EVALUATION OF *SACCHAROMYCES CEREVISIAE* STRAINS FROM HONEY BY-PRODUCTS BY THEIR PERFORMANCE AS STARTERS IN THE WINE INDUSTRY

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INTRODUCTION The species *Saccharomyces cerevisiae*, in addition to being the most used starter strain in the world for the production of fermented alcoholic beverages (wine, sparkling wine, beer, etc.) and other foods [1], is the most important yeast from a wine point of view. Fleet [2] showed that the indigenous strains of *S. cerevisiae* are much better suited to grow in grape must than any other inoculated strain. The yeast strains, in fact, differ mainly in both their fermentation performance and their ability to contribute to the bouquet of wine [3]. The objective of this research was to isolate non-conventional yeasts of the *S. cerevisiae* species from ecological niches other than that of wine and grapes, in order to identify new microbial starters to be applied in alcoholic fermentation processes for the production of sparkling wine base.

MATERIALS AND METHODS **Strain typing of *S. cerevisiae* isolates** Intraspecific characterization of the isolates belonging to *S. cerevisiae* species was carried out through two techniques: interdelta analysis with primers delta 12 and delta 21 [4] and microsatellite multiplex PCR based on the analysis of polymorphic microsatellite loci named SC8132X, YOR267C and SCPTSY7 [5]. The PCR products were analyzed on agarose gel 2.0% (w/v) in 1 X TBE buffer and visualized as above reported.

***In vitro* tests and microfermentation of sparkling base wine** The strains were tested for their growth in synthetic grape must [6] adjusted as follows: pH 3.1, 3.0, 2.9 and 2.8; total titratable acidity at 6.0, 8.0 and 10.0 g/L of tartaric acid; total acidity with values of malic acid at 2.0 and 2.5 g/L. The strains showing the best technological performances [low production of H_2S and acetic acid, resistance to ethanol up to 12% (v/v) and KMBS, ability to grow at low temperatures, growth in suspended form and low foam production)] were further characterized for their potential in fermenting grape must to be used for production of sparkling base wines.

Vinification and sampling The best four selected strains were employed into experimental vinification at Cantine Europa Soc. Cop. Agr. (Petrosino, Trapani). Grillo grapes were harvested at the initial stage of sugar maturity. Grapes were crushed, destemmed and added with 50 mg/q of sulphur dioxide (potassium metabisulfite). The must bulk was further added with pectolytic enzymes 40 mg/L, and clarified through floating with nitrogen. The clarified must was then divided into five tanks, representing five independent experimental trials (**Fig. 1**). After clarifying, when must reached the temperature of 16 °C, trials were inoculated with the selected *S. cerevisiae* strains: must from trial-1 to trial-4 were added with strain SPF21, SPF42, SPF52 and SPF159, respectively. As control, the Trial C was inoculated with GR1 strain. The alcoholic fermentations were carried out in steel tanks (10 hL volume capacity), at 15 °C. When total residual sugars were found lower than 2 g/L, wines were transferred into other steel tanks and added with 80 mg/L of sulphur dioxide, then were filled with nitrogen to avoid oxidation. After filtering, wines were bottled and stored at 18 °C for one month. Samples were collected just before and after the starter inoculation into grape must, and during AF at day 3, 6, 9 and 18. Further samples were collected at the end of sur lies ageing and at bottling phase.

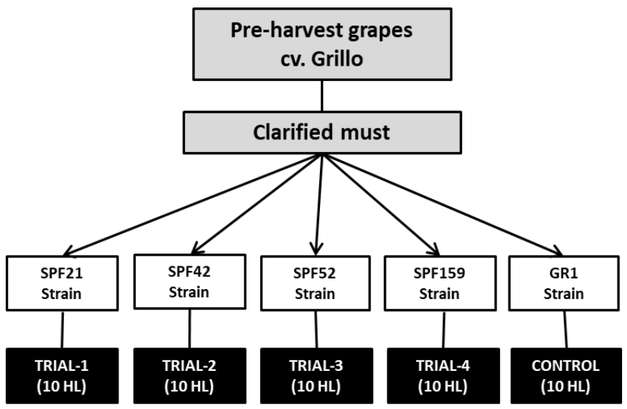

Fig. 1. Experimental design of industrial vinification.

