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Biocontrol activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius* and their ability to remove ochratoxin A from grape juice

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ABSTRACT

Aspergillus spp. infection of grape may lead to ochratoxin A (OTA) contamination in processed beverages such as wine and grape juice. The aim of the current study was to evaluate the biocontrol potential of two non-fermenting (*Cyberlindnera jadinii* 273 and *Candida friedrichii* 778) and two low-fermenting (*Candida intermedia* 235 and *Lachancea thermotolerans* 751) yeast strains against the pathogenic fungus and OTA-producer *Aspergillus carbonarius*, and their ability to remove OTA from grape juice. Two strains, 235 and 751, showed a significant ability to inhibit *A. carbonarius* both on grape berries and in in vitro experiments. Neither their filtrate nor their autoclaved filtrate culture broth was able to prevent consistently pathogen growth. Volatile organic compounds (VOCs) produced by all four selected yeasts were likely able to consistently prevent pathogen sporulation in vitro. VOCs produced by the non-fermenting strain 778 also significantly reduced *A. carbonarius* vegetative growth. Three yeast strains (235, 751, and 778) efficiently adsorbed artificially spiked OTA from grape juice, while autoclaving treatment improved OTA adsorption capacity by all the four tested strains. Biological control of *A. carbonarius* and OTA-decontamination using yeast is proposed as an approach to meet the Islamic dietary laws concerning the absence of alcohol in *halal* beverages.

1. Introduction

Table grape (*Vitis vinifera* L.) is one of the most economically important marketed fruit commodities (Jiang et al., 2014). Each year 30–40% of table grapes are lost due to the lack of efficient methods to prevent pre- and post-harvest diseases (Abeer et al., 2013; Prusky, 2011). Several fungi, mainly belonging to the genera *Aspergillus*, *Alternaria*, *Cladosporium*, *Rhizopus*, and *Penicillium*, are opportunistic pathogens, able to invade ripe berries after injury (Nally et al., 2013; Rooney-Latham et al., 2008), however various yeasts and bacteria are also associated with so-called sour rot.

Black *Aspergilli* are widespread in vineyards and may not only cause rot on berries but are also the main source of ochratoxin A (OTA) contamination. OTA is usually recovered on food of vegetable origin and its presence in raw ingredients may lead to severe contamination in processed beverages such as wine and grape juice (Battilani et al., 2003; Mulè et al., 2006; Zimmerli and Dick, 1996). Among black *Aspergilli*, *Aspergillus carbonarius* (Bainier) Thom is considered the most dangerous one, having the highest potential for OTA production in grape (Battilani et al., 2003, 2004a; Cabañes et al., 2002).

OTA has nephrotoxic, teratogenic, hepatotoxic, and carcinogenic effects in mammals (Rodriguez et al., 2011). Therefore, as a preventive measure, the European Union set the maximum permitted levels of OTA in wine and grape juice at 2 $\mu g \cdot kg^{-1}$ (Commission regulation No. 123/2005 amending Regulation No. 446/2001).

Biological control of plant pathogens with antagonistic microorganisms is an efficient alternative to synthetic fungicides in reducing postharvest diseases and product loss (Janisiewicz and Korsten, 2002; Meng et al., 2010; Tian, 2006). Yeasts are considered among the most efficient antagonists in biocontrol strategies (Droby et al., 2009). Because of their role in winemaking processes, they may also represent an important tool in the biological removal of OTA from natural juice (Bejaoui et al., 2004; De Felice et al., 2008; Delage et al., 2003; Petruzzi et al., 2013; Rodriguez et al., 2011). However, the potential rapid increase in ethanol concentration in must and juice due to the fermentation process by yeast represents a significant problem for their use as biocontrol agents, reducing their efficacy as antagonists and their ability

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to remove OTA (Cubaiu et al., 2012). This hindrance may be overcome, at least in non-alcoholic grape juices, by using non- or low-fermenting yeast strains.

