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Full Paper

Ochratoxin A adsorption phenotype: An inheritable yeast trait

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This study aimed to evaluate the inheritance of the trait *ochratoxin A adsorption* in two wine strains of *Saccharomyces cerevisiae* and their 46 descendants. Each strain was inoculated in triplicate in test tubes containing 10 ml of must obtained from the Calabrian *Zibibbo* white grape variety, artificially contaminated with ochratoxin A to reach a total content of 4.10 ng/ml. The microvinification trials were performed at 25°C. After 30 days, ochratoxin A values ranged from 0.74 to 3.18 ng/ml, from 0.01 to 2.69 ng/ml, and from 0.60 to 2.95 ng/ml respectively in wines, in lees after washing, and in the saline solution used to wash the lees. The analysis of OTA in wines was performed to find the residual toxin content after yeast activity, thus obtaining technological evidence of yeast influence on wine detoxification. The analysis of OTA in lees after washing was performed to distinguish the OTA linked to cells. The analysis of OTA in the saline solution used to wash the lees was performed to distinguish the OTA adsorbed on yeast cell walls and removed by washing, thus focusing on the adsorption activity of wine yeast through electrostatic and ionic interactions between parietal mannoproteins and OTA. Ploidy of the two parental strains was controlled by flow cytometry. Results demonstrated that the *ochratoxin A adsorption* is genetically controlled and is a polygenic inheritable trait of wine yeasts. The majority of the descendants are characterized by a great and significant diversity compared to their parents. Both the parental strains had genome sizes consistent with their being diploid, so validating the observed results. These findings constitute an initial step to demonstrate the mechanisms of inheritance and establish breeding strategies to improve the *ochratoxin A adsorption* trait in wine yeasts. This will allow a decrease in the ochratoxin A content of contaminated musts during winemaking, by using genetically improved wine yeasts.

Key Words——inheritance analysis; microvinification; ochratoxin A adsorption; *Saccharomyces cerevisiae*; wine yeast

Introduction

Ochratoxin A (OTA) is a very dangerous secondary metabolite, frequently noticed in various foods and beverages, including wines; OTA is bio-produced by a few species belonging to the genera *Aspergillus* and *Penicillium*. Since 1996, OTA has been reported in grapes, grape juices and wines (Zimmerli and Dick, 1996). In alcoholic beverages, OTA is formed prior to

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alcoholic fermentation, during which OTA is partially removed or degraded (Chu et al., 1975). Interestingly, the decrease in OTA is yeast strain-dependent (Scott et al., 1995). *Saccharomyces cerevisiae* strains are widely used in the wine industry. It would be worthwhile to study the diversity of the *S. cerevisiae* genome (Naumov et al., 2001) and to perform the genetic characterization (Oda et al., 2010) of some of the strains which have been developed for novel applications. Different decontamination procedures based on *Saccharomyces* strains have recently been proposed for OTA removal (Bejaoui et al., 2004; Caridi et al., 2006a; Piotrowska and Zakowska, 2000). Yeast mannoproteins play an important role in the OTA removal process (Ringot et al., 2005). Remarkable differences among wine yeasts — both *Saccharomyces* (Caridi et al., 2006b) and non-*Saccharomyces* (Cecchini et al., 2006) — have been reported in the OTA sequestering activity during winemaking. This may depend on the differing mannosylphosphate content of wine yeast mannoproteins, but also on dissimilar fermentation, cell sedimentation dynamics, cell dimension, and flocculence. At present, no study has been carried out to analyze the inheritable nature of the *OTA adsorption* phenotype. However, genomic strategies may be very useful to obtain a further enhancement of the adsorption activity of wine yeasts. Based on these elements, it is possible to delineate the enological trait of *OTA adsorption*. Homothallism among wine yeasts is the wild-type condition. Homothallic yeasts remain haploid only transiently (Bakalinsky and Snow, 1990). Diploid wine yeasts are able to segregate their various traits in the spore formation phase, which is age-dependent (Sando et al., 1973), allowing any diversity observed in their descendants to be explained as a real consequence of the trait segregation. This work aims to evaluate the inheritance of the trait of *OTA adsorption* during winemaking, testing two wine strains of *S. cerevisiae* and their 46 descendants. This trait was determined by analysis of the OTA content in wines, in lees after washing, and in the saline solution used to wash the lees.

