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In-vitro screening of *Saccharomyces* strains for ochratoxin A removal from liquid medium

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Abstract - The aptitude of twenty *Saccharomyces sensu stricto* strains to remove ochratoxin A from a synthetic medium containing 1.1 ng/mL, about half of the European Community limit, was evaluated using four to six mg of biomass (wet weight)/mL. Seven strains show high levels of ochratoxin A removal, 0.72-1.10 ng/mL, equivalent to 66-100% of the available toxin, and unitary removing activity of 14.31-27.24 pg/mg of biomass. Further research will be carried out to study the mechanism of OTA removal and to confirm the ability of the most efficacious strains of *Saccharomyces sensu stricto* to remove OTA from contaminated wort and grape must during alcoholic fermentation.

Key words*:* detoxification; ochratoxin A removal; *Saccharomyces sensu stricto*; wine.

INTRODUCTION

Ochratoxin A (OTA) is a potently nephrotoxic, carcinogenic, teratogenic, and immunotoxic mycotoxin, that frequently contaminates various foods and beverages and poses a serious threat to human health (Galvano *et al.,* 2005).

Different approaches to the risk assessment of OTA by diverse bodies have led to variable estimates of a tolerable daily intake (Walker *et al.,* 2002). In 1996, the Joint Expert Committee on Food Additives of the World Health Organization and the Food and Agriculture Organization, on the basis of the nephrotoxicity of OTA, proposed a provisional tolerable daily intake of 14 ng/kg bw (Anon, 1996). More cautious values (5 ng/kg bw) have been proposed by the Working Group of the Nordic Council of Ministers (Olsen *et al.,* 1991) and by the European Commission (Anon, 1998) whereas the Canadian authority proposed an OTA tolerable daily intake in the range 1.2-5.7 ng/kg bw (Kuiper-Goodman, 1996).

OTA occurrence in wines is related to the growth of *Aspergillus carbonarius* and other black aspergilli on grapes during cultivation (Cabañes *et al.,* 2002; Battilani *et al.,* 2003). According to the literature, the concentration of OTA in alcoholic beverages and their substrata is spread over a wide range, and vary from 0 to 13.08 ng/g in grape berries (Battilani *et al.,* 2003), up to 9.8 ng/mL in grape juice (Arici *et al.,* 2004), up to 0.205 ng/mL in beer (Araguás *et al.,* 2005), and up to 7.63 ng/mL in wine (Visconti *et al.,* 1999).

Prevention of contamination at source is an important but not sufficient strategy. One of the most effective strategies for controlling mycotoxin hazards is the use of specific materials that adsorb mycotoxins, thus avoiding or limiting their bioavailability in the gastro-intestinal tract (Yiannikouris *et al.,* 2003).

Increasing interest has been recently generated by the possibility of using microbiological-binding agents to remove mycotoxins (Galvano *et al.,* 2001).

Biodegradation by yeasts of OTA (Piotrowska and Zakowska, 2000) and a variety of decontamination procedures for the removal of OTA using yeasts (Scott *et al.,* 1995; Bejaoui *et al.,* 2004; Caridi *et al.* 2004, 2005a, 2005b), yeast cell walls or yeast cell wall extracts (Ringot *et al.,* 2005) have been reported.

The aim of this work was to evaluate the aptitude of wine yeasts to remove OTA from a synthetic medium. We decided to investigate the aptitude of twenty strains of *Saccharomyces sensu stricto*, previously selected for winemaking, to remove OTA in vitro. We settled to test a concentration of 1.1 ng of OTA/mL, about half of the European Community limit (2.0 ppb) for wines produced from the 2005 harvest onwards (Anon, 2005). We decided, however, in order to give emphasis to the yeast's variability, to utilise a biomass quantity of 4-6 mg/mL (wet weight), higher than that usually occurring during alcoholic fermentation.

MATERIALS AND METHODS

Methodology. Twenty strains of *Saccharomyces sensu stricto*, selected for winemaking and belonging to the Department STAFA collection (Reggio Calabria, Italy), were grown on Sabouraud Dextrose (4%) agar at 28 °C for 2 d. Aliquots of

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40-60 mg of biomass of each strain were taken in triplicate using 10 µL-inoculating loops, exactly weighed, and suspended in test tubes containing 10 mL of physiological sterile saline and 1.1 ng of OTA/mL. Test tubes were kept at room temperature (20 °C) for 15 d without shaking and then the OTA content of the solutions was assayed. The removed toxin was calculated by subtracting the amount of toxin in each test tube from the amount found in the control test tubes, where no biomass was added. The unitary removing activity was calculated as pg of OTA/mg of biomass, expressed as wet weight.