Physico-chemical analysis All samples were subjected to physico-chemical analysis as follows: total titratable acidity (TTA), pH, volatile acidity (VA), reducing sugars, glucose, fructose, ethanol, glycerol, malic and lactic acids were determined throught Winescan (FOSS, Hillerød, Denmark) calibrated following EEC 2676 standard procedure [7] and Enzymatic Analyzer System (r-Biopharm).

Microbiological monitoring Samples were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Decimal dilutions were spread-plated (0.1 mL) onto Wallerstein Laboratory (WL) nutrient agar (Oxoid, Basingstoke, UK) and incubated at 28 °C for 48–72 h to determine total yeast (TY) counts. All analyses were carried out in duplicate. Yeasts were isolated and subjected to genetic characterisation to verify the starter strains dominance.

Sensory analysis The evaluation of the sensory profiles of wines was performed using a descriptive method [8].

RESULTS: The 552 isolates ascribed to *S. cerevisiae* species were further investigated at strain level by PCR fingerprinting analysis. A high values of dissimilarity were found among the 98 strains identified. The 98 *S. cerevisiae* strains were screened for their oenological characters (**Tab. 1**). From previous technological tests, 16 strains were used as starter to ferment grape must for sparkling base wines at 10 and 15 °C in present of 100 mg/L of KMBS. The results of the fermentation kinetics in terms of FP and FR (**Fig. 2a** and **2b**). Regarding to chemical parameters, there were no significant differences among experimental trials respect to GR1 control **(Tab. 2).** *Sensory analysis of experimental sparkling base wines.* The bottled wines were evaluated by sensory analysis and the results are reported in **Tab. 3**. Trials inoculated with SPF strains differed significantly from the GR1 control with regard to the majority of odour and taste descriptors. In particular, the highest values of odour intensity as well as odour and taste complexity were displayed by Trial-3 and Trial-4. The wines fermented with SPF strains showed also the lowest values of acid and astringent (taste) than control. Trial-3 showed more odour complexity than other trials. No off-odours and off-flavours were detected in all samples analyzed.

Tab. 1. Technological screening of 98 *S. cerevisiae* strains.