Materials and Methods

Strains and methodology. The study was carried out using two wine strains of *S. cerevisiae*, named TP5 and TT173, and 46 single-spore cultures of which 32 were obtained from 12 asci of the strain TP5 and 14 from 8 asci of the strain TT173. The two parental strains had been isolated from native microflora of wine fermentations and already selected for enology (Caridi et al., 1999). The strain TT173 had also been tested for OTA removing capacity in saline solution (Caridi et al., 2006a) and during winemaking (Caridi et al., 2006b). The 46 single-spore cultures were isolated by a micromanipulator and tested for the enological trait of *wine color adsorption* to evaluate its inheritance (Caridi et al., 2007). The *OTA adsorption* phenotype study was carried out by microvinification trials, using must from the Calabrian *Zibibbo* white grape variety of *Vitis vinifera*. The grapes were destemmed, crushed and pressed to obtain the must (pH 3.40, 20° brix). The 48 wine yeasts were inoculated (1 ml) in triplicate in test tubes containing 10 ml of pasteurized must, which had been artificially contaminated with OTA to reach a total content of 4.10 ng/ml. The microvinification trials were performed in a 130-L refrigerated thermostat at 25°C. Carbon dioxide production was measured weekly by weight loss to determine the end of fermentation. When the CO_2 production finished (28–30 days), fermentation was considered completed (residual sugar $\leq$ 1 g/L) and the samples were analyzed for residual OTA. In this study no other analytical parameter was determined since the 48 yeast strains had been previously studied for their activity on wine color parameters (Caridi et al., 2007) and for other basic traits (unpublished data). To determine the OTA adsorbed by yeasts on cell walls, the lees were washed twice with saline solution, 0.85% (m/v) NaCl, and the toxin content both on the lees and in the saline solution was determined. Genetic analysis was performed on the two parental strains in order to control their ploidy. Genome sizes were estimated by flow cytometry; the basic procedure followed was that of Paulovich and Hartwell (1995) as described by Naumova et al. (2005). The laboratory strain BY 474i was used as a standard haploid yeast and assigned a relative fluorescence value of 1.0. The diploid strain Zymaflore F15 and the aneuploid strain Anchor NT50 (Bradbury et al., 2006) were used as control strains. All the data were analyzed using StatGraphics Centurion XVI for Windows XP from StatPoint.

Determination of the OTA content in wines, in the lees after washing by saline solution and in the saline solution used to wash lees. The HPLC analyses were performed using LC-10AD pumps and the RF-10Axl (Shimadzu, Japan) fluorescence detector, set to an ex-

citation wavelength of 333 nm and emission wavelength of 460 nm. Data acquisition and handling were made by a system control SLC10A with software VP5 (Shimadzu, Japan). A Synergi (Phenomenex, USA) C18 (250 mm × 4.6 mm, 5 μm) column was used. The HPLC conditions were set up using elution at a constant flow of 1 ml/min and CH_3CN (1% acetic acid)-H_2O (1% acetic acid) (50 : 50, v/v) as the starting eluent system. The starting ratio was linearly modified to 100% CH_3CN in 15 min. From the 15th to 18th min the pumps were taken back to starting conditions and then the isocratic conditions were taken for 5 min. Eluent was freshly prepared and filtered (0.22 μm) before use. All samples were filtered through a 0.22 μm syringe filter (Millipore, Bedford, MA, USA) prior to injection (20 μl) into the HPLC column by 250 ml syringe (Hamilton, Switzerland). Mycotoxin identification was performed by comparing retention times and UV spectra of purified samples with a pure OTA standard. A further confirmation was performed by co-injecting samples together with an OTA standard solution. The average retention time for OTA (4.5 min, R.S.D. 1.5%) was obtained with 10 consecutive injections of the same OTA working solution within the same day. This retention time enhanced the chromatographic resolution of the OTA peak from other matrix interferences. The calculated instrumental detection limit and quantification limit for OTA under these conditions were 0.2 (10 ng/L, S/N 3) and 2 pg (100 ng/L), respectively. Mycotoxin quantification was carried out by comparing peak areas of investigated samples to the calibration curve, ranging from 0.1 to 100 μg/L, of authentic OTA standards. All the analyzed samples were prepurified prior to injection by Ochraprep immunoaffinity columns (R-Biopharm Rhône, Glasgow, Scotland) and eluted at a flow rate of 1–2 drops/s. The column was washed with 20 ml of water and then eluted with 1.5 ml of methanol (2% acetic acid) and 1.5 ml of pure water. The OTA adsorbed on yeast cell walls was determined as follows: cells were harvested by centrifugation (centrifuge Juan model CR3i) and washed twice (4,000 rpm for 10 min at 4°C) with saline solution. The two aliquots of saline solution were combined and analyzed as described above for wines. The OTA content in lees after the washing was determined as follows: cells were harvested by centrifugation (4,000 rpm for 10 min at 4°C) sonicated in the saline solution for 30 min and washed twice with saline solution. The pellet was suspended in 3 ml of saline solution, 3 ml of ethyl acetate were added and, after mixing and centrifugation (4,000 rpm for 10 min at 4°C), 1 ml of the top phase was evaporated by Rotavapor (system Juan model RC60), re-suspended in 1 ml of methanol and analyzed by injecting 20 μl into the HPLC column with a 100 μl syringe (Hamilton, Switzerland).