Islamic laws (Quran V: 90–91) forbid Muslim populations from drinking alcoholic beverages and consuming food prepared with alcohol, even in a small amount (Regenstein et al., 2003). Nonetheless, alcohol is common in many biological systems: e.g. fresh fruits and/or their essences and juices can contain traces of alcohol (Riaz and Chaudry, 2003). Whenever alcohol is naturally present in food, it does not invalidate its permissible (*halal*) status. Even if a global standard limit for *halal* certified food is not allowable, ingredients containing an average of 0.5% or even 0.75% residual alcohol are generally considered as acceptable (Anis Najiha et al., 2010), although these limits may vary according to countries and religious groups.

The objective of this study was to evaluate the biocontrol potential of four selected yeast strains (two non-fermenting and two low-fermenting) against *A. carbonarius*, and their ability to remove OTA from grape juice. This biological treatment is proposed to meet the target of the Islamic dietary laws concerning the absence of residual alcohol in *halal* beverages.

2. Materials and methods

2.1. Strain identification

Four yeast strains from the DISAABA (Dipartimento di Agraria, Università di Sassari, Italy) collection were selected based on their ability to efficiently prevent green rot caused by *Penicillium expansum* Link on apple fruit (Fiori et al., unpublished results). Strains were identified by sequencing the ITS1 and ITS2 regions of ribosomal DNA as belonging to: 253, *Candida intermedia* (Cif. & Ashford) Langeron & Guerra; 273, *Cyberlindnera jadinii* (Sartory, R. Sartory, Weill & J. Mey.) Minter; 751, *Lachancea thermotolerans* (Filippov) Kurtzman, and 778, *Candida friedrichii* Uden & Windisch.

2.2. Evaluation of the fermenting activity

To check the capacity of the four yeast strains to produce and release alcohol in commercial grape juice, a micro-fermentation experiment was carried out. The four yeasts plus a *Saccharomyces cerevisiae* Meyen *ex* E.C. Hansen commercial strain (Lalvin EC-1118; Lallemand, Montreal Quebec, Canada) chosen as a positive control, were grown overnight at 25 °C in 20 mL YPD broth (1% yeast extract, 2% bacteriological peptone, 2% dextrose; Sigma-Aldrich, St. Louis, MO, USA). Cells were recovered by centrifugation, washed, resuspended in Ringer solution (0.9% NaCl) and counted with a Thoma hemocytometer. Fermentation was performed in sterile flasks containing 50 mL of commercial grape juice inoculated with yeasts (final concentration of 5×10^6 cells/mL). Uninoculated grape juice served as a negative control and each sample was replicated twice. All the flasks were corked with Müller valves filled with 4 mL of sulfuric acid to avoid liquid evaporation from juices. From day 0 to day 15 the weight loss in each flask, due to the release of carbon dioxide by fermentation, was recorded.

2.3. In vitro biocontrol experiments

Yeast cells were grown overnight and counted as described previously. Part of the overnight culture broth was filtered through 0.22 μm Millex® filters (Millipore, Cork, Ireland) while an aliquot was both filtered and autoclaved (20 min at 121 °C). Petri plates (diameter of 90 mm) containing YPD agar (2%) or YESA (2% yeast extract, 15% sucrose, 2% agar; Sigma-Aldrich) media were inoculated with each yeast strain as follows: (i) topped with soft agar (0.7%) enriched with 1 mL of yeast suspension (10^6 CFU/mL), (ii) spread with 300 μL of filtered culture broth, and (iii) spread with 300 μL of filtered and sterilized culture broth.

A spore suspension (10^5 spores/mL) of *A. carbonarius* Bainier Thom. MPVA566 (courtesy of Professor P. Battilani, Università Cattolica del Sacro Cuore, Piacenza, Italy), grown on a PDA (potato dextrose agar; Sigma-Aldrich) medium at 25 °C for 1 week, was prepared in distilled sterile water containing 0.1% Tween 20 (Sigma) to prevent spore clumping. Three aliquots (each of 10 μL) of the suspension were separately spotted in each plate. Experiment was performed in triplicate and the plates were sealed with Parafilm®, stored at 25 °C, and the average diameter of fungal colonies was measured after 4 days of growth and compared with controls grown in the absence of living yeast, filtered, or filtered and autoclaved culture broth.

