J33 strain.

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