Results and Discussion

The present work aimed to explore the different *OTA adsorption* phenotypes of the parental strains and their progeny. Therefore, the analysis of OTA in wines was performed to find the residual toxin content after yeast activity, thus obtaining important technological evidence of the influence of yeast on wine detoxification. Moreover, the analysis of OTA in lees after washing was performed to distinguish the OTA linked to cells. Lastly, the analysis of OTA in the saline solution used to wash the lees was performed to distinguish the OTA adsorbed on yeast cell walls and removed by washing, thus focusing on the adsorption activity of wine yeast through electrostatic and ionic interactions between parietal mannoproteins and OTA. The residual OTA in wines varied from 0.74 to 3.18 ng/ml. The OTA linked to the lees varied from 0.01 to 2.69 ng/ml. The OTA adsorbed on yeast cell walls varied from 0.60 to 2.95 ng/ml. The majority of the descendants exhibited significant ($p < 0.05$) differences from their parental strains (Table 1); different distribution patterns of residual OTA in wines, OTA linked to cells, and adsorbed OTA on cell walls were shown for each parental strain. As regards the wine, strain TT173 showed a majority of descendants with significantly different values, while strain TP5 had the least number of descendants with significantly different values. As regards the OTA adsorbed on cell walls, all the progeny of strain TP5 exhibited significantly different values compared to the parental strain, strain TT173 showed a majority of descendants with significantly different values. As regards the OTA linked to cells, the overwhelming majority of the progeny of strain TT173 exhibited significantly different values compared to the parental strain, while strain TP5 showed less than 50% of descendants with significantly different values. It seems clear that within a tetrad no simple segregation pattern exists, suggesting the polygenic nature of the studied trait and the three parameters. In this context, for each parental strain we considered its progeny as a whole that exhibits a continuous range of variation. In Tables

Table 1. *Ochratoxin A adsorption* phenotype of two wine strains and their progenies studied by microvinification trials.

Parameters	TP5				TT173			
		Progeny				Progeny		
	Parent	Mean	Range	%[a]	Parent	Mean	Range	%[a]
Residual OTA in wines (ng/ml)	1.42	1.35	0.74–1.86	15.62	1.60	1.81	1.32–3.18	57.14
Adsorbed OTA on cell walls (ng/ml)	2.64	0.96	0.60–2.95	100.00	1.14	0.98	0.72–1.75	78.57
OTA linked to cells (ng/ml)	0.12	0.28	0.01–2.69	46.87	0.17	0.74	0.06–2.07	85.71

[a]Percentage of descendants included in homogeneous groups ($p<0.05$ according to *Least Significant Difference* analysis) that do not include their parental strain.

Table 2. Distributions of the yeast strain TP5 and its progeny based on the ascending order of the parameter values: (1) *residual OTA in wine*, (2) *adsorbed OTA on cell wall*, and (3) *OTA linked to cells*. The values are expressed as ng OTA/ml and correspond to mean, standard deviation, and homogeneous group, according to *Least Significant Difference* analysis ($p<0.05$).