Strain code	10°C[a]	15°C[b]	Ethanol[c]	KMBS[d]	H_2S[e]	Strain code	10°C[a]	15°C[b]	Ethanol[c]	KMBS[d]	H_2S[e]
SPF 1	+	+	3	n.d.	n.d.	SPF 52	+	+	3	2	2
SPF 2	+	+	3	n.d.	n.d.	SPF 53	+	+	2	n.d.	n.d.
SPF 3	+	+	3	n.d.	n.d.	SPF 54	+	+	2	n.d.	n.d.
SPF 4	+	+	3	n.d.	n.d.	SPF 67	+	+	2	n.d.	n.d.
SPF 5	+	+	3	n.d.	n.d.	SPF 68	+	+	2	n.d.	n.d.
SPF 6	+	+	2	n.d.	n.d.	SPF 69	+	+	2	n.d.	n.d.
SPF 7	+	+	2	n.d.	n.d.	SPF 148	+	+	2	n.d.	n.d.
SPF 8	+	+	3	n.d.	n.d.	SPF 149	+	+	2	n.d.	n.d.
SPF 9	+	+	3	n.d.	n.d.	SPF 152	+	+	2	n.d.	n.d.
SPF 10	+	+	2	n.d.	n.d.	SPF 157	+	+	2	n.d.	n.d.
SPF 11	+	+	2	n.d.	n.d.	SPF 158	+	+	3	2	2
SPF 12	+	+	2	n.d.	n.d.	SPF 159	+	+	3	2	1
SPF 13	+	+	3	n.d.	n.d.	SPF 162	+	+	3	2	1
SPF 14	+	+	3	2	0	SPF 163	+	+	3	2	0
SPF 16	+	+	3	2	0	SPF 164	+	+	3	n.d.	n.d.
SPF 17	+	+	3	2	1	SPF 165	+	+	3	n.d.	n.d.
SPF 19	+	+	3	n.d.	n.d.	SPF 166	+	+	3	n.d.	n.d.
SPF 20	+	+	3	n.d.	n.d.	SPF 167	+	+	3	n.d.	n.d.
SPF 21	+	+	3	2	2	SPF 168	+	+	2	n.d.	n.d.
SPF 22	+	+	2	n.d.	n.d.	SPF 169	+	+	3	n.d.	n.d.
SPF 24	+	+	2	n.d.	n.d.	SPF 170	+	+	2	n.d.	n.d.
SPF 25	+	+	3	n.d.	n.d.	SPF 171	+	+	3	n.d.	n.d.
SPF 26	+	+	2	n.d.	n.d.	SPF 172	+	+	2	n.d.	n.d.
SPF 27	+	+	2	n.d.	n.d.	SPF 174	+	+	3	2	3
SPF 28	+	+	2	n.d.	n.d.	SPF 177	+	+	2	n.d.	n.d.
SPF 29	+	+	2	n.d.	n.d.	SPF 178	+	+	2	n.d.	n.d.
SPF 30	+	+	2	n.d.	n.d.	SPF 179	+	+	2	n.d.	n.d.
SPF 31	+	+	2	n.d.	n.d.	SPF 181	+	+	2	n.d.	n.d.
SPF 33	+	+	2	n.d.	n.d.	SPF 186	+	+	2	n.d.	n.d.
SPF 34	+	+	3	n.d.	n.d.	SPF 187	+	+	2	n.d.	n.d.
SPF 35	+	+	3	n.d.	n.d.	SPF 189	+	+	2	n.d.	n.d.
SPF 36	+	+	2	n.d.	n.d.	SPF 190	+	+	2	n.d.	n.d.
SPF 37	+	+	3	n.d.	n.d.	SPF 191	+	+	2	n.d.	n.d.
SPF 38	+	+	3	n.d.	n.d.	SPF 192	+	+	3	n.d.	n.d.
SPF 39	+	+	3	n.d.	n.d.	SPF 194	+	+	2	n.d.	n.d.
SPF 40	+	+	3	2	2	SPF 195	+	+	2	n.d.	n.d.
SPF 41	+	+	3	2	1	SPF 196	+	+	2	n.d.	n.d.
SPF 42	+	+	3	2	2	SPF 197	+	+	2	n.d.	n.d.
SPF 43	+	+	2	n.d.	n.d.	SPF 198	+	+	2	n.d.	n.d.
SPF 44	+	+	2	n.d.	n.d.	SPF 199	+	+	2	n.d.	n.d.
SPF 46	+	+	2	n.d.	n.d.	SPF 200	+	+	2	n.d.	n.d.
SPF 47	+	+	2	n.d.	n.d.	SPF 201	+	+	2	n.d.	n.d.
SPF 48	+	+	2	n.d.	n.d.	SPF 206	+	+	2	n.d.	n.d.
SPF 49	+	+	3	1	0	SPF 208	+	+	2	n.d.	n.d.
SPF 50	+	+	3	1	1	SPF 209	+	+	2	n.d.	n.d.
SPF 51	+	+	3	2	2	GR1	+	+	3	2	2

[a] +, growth; –, not growth at 10 °C in YPD broth.
[b] +, growth; – , not growth at 15 °C in YPD broth.
[c] 0, 0%(v/v); 1, 12% (v/v); 2, 14% (v/v); 3, 16% (v/v) of ethanol contained in MESA plates at which strains showed growth.
[d] 0, no growth in the plates; 1, 15 g/hl of MBSK + 16% ethanol; 2, 25 g/hl of MBSK + 16% (v/v) of ethanol contained into MESA plates at which strains showed growth.
[e] Colour of colony on Biggy agar plates: 0 = white; 1 = beige; 2 = light brown; 3 = brown; 4 = dark brown; 5 = black.

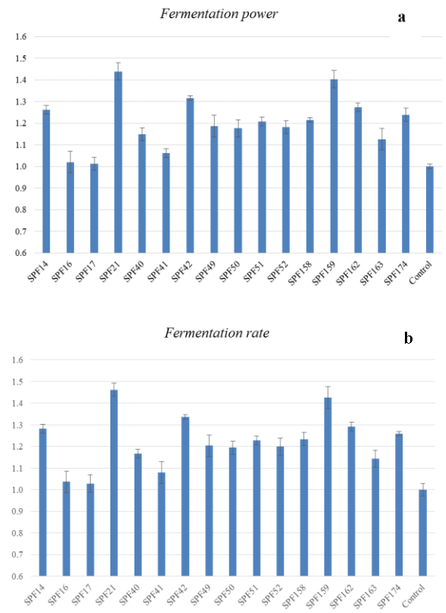

Fig.2. Fermentation power (a) and fermentation rate (b) calculated during AF and related to values measured for the control strain (GR1)

Tab. 2. Chemical parameters during industrial vinifications.