2.4. In vitro evaluation of volatile organic compounds (VOCs)

To ascertain whether VOCs produced by the antagonistic yeast strains may have a role in *A. carbonarius* inhibition, yeast and pathogen cell suspensions were prepared as described previously, by using two distinct *A. carbonarius* strains MPVA566 and AN6 (isolated from grape, courtesy of Professor G. Romanazzi and Dr. E. Feliziani, Università Politecnica delle Marche, Ancona, Italy).

YPD agar (2%) plates were inoculated by evenly streaking 100 μL of yeast cell suspension (10^8 CFU/mL) using a sterile spreader and incubated for 24 h at 25 °C. The plate lid was then replaced by a Petri dish containing PDA point-inoculated with 20 μL of pathogen spore suspension (10^7 conidia/mL), sealed with Parafilm® and examined after 5 days of incubation at 25 °C by evaluating the radial growth and the morphology of *A. carbonarius* colonies. Three replicates for each fungus–yeast combination were set and each experiment was repeated twice.

2.5. Biocontrol experiments on detached grape berries

For each yeast strain, four grape (cv. Italia) bunches, each bearing five mature berries, were prepared, disinfected with 1% sodium hypochlorite for 10 min and rinsed twice with sterile distilled water. Berries were wounded with a sterile needle (2 mm diameter, 1 wound/berry) and whole bunches were dipped into a 50 mL suspension of each antagonist (10^8 CFU/mL). Bunches were then air-dried and subsequently sprayed until runoff (1 mL/bunch) with an *A. carbonarius* spore suspension (10^7 conidia/mL) by using a hand sprayer. After inoculation, grape bunches were placed in plastic boxes ($16 \times 26 \times 10$ cm) for 6 days and incubated at 25 °C in the dark under high relative humidity ($95 \pm 5\%$). After 6 days berries were checked and a 0–100 disease index was assigned to them according to the percentage (0, 25, 50, 75 and 100%) of berry surface covered by mold.

2.6. VOC experiments on artificially inoculated grape berries

To check whether VOCs produced by the antagonistic yeast strains may inhibit *A. carbonarius* growth on grape berries, bunches were prepared, wounded and inoculated with the pathogen as previously described. The grape bunches were then placed into plastic boxes whose lids were internally supplied with three open YPD agar (2%) plates inoculated by streaking 100 μL of yeast cell suspension (10^8 CFU/mL) and previously incubated for 24 h at 25 °C. Plastic boxes were sealed with Parafilm® and cellophane and stored for 6 days at 25 °C under high relative humidity ($95 \pm 5\%$). A disease index was then assigned to inoculated berries as previously reported.

2.7. Reduction of OTA from grape juice

To evaluate the ability of the four yeast strains to remove ochratoxin A from grape juice, 24 mL of a commercial grape juice (Vitalift, Lidl Stiftung & Co. KG, Neckarsulm, Germany) was poured into sterile flasks (100 mL volume) and spiked with 20 ng/mL of OTA, obtained by a nitrile acetate standard (Sigma-Aldrich) which was preliminarily