Analytical methods. HPLC analyses were performed using LC-10AD pumps and a RF-10Axl fluorescence detector (Shimadzu, Japan), set to an excitation wavelength of 333 nm and emission wavelength of 460 nm. Data acquisition and handling were made by system control SLC10A with VP5 software (Shimadzu). A Jupiter C18 (250 x 4.6 mm, 5 µm) column (Phenomenex, USA) was used. HPLC conditions were set up using an elution at a constant flow of 1 mL/min and acetonitrile:water:acetic acid (49:49:1) as the starting eluent system. The starting ratio was linearly modified to 100% acetonitrile 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 prepurified by Ochratest immunoaffinity columns (Vicam, USA) according to manufacturing protocol, filtered through a 0.20 µm syringe filter (RC 0.20 µm), and 100 µL injected (loop of 20 µL) into the HPLC column by 250 µL syringe (Hamilton, Switzerland). Mycotoxin identification was performed by comparing retention times and UV maximum absorption of purified samples to pure OTA standard. A further investigation was performed co-injecting samples together with OTA standard solution. The average retention time for OTA (4.5 min, RSD 1.5%) was obtained with 10 consecutive injections of the same OTA working solution within the same day. These conditions enhance the chromatographic resolution of the OTA peak from other matrix interferences that may invalidate identification and/or quantification steps. The calculated instrumental detection limit and quantification limit for OTA under these conditions were 0.2 pg (10 ng/L) and 2 pg (100 ng/L) with a ratio signal to noise (S/N) 3 and 5, respectively. Mycotoxin quantification was carried out by comparing peak areas of investigated samples to the calibration curve of OTA standard solution, ranging from 0.1 to 100 µg/L. Recovery experiments were performed, artificially contaminating physiological saline with stock OTA solution at two different levels (500 and 1000 ng/L), and then extracted using the above described protocol. These experiments were performed three times for two consecutive days and the values were 100% for both selected concentrations. Mean and standard deviation were calculated using three replicates.

RESULTS AND DISCUSSION

Table 1 shows the list of the 20 wine yeasts, the percentage and level of removed OTA, and the unitary removing activity. The capacity of the tested yeasts to remove OTA varies from 0 to 100% (OTA content of 1.1 ng/mL).

TABLE 1 – Percentage and level (mean and standard deviation) of removed ochratoxin A (OTA) and unitary removing activity of the 20 yeast strains

Strain	Percentage of removed OTA (%)	Removed OTA (ng/mL)		Unitary removing activity (pg/mg of biomass wet weight)	
		Mean	SD	Mean	SD
Sc2717	0	0.00	0.00	0.00	0.00
Sc1661	5	0.05	0.09	1.12	1.95
Sc708	6	0.06	0.10	1.12	1.94
Sc2659	7	0.08	0.07	1.83	1.59
Sc1864	8	0.09	0.16	1.53	2.64
1042	18	0.20	0.19	4.24	4.13
TT77	21	0.23	0.20	5.00	4.38
Sc1304	33	0.37	0.08	7.93	1.23
Sc1766	42	0.46	0.13	10.03	3.90
Sc2640	47	0.52	0.13	11.74	4.12
Sc560	51	0.56	0.48	11.63	10.38
TT254	53	0.59	0.02	15.11	1.88
Sc226	57	0.63	0.25	13.37	4.32
Sc2621	66	0.72	0.34	14.31	6.14
12233	86	0.94	0.27	18.49	5.16
Sc45	100	1.10	0.00	21.91	1.57
Sc1483	100	1.10	0.00	22.03	0.76
Sc254	100	1.10	0.00	26.82	1.76
TT173	100	1.10	0.00	27.22	0.82
Sc2489	100	1.10	0.00	27.24	3.27
Control	0	0.00	0.00	0.00	0.00

OTA content of physiological sterile saline: 1.1 ng/mL. Yeast strains are ordered based on their increasing aptitude to remove OTA.

Five strains of *Saccharomyces* show OTA removal (0.00-0.09 ng/mL equivalent to 0-8% of the available OTA) and unitary removing activity (0.00-1.83 pg of OTA/mg of biomass) at low levels.

Eight strains of *Saccharomyces* show OTA removal (0.20-0.63 ng/mL equivalent to 18-57% of the available OTA) and unitary removing activity (4.24-15.11 pg of OTA/mg of biomass) at medium levels.

Seven strains of *Saccharomyces* show OTA removal (0.72-1.10 ng/mL equivalent to 66-100% of the available OTA) and unitary removing activity (14.31-27.24 pg of OTA/mg of biomass) at high levels. It is interesting to note that five strains of *Saccharomyces*, i.e. Sc45, Sc1483, Sc254, TT173, and Sc2489 have entirely removed the available OTA in all the three replicates. These strains could be checked in saturation tests to verify their maximum capacity to remove OTA.

OTA in wine is a great problem and many materials have been tested for its removal. The need for an efficient adsorption agent appears to be of particular importance considering the decision of the European Community to fix a maximum limit of 2.0 ppb of OTA for wines (Anon, 2005). In this context, yeasts represent a very promising biomaterial, also considering their role in the vinification process. Our results demonstrate the possibility of removing OTA with expressly selected wine yeasts. Further research will be carried out to study the mechanism of OTA removal and to confirm the ability of the most efficacious strains of *Saccharomyces sensu stricto* to remove OTA from contaminated wort and grape must during alcoholic fermentation.

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