Residual OTA in wine			Adsorbed OTA on cell wall			OTA linked to cells		
Yeast strains	Mean ± standard deviation	Homogeneous group	Yeast strains	Mean ± standard deviation	Homogeneous group	Yeast strains	Mean ± standard deviation	Homogeneous group
TP5-2A	0.74±0.42	a	TP5-8A	0.60±0.08	a	TP5-9B	0.01±0.00	a
TP5-7B	0.95±0.05	ab	TP5-11C	0.68±0.09	ab	TP5-9D	0.01±0.00	a
TP5-3A	1.04±0.05	abc	TP5-3A	0.75±0.04	abc	TP5-4B	0.02±0.01	a
TP5-1A	1.11±0.01	bcd	TP5-12C	0.76±0.10	bcd	TP5-10A	0.02±0.01	a
TP5-2C	1.11±0.01	bcd	TP5-10A	0.77±0.05	bcde	TP5-4D	0.02±0.01	a
TP5-3C	1.18±0.03	bcde	TP5-12D	0.77±0.09	bcde	TP5-1A	0.03±0.01	a
TP5-4B	1.18±0.02	bcde	TP5-2C	0.77±0.04	bcde	TP5-10C	0.03±0.00	ab
TP5-8D	1.19±0.02	bcde	TP5-9B	0.78±0.04	bcdef	TP5-10B	0.03±0.00	ab
TP5-8B	1.19±0.01	bcdef	TP5-7A	0.78±0.01	bcdef	TP5-3C	0.03±0.01	ab
TP5-9D	1.22±0.03	bcdefg	TP5-8C	0.80±0.01	bcdef	TP5-9C	0.04±0.00	abc
TP5-9C	1.22±0.01	bcdefg	TP5-3C	0.80±0.07	bcdef	TP5-12A	0.06±0.01	abc
TP5-9B	1.26±0.11	bcdefgh	TP5-11A	0.80±0.11	bcdef	TP5-4A	0.06±0.01	abc
TP5-8A	1.28±0.09	cdefghi	TP5-9C	0.82±0.02	bcdefg	TP5-8C	0.06±0.02	abc
TP5-4D	1.30±0.12	cdefghij	TP5-7B	0.86±0.02	cdefg	TP5-8D	0.07±0.01	abcd
TP5-10C	1.30±0.07	cdefghij	TP5-6D	0.87±0.01	cdefgh	TP5-2C	0.08±0.01	abcde
TP5-11A	1.31±0.04	cdefghij	TP5-10D	0.87±0.02	cdefgh	TP5-8B	0.10±0.00	bcde
TP5-4A	1.33±0.03	cdefghijk	TP5-4D	0.88±0.05	cdefgh	TP5-2A	0.10±0.01	bcde
TP5-8C	1.35±0.15	cdefghijk	TP5-1A	0.89±0.06	cdefgh	TP5-11B	0.11±0.01	cdef
TP5-5B	1.35±0.05	cdefghijk	TP5-9D	0.90±0.01	cdefgh	TP5-1C	0.12±0.02	cdefg
TP5-10A	1.37±0.02	cdefghijk	TP5-8B	0.91±0.05	defgh	***TP5***	0.12±0.01	cdefg
TP5-5A	1.38±0.09	defghijk	TP5-5A	0.92±0.11	efghi	TP5-5B	0.14±0.02	defgh
TP5	1.42±0.06	defghijk	TP5-4A	0.92±0.02	efghij	TP5-12C	0.14±0.01	defgh
TP5-7A	1.46±0.04	efghijkl	TP5-8D	0.93±0.02	fghij	TP5-1D	0.14±0.01	defgh
TP5-10B	1.46±0.01	efghijkl	TP5-11B	0.98±0.12	ghijk	TP5-10D	0.15±0.02	efgh
TP5-12C	1.52±0.06	fghijkl	TP5-2A	1.02±0.03	hijk	TP5-3A	0.19±0.01	fghi
TP5-11B	1.53±0.04	ghijklm	TP5-4B	1.02±0.04	hijk	TP5-8A	0.19±0.02	ghi
TP5-10D	1.59±0.09	hijklm	TP5-1C	1.08±0.04	ijk	TP5-11A	0.20±0.01	ghi
TP5-11C	1.59±0.03	hijklm	TP5-5B	1.08±0.01	jk	TP5-6D	0.20±0.00	hi
TP5-6D	1.61±0.08	ijklm	TP5-10C	1.10±0.07	k	TP5-7A	0.25±0.00	i
TP5-1C	1.62±0.19	jklm	TP5-10B	1.31±0.09	l	TP5-12D	0.49±0.02	j
TP5-12D	1.66±0.07	klm	TP5-1D	1.44±0.08	l	TP5-5A	0.89±0.01	k
TP5-1D	1.79±0.01	lm	***TP5***	2.64±0.11	m	TP5-11C	2.36±0.05	l
TP5-12A	1.86±0.08	m	TP5-12A	2.95±0.24	n	TP5-7B	2.69±0.21	m

Table 3. Distributions of the yeast strain TT173 and its progeny based on the ascending order of the parameter values: (1) *residual OTA in wine*, (2) *adsorbed OTA on cell wall*, and (3) *OTA linked to cells*. The values are expressed as ng OTA/ml and correspond to mean, standard deviation, and homogeneous group, according to *Least Significant Difference* analysis ($p<0.05$).

Residual OTA in wine			Adsorbed OTA on cell wall			OTA linked to cells		
Yeast strains	Mean ± standard deviation	Homogeneous group	Yeast strains	Mean ± standard deviation	Homogeneous group	Yeast strains	Mean ± standard deviation	Homogeneous group
TT173-6C	1.32±0.15	a	TT173-1A	0.72±0.04	a	TT173-4D	0.06±0.01	a
TT173-1A	1.37±0.02	a	TT173-2C	0.72±0.08	a	TT173-1A	0.08±0.00	ab
TT173-5B	1.49±0.01	b	TT173-5A	0.73±0.08	a	TT173-1C	0.11±0.01	bc
TT173-1D	1.49±0.00	b	TT173-5B	0.77±0.12	a	TT173-5B	0.15±0.01	cd
TT173	1.60±0.07	bc	TT173-1C	0.79±0.04	a	TT173-5A	0.16±0.01	cd
TT173-8A	1.65±0.06	c	TT173-3B	0.86±0.09	ab	***TT173***	0.17±0.01	d
TT173-3C	1.67±0.01	cd	TT173-6D	0.86±0.06	ab	TT173-6C	0.34±0.02	e
TT173-3B	1.67±0.02	cde	TT173-8A	0.87±0.03	ab	TT173-6D	0.45±0.01	f
TT173-2C	1.68±0.02	cde	TT173-7C	0.96±0.09	b	TT173-7C	0.54±0.02	g
TT173-1C	1.78±0.03	de	TT173-3C	0.97±0.10	bc	TT173-8A	0.65±0.01	h
TT173-5A	1.79±0.01	e	TT173-1D	1.02±0.13	bc	TT173-4B	1.05±0.06	i
TT173-6D	1.96±0.02	f	***TT173***	1.14±0.08	cd	TT173-3C	1.55±0.01	j
TT173-7C	2.05±0.05	f	TT173-6C	1.28±0.08	de	TT173-1D	1.57±0.02	j
TT173-4B	2.18±0.02	g	TT173-4B	1.44±0.06	e	TT173-2C	1.57±0.03	j
TT173-4D	3.18±0.03	h	TT173-4D	1.75±0.07	f	TT173-3B	2.07±0.03	k