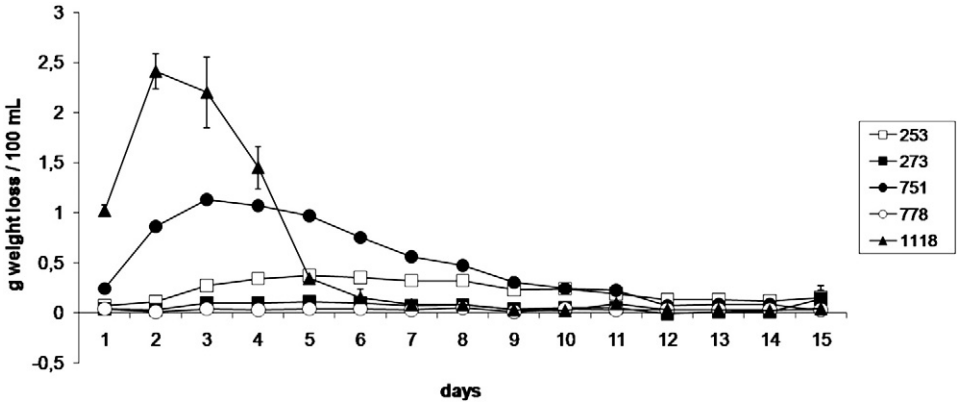

Fig. 1. Fermentation activity of four yeast strains (253, *Candida intermedia*; 273, *Cyberlindnera jadinii;* 751, *Lachancea thermotolerans*, and 778, *Candida friedrichii*) compared to the activity of the strong fermenting *Saccharomyces cerevisiae* strain (EC-1118). Results are expressed as weight loss (g) during 15 day incubation in grape juice at 25 °C due to CO_2 release in 100 mL.

evaporated and resuspended in sterile distilled water. One milliliter of yeast cell suspension (10^8 CFU/mL) was added to the OTA-amended grape juice and the flasks were then incubated in the dark at 25 °C under constant agitation (100 rpm) for 8 days. The same experiment was also conducted by adding 1 mL of autoclaved yeast cell suspension (10^{10} CFU/mL) to 19 mL of OTA-amended grape juice (20 ng/mL) to check whether inactivated yeast cells could still remove OTA from grape juice. Yeast cells were separated after 8 days by centrifuging at 40,000 rpm (29,000 ×*g*) for 20 min, and both yeast pellet and a 5 mL sample of the grape juice were subject to OTA extraction and purification for subsequent HPLC analysis.

2.8. OTA purification and HPLC analysis

OTA was extracted from grape samples and purified according to the protocol described in the MycoSep® Ochra Push-through kit (Romer Labs®, Union, MO, USA). The purified extract was then resuspended in the HPLC mobile phase.

OTA was extracted from yeast pellet by directly adding 4 mL of methanol to the pellet and by mixing vigorously the suspension for 30 min. After centrifugation (29,000 ×*g*, 20 min), the supernatant was separated and evaporated under nitrogen atmosphere, then resuspended in the HPLC mobile phase to undergo HPLC analysis. The whole procedure was repeated thrice in order to achieve quantitative extraction from the pellet. OTA extraction was carried out in triplicate and the experiments were repeated at least twice.

OTA determination was performed on an Agilent 1200 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump with integrated vacuum degasser (G1311C), autosampler (G1329B), column oven (G1316A) and fluorescence detector (G1321A). The separation, identification and quantification steps of OTA were carried out according to the EN 14133:2009 method. Briefly, the separation was performed on a Zorbax column SB-C18, 4.6 × 150 mm, 5-Micron (Agilent, Santa Clara, CA, USA), under isocratic conditions. The mobile phase was a mixture of acetonitrile, water and acetic acid (49.5:49.5:1 v/v/v, respectively). The flow was set to 1 mL/min and the column oven temperature was set to 25 °C. The excitation and the emission wavelengths were set to 333 and 460 nm, respectively. OTA was identified by comparing the retention time of the relevant peak on samples with the one from the standard (5.9 min). The quantification of OTA was performed using a linear external calibration in a range from 0.6 to 60 ng/mL, with a mean correlation coefficient of 0.99995.

Table 1
Biocontrol activity of low- or non-fermenting yeast strains against *Aspergillus carbonarius* MPVA566 on YPD and YES agar media amended with living yeast cells, culture filtrate or autoclaved culture filtrate. Results are expressed as colony diameter (mm) ± standard error after 4 days of incubation at 25 °C. Values in each column followed by one ($P \leq 0.05$) or two ($P \leq 0.01$) asterisks are significantly different from the control (44.9 ± 0.1 mm on YPD; 47.9 ± 0.5 mm on YES) according to Dunnett's test.