	Chemical parameters							
	pH	Total acidity (g/L)	Volatile acidity (g/L)	Reducing sugars (g/L)	Ethanol (% v/v)	Glicerol (g/L)	Malic Acid (g/L)	Lactic acid (g/L)
Must	3.25±0.01	7.49±0.01	0.08±0.01	152.00±0.02	1.67±0.03	2.64±0.01	2.47±0.02	0.00±0.02
SPF21	3.29±0.02	6.84±0.02	0.17±0.03	0.30±0.01	10.30±0.01	5.53±0.02	1.50±0.03	0.26±0.01
SPF42	3.20±0.01	6.81±0.03	0.18±0.01	0.70±0.01	10.08±0.01	4.98±0.01	1.63±0.01	0.30±0.02
SPF52	3.23±0.02	6.78±0.03	0.16±0.03	0.60±0.01	10.29±0.02	5.35±0.01	1.67±0.03	0.27±0.02
SPF159	3.23±0.01	6.51±0.01	0.21±0.02	0.10±0.01	10.94±0.01	5.37±0.03	1.57±0.01	0.33±0.02
GR1 (control)	3.29±0.02	6.86±0.01	0.15±0.01	0.20±0.01	10.91±0.03	5.62±0.02	1.80±0.01	0.35±0.01

Tab. 3. Sensory analysis.

Descriptors	Sensory analysis				
	Trial-1 (SPF21)	Trial-2 (SPF42)	Trial-3 (SPF52)	Trial-4 (SPF159)	Control (GR1)
Colour intensity	2.80±0.14	2.74±0.13	2.65±0.17	2.76±0.10	2.20±0.20
Odour:					
Intensity	5.40±0.10	5.65±0.10	5.86±0.19	5.67±0.11	5.60±0.10
Complexity	6.43±0.13	6.35±0.11	6.98±0.13	6.85±0.13	5.96±0.17
Fresh fruits	4.96±0.15	5.10±0.16	5.84±0.15	5.23±0.15	6.51±0.10
Dried fruits	4.03±0.17	3.02±0.14	2.20±0.18	3.30±0.19	3.45±0.13
Flowers	5.37±0.11	5.41±0.19	5.84±0.10	4.67±0.13	4.35±0.10
Aromatic herbs	3.74±0.19	3.65±0.14	3.23±0.15	3.84±0.11	5.68±0.20
Spices	5.32±0.20	5.45±0.12	6.25±0.17	4.21±0.10	3.22±0.19
Taste:					
Sweet	3.26±0.10	3.75±0.17	3.94±0.10	3.24±0.12	2.95±0.15
Hot	1.20±0.15	1.30±0.12	1.25±0.14	1.30±0.10	0.90±0.16
Acid	3.52±0.12	3.60±0.10	3.10±0.12	3.25±0.10	4.42±0.10
Astringent	5.20±0.18	5.80±0.10	5.06±0.11	5.32±0.12	6.84±0.13
Bitter	3.13±0.15	3.24±0.18	3.10±0.16	3.54±0.11	3.16±0.10
Complexity	5.43±0.10	5.25±0.20	5.78±0.10	5.65±0.19	4.96±0.11

CONCLUSIONS Microbial ecology of honey and its by-products is rich in *Saccharomyces* spp., with high fermentation capacity, and potentially applicable in alcoholic fermentation. Technological screening, applied on 98 strains, showed that four of which (SPF 21, SPF 42, SPF 52 and SPF 159) might potentially be used as starter cultures in wine system. Application to wine industry showed that SPF strains did not produce off-odours and/or off-flavours, whereas they improved quality of final product, since panellists found a significant increase of complexity and intensity characteristics of bottled wines. Use of non-conventional yeasts, in alcoholic fermentation of wine, might be a valid alternative to characterize wine and improve typicality of final products, which is requested by expert wine consumers.

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