2 and 3 the distributions of the yeast strain TP5 and TT173 (respectively) and their progeny based on the ascending order of the parameter values for residual OTA in wine, adsorbed OTA on cell wall, and OTA linked to cells have been reported. The respective positions of parental strains within the descendant distribution were different among both parameters and parental strains. In particular, considering the residual OTA in wines, the parental strains TP5 and TT173 were, respectively, the 22nd out of 33 (Table 2) and the 5th out of 15 (Table 3) among their progeny. Regarding the OTA adsorbed on cell walls, the two wine yeasts were positioned 32nd (Table 2) and 12th (Table 3). For the OTA linked to cells, the parental strains were, respectively, the 20th (Table 2) and the 6th (Table 3) among their progeny. It is interesting to note that progeny deriving from the same ascus occasionally were included in the same homogeneous group according to *Least Significant Difference* analysis ($p<0.05$), such as for the three single-spore cultures deriving from the ascus 9 — TP5 progeny — regarding the residual OTA in wine (Table 2). This phenomenon may be exploited by trying to cross these neighboring progenies in order to strengthen the strain traits. In order to better appreciate the link that exists among the three parameters (1) residual OTA in wine, (2) adsorbed OTA on cell walls, and (3) OTA linked to cells, the related dot plots have been elaborated for the yeast strain TP5 and its progeny (Fig. 1) and for the yeast strain TT173 and its progeny (Fig. 2). The *OTA adsorption* trait segregates through progenies showing an amplitude of distribution that is peculiar for each strain. According to Marullo et al. (2004) the relations of dominance/recessivity between alleles involved in the control of a particular trait are different from one strain to another. For each parameter, there are a congruous number of descendants significantly different from their parents. This may allow the possibility of improving wine yeast as regards the *OTA adsorption* trait with the goal of identifying strains with improved OTA adsorption activity. It is interesting to note that the residual OTA in wine, the adsorbed OTA on cell walls, and the OTA linked to cells exhibited a large and not fully correlated variability. Based on Spearman test analysis, a significant (p-value 0.0026) positive correlation between residual OTA content in wines and OTA linked to cells was observed, but no more significant correlation between the other parameters tested in the present study (Table 4). In order to know if there is a correlation between ability to adsorb wine color in red winemaking and ability to adsorb OTA, a further statistical analysis was performed, exploiting previous data