Treatment	*Aspergillus carbonarius* colony diameter (mm)		
	+ Living yeast	+ Filtrate	+ Autoclaved filtrate
YPD			
C. intermedia 253	0**	45.9 ± 0.2	45.7 ± 0.3
C. jadinii 273	15.2 ± 0.2**	45.0 ± 0.3	45.2 ± 0.4
L. thermotolerans 751	0**	47.2 ± 0.3**	47.2 ± 0.4**
C. friedrichii 778	13.7 ± 0.6**	46.1 ± 0.5 *	47.0 ± 0.3**
YES			
C. intermedia 253	0**	40.6 ± 0.2**	43.9 ± 0.3**
C. jadinii 273	0**	42.8 ± 0.3**	45.1 ± 0.3**
L. thermotolerans 751	0**	44.7 ± 0.3**	43.4 ± 0.3**
C. friedrichii 778	0**	47.8 ± 0.4	46.8 ± 0.3

2.9. Statistical analysis

Data from each experiment were subjected to arcsine square root transformation prior to one-way analysis of variance followed by multiple comparison by Dunnett's test, using Minitab® for Windows release 12.1.

3. Results

3.1. Micro-fermentation assay

In micro-fermentation assays the four yeast strains displayed different fermenting behaviors (expressed as CO_2 loss (g) in 100 mL) compared to the commercial *S. cerevisiae* strain EC-1118. The antagonistic strains *C. intermedia* 253 and *L. thermotolerans* 751 revealed a very low fermentation activity that was limited to the first 5 days of incubation, while strains *C. jadinii* 273 and *C. friedrichii* 778 had no fermentation activity (Fig. 1).

3.2. In vitro biocontrol activity of living yeasts against A. carbonarius

On YPD agar only living cells were able to significantly prevent pathogen development (Table 1). On YPD enriched with filtered or filtered and subsequently sterilized culture broth, *A. carbonarius* was not inhibited: in fact, when sterile culture broth from strains *L. thermotolerans* 751 and *C. friedrichii* 778 was added to YPD agar, *A. carbonarius* colony growth was significantly higher compared to the control plates (Table 1).

On YES agar medium, the presence of living yeast cells prevented *A. carbonarius* growth, while the pathogen was only slightly – albeit significantly – inhibited by the filtered/autoclaved culture broth from

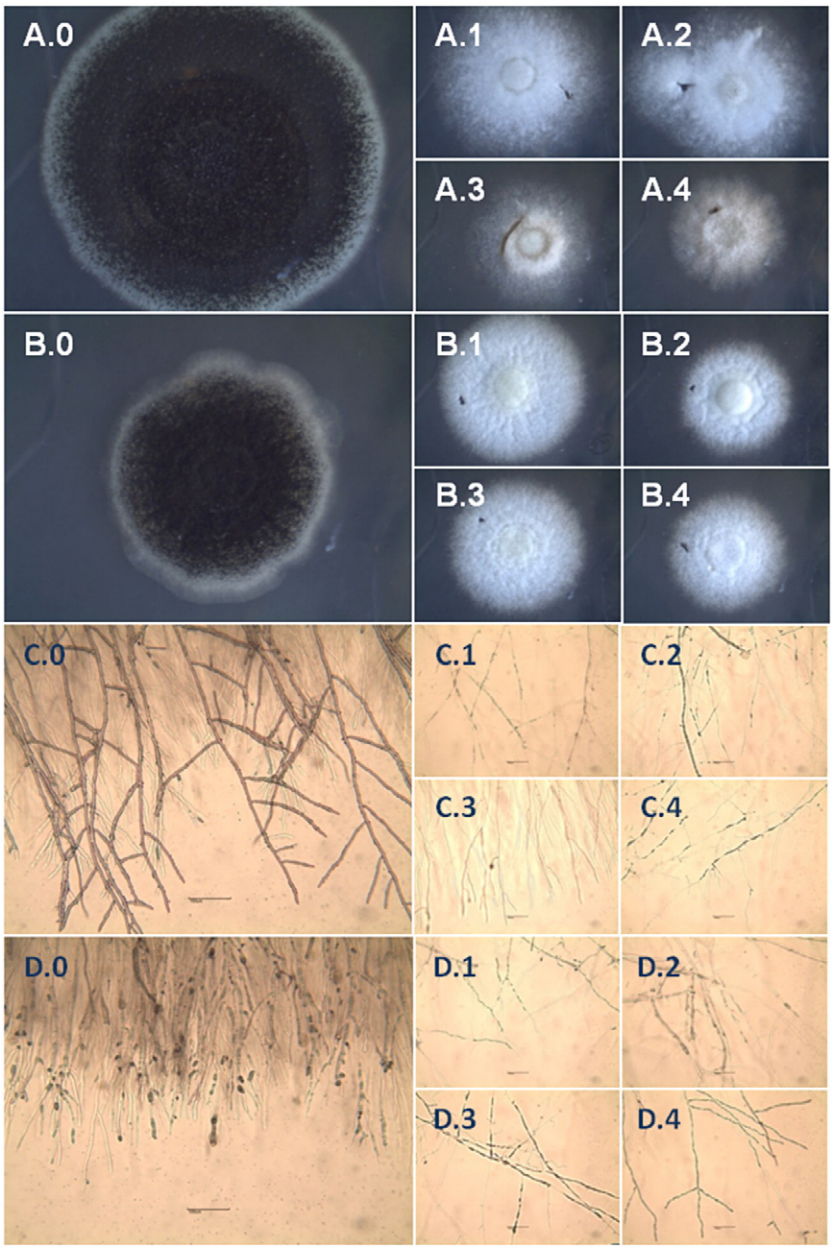

Fig. 2. Colony growth and morphology and microscopic detail (10×) of the colony border of *Aspergillus carbonarius* strains MPVA566 (A and C) and AN6 (B and D) grown (0 = control) in an environment saturated by VOCs released by yeast strains 253 (1), 273 (2), 751 (3), and 778 (4).

yeast strains *C. intermedia* 253, *C. jadinii* 273 and *L. thermotolerans* 751 (Table 1).

3.3. Effect of yeast VOCs on A. carbonarius in vitro

Only VOCs produced by *C. friedrichii* 778 reduced *A. carbonarius* MPVA566 and *A. carbonarius* AN6 growth significantly, resulting in 58.3% ($P \leq 0.01$) and 33.7% ($P \leq 0.05$) reduction of colony diameter, respectively, compared to the controls. All the other yeast/pathogen pairings did not differ from the control in terms of colony development. Nonetheless, *A. carbonarius* colonies exposed to yeast VOCs did not sporulate, and were characterized by white mycelium; the colony border was undefined, with elongated and scattered hyphae compared to unexposed control (Fig. 2). Single hyphal tips and mycelium fragments were then transferred on fresh PDA and after 5 days of growth at 25 °C, typical black sporulating colonies were evident (not shown), suggesting that the anti-sporulating effect is reversible.

3.4. Effect of living yeast cells and yeast VOCs on grape rot caused by A. carbonarius

Three out of four tested yeasts, namely *C. intermedia* 253, *L. thermotolerans* 751 and *C. friedrichii* 778, reduced significantly the incidence of infection by *A. carbonarius* on detached grape berries (Table 2). No significant inhibition of grape infection was observed upon exposure to VOCs produced by any of the tested yeast strains (Table 2).

3.5. OTA adsorption by antagonistic yeast in grape juice

When living yeast cells were incubated in OTA-amended grape juice for 8 days, *C. intermedia* 253, *L. thermotolerans* 751, and *C. friedrichii* 778 were able to significantly reduce OTA content in OTA-amended grape juice by 73, 75, and 70%, respectively, while *C. jadinii* 273 had no significant adsorption effect (Table 3). Autoclaved cells derived from

Table 2
Biological control activity of living yeast cells and of volatile organic compounds (VOCs) against *Aspergillus carbonarius* MPVA566 on detached grape berries. Results are expressed as percentage (± standard error) of berry surface covered by mold. Values in each column followed by one ($P \leq 0.05$) or two ($P \leq 0.01$) asterisks are significantly different from inoculated control according to Dunnett's test.