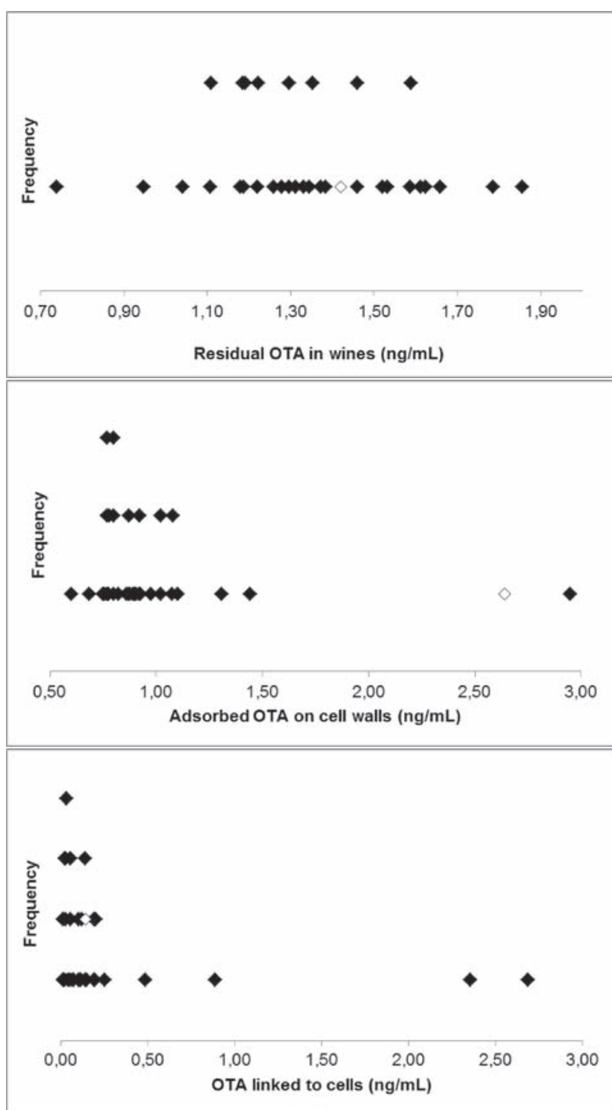


Fig. 1. Dot plots of the three parameters for the yeast strain TP5 and its progeny.
White symbol is the parental strain.

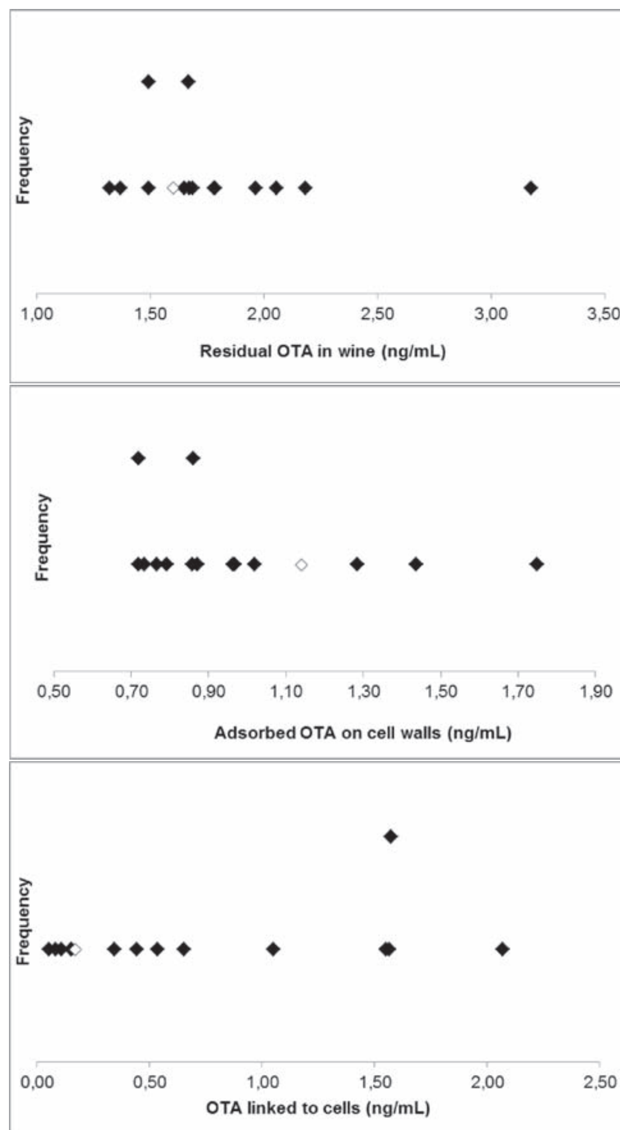
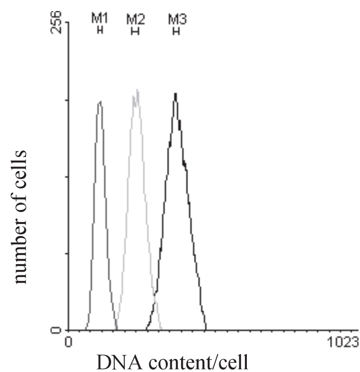


Fig. 2. Dot plots of the three parameters for the yeast strain TT173 and its progeny.
White symbol is the parental strain.

obtained studying the wine color adsorption trait in these populations (Caridi et al., 2007). As expected, significant positive correlations based on Spearman test analysis were observed between (a) residual OTA content and red component of the yeast biomasses, (b) residual OTA content and green component of the yeast biomasses, and (c) residual OTA content and blue component of the yeast biomasses. Consistently, significant negative correlations based on Spearman test analysis were observed between (a) residual OTA content and 420 nm absorbance of the red wines, (b) residual OTA content and 620 nm absorbance of the red wines, (c) residual OTA content and color intensity of the red wines, (d) OTA linked to cells and 420 nm absorbance of the red wines, (b) OTA linked to cells and 620 nm absorbance of the red wines, (c) OTA linked to cells and color intensity of the red wines (Table 4). The flow cytometric analysis allowed the genomic size of the parental strains to be checked in order to ensure that they are diploid. In Fig. 3 the flow cytometric profiles of haploid, diploid, and aneuploid *S. cerevisiae*, used as control strains, are shown. Figures 4 and 5 show in duplicate the flow cytometric profiles of strain TP5 and strain TT173, respectively. Both the profiles of the parental strains had genome sizes consistent with their being diploid, thus validating the