Treatment	Infected grape berry surface (%)	
	1st experiment	2nd experiment
Living yeast cells		
Control	87.5 ± 4.8	75.0 ± 11.7
C. intermedia 253	32.5 ± 7.5**	17.5 ± 8.0**
C. jadinii 273	67.5 ± 4.8	42.5 ± 10.7
L. thermotolerans 751	20.0 ± 8.2**	27.5 ± 14.3*
C. friedrichii 778	37.5 ± 2.5**	27.5 ± 10.4*
VOCs		
Control	87.5 ± 12.5	95.0 ± 5.0
C. intermedia 253	85.0 ± 9.6	80.0 ± 7.1
C. jadinii 273	80.0 ± 8.2	92.5 ± 7.5
L. thermotolerans 751	95.0 ± 5.0	85.0 ± 6.4
C. friedrichii 778	57.5 ± 16.5	95.0 ± 5.0

C. friedrichii 778, *C. intermedia* 253, *C. jadinii* 273, and *L. thermotolerans* 751 reduced OTA concentration by 72, 74, 82, and 84%, respectively.

4. Discussion

The *halal* dietary laws indicate which foods are considered as "lawful" (i.e., permissible) for Muslims. These rules are found in the Quran and in the Sunna, and are recorded in the books of Hadith, the Traditions (Regenstein et al., 2003). Alcohol represents one of the main concerns, as alcoholic food and beverage are totally prohibited in Islam (Anis Najiha et al., 2010). The Islamic Food and Nutrition Council of America (IFANCA) has adopted a standard of 0.5% alcohol content in ingredients and 0.2% in finished products (Al-Mazeedi et al., 2013). Therefore, any attempt to reduce mycotoxin contamination in food and beverages by biological means should assure that no traces of ethanol are generated during the process.

Grape infection by *Aspergillus* spp. occurs immediately prior to or during harvesting, transportation and storage of harvested grape bunches (Battilani et al., 2004b; Magan and Aldred, 2005; Martinez-Rodriguez and Carrascosa, 2009; Zimmerli and Dick, 1996). During the wine making process, OTA is not completely released during the crushing of the berries: maceration can cause an increase in OTA content estimated at around 20% (Battilani et al., 2004b), while OTA content tends to diminish during the yeast and malolactic fermentations, probably due to adsorption on the yeast surface, or to degradation by lactic bacteria (Amézqueta et al., 2009; Bejaoui et al., 2004; Cecchini et al., 2006). On the contrary, in grape juice production OTA contamination may reach significant levels, since the activity of fermenting yeast and lactic bacteria is absent (Rosa et al., 2004).

Table 3
Ochratoxin A (OTA) reduction on grape juice by living yeast cells. Yeast cells were inoculated in 25 mL of grape juice artificially amended with OTA (20 ng/mL) and incubated in the dark at 25 °C under constant agitation (100 rpm) for 8 days, then juice and cell pellets were separated to measure OTA reduction and adsorption, respectively. Results obtained from HPLC analysis are expressed as ng/mL of OTA ± standard error, and referred to the initial volume of 25 mL. Values in each column followed by one ($P \leq 0.05$) or two ($P \leq 0.01$) asterisks are significantly different from inoculated control according to Dunnett's test.

Treatment	OTA content (ng/mL ± SE)	
	Grape juice	Yeast cells
Control	16.4 ± 1.4	/
C. intermedia 253	5.5 ± 0.3**	7.4 ± 0.5
C. jadinii 273	13.0 ± 1.6	1.8 ± 0.6
L. thermotolerans 751	4.9 ± 0.0**	11.3 ± 1.6
C. friedrichii 778	5.9 ± 0.4**	9.7 ± 0.5

Our aim was to develop a new biocontrol approach to prevent OTA contamination that could meet Islamic laws concerning the presence of alcohol in *halal* beverages. In the present investigation, we have selected four antagonistic yeast strains with no or low-fermenting capacity, that are able to control the pathogenic fungus and OTA-producer *A. carbonarius* in grape.

The two low-fermenting yeast strains, *C. intermedia* 235 and *L. thermotolerans* 751, showed a significant antagonistic activity against *A. carbonarius* both on grape berries and in in vitro experiments. Since their filtrate and autoclaved filtrate culture broth were not able to prevent consistently the pathogen growth, it is likely to assume that the main mechanism behind the biocontrol efficiency of these strains consists in the competition for space and nutrients rather than to the release of diffusible antifungal compounds.

Accumulation of carbon dioxide and decreased levels of oxygen may also play a role in reducing fungal growth or sporulation (Schalchli et al., 2011). Since these parameters were not evaluated in our experiments, it is not possible to completely rule out the role of competition for oxygen in the yeast — *A. carbonarius* interaction. Nonetheless, volatile organic compounds (VOCs) produced by all four selected yeasts were likely able to inhibit sporulation of *A. carbonarius* in vitro. VOCs are low molecular weight metabolites that may contribute to pathogen control (Fialho et al., 2010); they play a role in changing protein expression (Humphris et al., 2002) and enzymatic activity (Wheatley, 2002) of fungi and their efficacy may depend on the target pathogen (Mari et al., 2012). Antifungal VOC production by yeasts may represent an important tool for postharvest management, especially under airtight environment (Huang et al., 2011).

VOCs produced by one of the two non-fermenting yeast strains, namely *C. friedrichii* 778, in addition to inhibiting sporulation by *A. carbonarius*, also reduced significantly its vegetative growth *in vitro*. Further investigation shall be focused on the characterization of the chemical composition of VOCs produced by *C. friedrichii* 778 compared to those produced by the other yeast strains, in order to identify the molecules putatively responsible for the inhibition of mycelium development.

To the best of our knowledge, this is the first report on yeast VOCs used against *A. carbonarius*.

Among the tested yeast, *C. intermedia* 235 and *L. thermotolerans* 751, along with *C. friedrichii* 778, presented a remarkable capacity to adsorb OTA on grape juice amended with this mycotoxin, while living cells of *C. jadinii* 273 did not display any significant absorption capacity. Autoclaving treatment provided OTA adsorption capacity to all the four tested strains: as previously observed for other yeast, this effect may be due to the boost of adsorption cell sites as a consequence of the heat treatment (Bejaoui et al., 2004; Péteri et al., 2007).

Compared to other detoxification methods, such as the use of organic and inorganic adsorbent materials, the biological control approach represents a more efficient preventive strategy to reduce OTA contamination, since selected antagonists may be applied both before harvesting to control grape infection by OTA-producing fungi and also during processing to act as OTA detoxifiers. Field experiments are being carried out to evaluate the competitive ability of low- and non-fermenting yeasts towards resident microflora, their fitness and resistance to multiple environmental stress (including agrochemicals). These features are a basic prerequisite for any biocontrol product to be commercially developed (Droby et al., 2009).

5. Conclusions

Our results show that selected non-fermenting or low-fermenting yeast strains may be efficiently developed as biocontrol agents in postharvest disease management of grape as well as processing aids in the

production of grape juice, to reduce mycotoxin contamination if OTA levels exceed the limits fixed by food regulation. Processing aids are commonly used in food industry as adjuvants in food processing and do not need to be reported on the label by law in many countries (Codex Alimentarius, 1981). The use of several processing aids may lead, even not intentionally, to the presence of non-admitted substances in the final product, hence invalidating the *halal* status of food (Al-Mazeedi et al., 2013). *Halal* food industry is among the biggest and fastest expanding niches in the food market, representing one fifth of global food trade (Azeez, 2013). Research and development on microorganisms to be adopted as biocontrol agents in fruit post-harvest or as biological adsorbent to remove mycotoxins from fruit juices should fulfill the request imposed by *halal* food market to guarantee that products meet religious standards. In this perspective, it is recommended to use low-fermenting yeast that does not release any or release only extremely low levels of alcohol below *halal* thresholds.

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