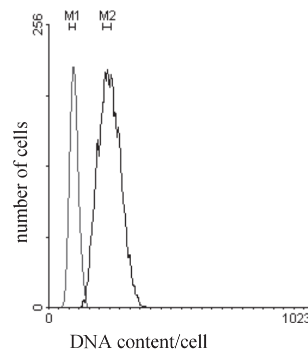
Table 4. Correlation among the three parameters for yeast strains TP5 and TT173 and their progeny based on Spearman test values.

Parameters		Residual OTA in wines	Adsorbed OTA on cell walls	OTA linked to cells
Residual OTA in wines	correlation		0.1619	0.4397
	p-value		0.2670	**0.0026**
Adsorbed OTA on cell walls	correlation	0.1619		−0.1462
	p-value	0.2670		0.3160
OTA linked to cells	correlation	0.4397	−0.1462	
	p-value	**0.0026**	0.3160	
Red component (yeast biomass)	correlation	0.4803	0.1024	0.1417
	p-value	**0.0010**	0.4825	0.3312
Green component (yeast biomass)	correlation	0.4483	0.1038	0.0817
	p-value	**0.0021**	0.4768	0.5752
Blue component (yeast biomass)	correlation	0.4526	0.1131	0.0950
	p-value	**0.0019**	0.4382	0.5151
Folin-Ciocalteu index (red wine)	correlation	0.1014	0.0899	0.1548
	p-value	0.4870	0.5375	0.2887
420 nm absorbance (red wine)	correlation	−0.3791	−0.1332	−0.3322
	p-value	**0.0094**	0.3612	**0.0228**
520 nm absorbance (red wine)	correlation	−0.1958	−0.1124	−0.2256
	p-value	0.1796	0.4412	0.1220
620 nm absorbance (red wine)	correlation	−0.4629	−0.1737	−0.3584
	p-value	**0.0015**	0.2337	**0.0140**
Color intensity (red wine)	correlation	−0.2881	−0.1392	−0.2942
	p-value	**0.0483**	0.3400	**0.0437**
Color tonality (red wine)	correlation	−0.0818	−0.0238	0.0064
	p-value	0.5748	0.8705	0.9648

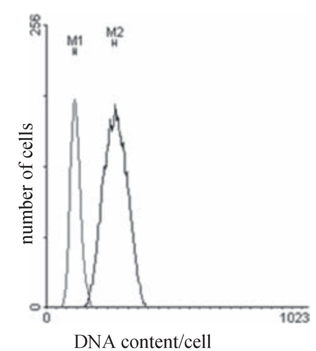


BY 474i (haploid M1)
Zymaflor F15 M2/M1 2.03
Anchor NT50 M3/M1 3.20

Fig. 3. DNA content per cell measured by flow cytometric analysis of the control strains of *S. cerevisiae* BY 474i (haploid), Zymaflore F15 (diploid), and Anchor NT50 (aneuploid).



BY 474i (haploid M1)
TP5 M2/M1 2.32



BY 474i (haploid M1)
TP5 M2/M1 2.39

Fig. 4. DNA content per cell measured by flow cytometric analysis of the laboratory yeast BY 474i (haploid) and the strains TP5 of *S. cerevisiae* (diploid).

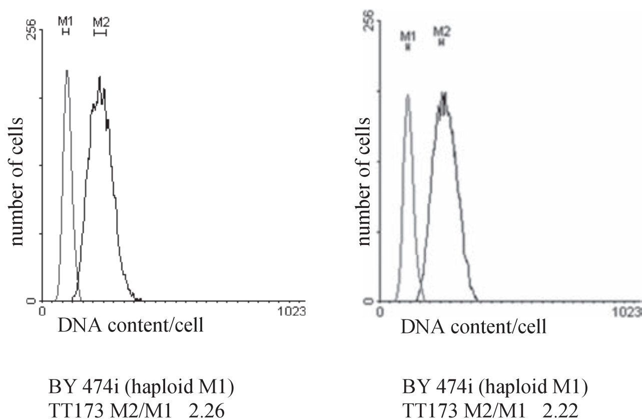


Fig. 5. DNA content per cell measured by flow cytometric analysis of the laboratory yeast BY 474i (haploid) and the strains TT173 of *S. cerevisiae* (diploid).

observed results.

Conclusions

The analysis of the progeny carried out with this study demonstrated that *OTA adsorption* is genetically controlled and, therefore, it is an inheritable trait of wine yeasts. It is interesting to note that the majority of the descendants are characterized by a great and significant diversity compared to their parents. These findings will be the basis of a future study to fully understand the mechanisms of inheritance; as such, they constitute an initial step for establishing breeding strategies in order to improve wine yeast as regards the *OTA adsorption* trait. This study may provide important applications in food safety since it will make it possible to drastically reduce, during winemaking, the OTA content of contaminated musts using wine yeasts improved by genomic strategies.

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References

Bakalinsky, A. T. and Snow, R. (1990) Conversion of wine strains of *Saccharomyces cerevisiae* to heterothallism. *Appl. Environ. Microb.*, **56**, 849–857.

Bejaoui, H., Mathieu, F., Taillandier, P., and Lebrihi, A. (2004) Ochratoxin A removal in synthetic and natural grape juices by selected oenological *Saccharomyces* strains. *J. Appl. Microbiol.*, **97**, 1038–1044.

Bradbury, J. E., Richards, K. D., Niederer, H. A., Lee, S. A., Rod Dunbar, P., and Gardner, R. C. (2006) A homozygous diploid subset of commercial wine yeast strains. *Anton. Leeuw. Int. J. G.*, **89**, 27–37.

Caridi, A., Crucitti, P., and Ramondino, D. (1999) Winemaking of musts at high osmotic strength by thermotolerant yeasts. *Biotechnol. Lett.*, **21**, 617–620.

Caridi, A., Galvano, F., Tafuri, A., and Ritieni, A. (2006a) In-vitro screening of *Saccharomyces* strains for ochratoxin A removal from liquid medium. *Ann. Microbiol.*, **56**, 135–137.

Caridi, A., Galvano, F., Tafuri, A., and Ritieni, A. (2006b) Ochratoxin A removal during winemaking. *Enzyme Microb. Tech.*, **40**, 122–126.

Caridi, A., Sidari, R., Solieri, L., Cufari, A., and Giudici, P. (2007) Wine colour adsorption phenotype: An inheritable quantitative trait loci of yeasts. *J. Appl. Microbiol.*, **103**, 735–742.

Cecchini, F., Morassut, M., Garcia Moruno, E., and Di Stefano, R. (2006) Influence of yeast strain on ochratoxin A content during fermentation of white and red must. *Food Microbiol.*, **23**, 411–417.

Chu, F. S., Chang, C. C., Ashoor, S. H., and Prentice, N. (1975) Stability of aflatoxin B-1 and ochratoxin A in brewing. *Appl. Microbiol.*, **29**, 313–316.

Marullo, P., Bely, M., Masneuf-Pomarede, I., Aigle, M., and Dubourdieu, D. (2004) Inheritable nature of enological quantitative traits is demonstrated by meiotic segregation of industrial wine yeast strains. *FEMS Yeast Res.*, **4**, 711–719.

Naumov, G. I., Naumova, E. S., and Korshunova, I. V. (2001) Genetic identification of cultured *Saccharomyces* yeasts from Asia. *J. Gen. Appl. Microbiol.*, **47**, 335–338.

Naumova, E. S., Naumov, G. I., Masneuf-Pomarède, I., Aigle, M., and Dubourdieu, D. (2005) Molecular genetic study of introgression between *Saccharomyces bayanus* and *Saccharomyces cerevisiae. Yeast,* **22**, 1099–1115.

Oda, Y., Mikumo, D., Leo, F., and Urashima, T. (2010) Discrimination of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* strains by the SUC2 gene sequences. *J. Gen. Appl. Microbiol.*, **56**, 355–358.

Paulovich, A. G. and Hartwell, L. J. (1995) A checkpoint regulates the rate of progression through S phase in *Saccharomyces cerevisiae* in response to DNA damage. *Cell*, **82**, 841–847.

Piotrowska, M. and Zakowska, Z. (2000) The biodegradation of ochratoxin A in food products by lactic acid bacteria and baker's yeast. *Food Biotechnol.*, **17**, 307–310.

Ringot, D., Lerzy, B., Bonhoure, J. P., Auclair, E., Oriol, E., and Larondelle, Y. (2005) Effect of temperature on in vitro ochratoxin A biosorption onto yeast cell wall derivatives. *Process Biochem.*, **40**, 3008–3016.

Sando, N., Maeda, M., Endo, T., Oka, R., and Hayashibe, M. (1973) Induction of meiosis and sporulation in differently

aged cells of *Saccharomyces cerevisiae. J. Gen. Appl. Microbiol.*, **19**, 359–373.

Scott, P. M., Kanhere, S. R., Lawrence, G. A., Daley, E. F., and Farber, J. M. (1995) Fermentation of wort containing added ochratoxin A and fumonisins B1 and B2. *Food Addit. Contam.*, **12**, 31–40.

Zimmerli, B. and Dick, R. (1996) Ochratoxin A in table wine and grape-juice: Occurrence and risk assessment. *Food Addit. Contam.*, **13**, 655–668.