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Original Article

بهینه سازی فعالیت کنترل زیستی جدایه های مخمرهای کم تخمیر در برابر *آسپرژیلوس نایجر* به منظور سم زدایی از آب انگور

آرش بابایی*[۱]، محسن دوزبخشان[۲]، حدیث طوافی[۱]

[۱]استادیار، گروه زیست شناسی، دانشکده علوم پایه، دانشگاه ملایر، ملایر، ایران، [۲]مربی، گروه زیست شناسی، دانشکده علوم پایه، دانشگاه ملایر، ملایر، ایران.

چکیده

سابقه و هدف: انگورهای آلوده به *آسپرژیلوس* می‌توانند موجب تولید اوخراتوکسین A در فراوری نوشیدنی‌هایی مانند شراب یا آب انگور شوند. هدف از این پژوهش ارزیابی توانایی زیستی ضد قارچی دو جدایه بومی مخمری با میزان تخمیر کم (A01 و G01) و سه سویه استاندارد *ساکارومیسس سرویزیه، کاندیدا گیلرموندی، مچنیکوویا آگاوس* علیه *آسپرژیلوس نایجر* و توانایی آن در حذف اوخراتوکسین A در انگور و محصولات آن بدون تولید الکل در حین فرایند بود.

مواد و روش‌ها: دو جدایه مخمری(A01 و G01) بومی به ترتیب از سیب‌ها و انگورهای بومی منطقه جداسازی شدند و بر روی محیط PDA کشت داده شدند. جدایه‌های بومی با روش تعیین توالی نواحی D1 و D2 و همچنین ITS1 و ITS2 از DNA ریبوزومی شناسایی شدند.

یافته‌ها: نتایج حاصل از تعیین توالی ژنوم مربوط به دو جدایه بومی نشان داد که هر دو توالی مربوط به *ساکارومیسس* می‌باشند. تمامی سویه‌ها توانایی قابل توجهی در ممانعت از رشد *آسپرژیلوس نایجر* بر روی دانه‌های انگور و نیز محیط کشت از خود نشان دادند. از طرف دیگر میزان تولید الکل مخمرها نیز بسیار ناچیز بود.

نتیجه گیری: کنترل زیستی *آسپرژیلوس نایجر* و گندزدایی اوخراتوکسین A با استفاده از مخمرهای کم تخمیر پیشنهاد دهنده روشی مطابق موازین اسلامی و نوشیدنی‌های حلال می‌باشد.

واژگان کلیدی: *آسپرژیلوس نایجر*، کنترل زیستی، آب انگور، اوخراتوکسین A.

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*) آدرس برای مکاتبه: ملایر، دانشگاه ملی ملایر، دانشکده علوم پایه.

تلفن: ۰۹۱۸۸۵۱۲۶۲۲ پست الکترونیک: a.babaei@sheffield.ac.uk

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Original Article

Biocontrol activity optimization of low-fermenting yeast isolates against *Aspergillus niger* to remove toxin from grape juice

Arash Babaei[1], Mohsen Duzbakhshan[2], Hadis Tavafi[1]

[1]Assistant Professor, Department of Biology, Faculty of Sciences, Malayer University, Malayer, Iran.
[2]Instructor, Department of Biology, Faculty of Sciences, Malayer University, Malayer, Iran.

Abstract

Background & Objectives: Grapes infected with *Aspergillus* can produce ochratoxin A (OTA) in the processing of beverages such as wine or grape juice. This study aimed to evaluate the antifungal biological potential of two low-fermenting native yeast isolates (A01 G01) and three standards (*Saccharomyces cerevisiae*, *Candida guilliermondii*, *Metschnikowia agaves*) yeast isolates against *Aspergillus niger* and their ability to remove OTA in grape juice and its products, without any considerable alcohol production during the process.

Material & Methods: Two native yeast isolates (A01 and G01) were obtained from Malayer apples and grapes, respectively, and inoculated on the PDA culture medium. Native isolates were identified by sequencing D1 and D2 and ITS 1 and ITS2 regions of the ribosomal DNA gene.

Results: The results of DNA sequencing identified both native isolates as Saccharomyces. All strains showed a significant ability in inhibition of *A. niger* growth both on grape berries and in culture media. Meanwhile, yeast isolates produced a trace amount of alcohol.

Conclusion: Biological control of *A. niger* and OTA-decontamination using yeast is proposed as an approach to meet the Islamic dietary laws regarding the absence of alcohol in halal beverages.

Keywords: *Aspergillus niger,* Biocontrol, Grape juice, Ochratoxin A.

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Correspondence to: Arash Babaei
Tel: +989188512622
E-mail: a.babaei@sheffield.ac.uk
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Introduction

Grape is one of the most important economically marketed fruits (1). Pre-and post-harvest infection of grapefruits by fungi results in significant economic loss every year (2, 3). Some species of *Aspergillus*, *Penicillium*, *Fusarium*, and *Rhizopus* are the main fungi that infect many foods. Most species of these fungi produce varieties of mycotoxins (4, 5).

Mycotoxins remain in raw agricultural crops (pre- and post-harvest condition) commonly. All these crops are mostly contaminated in the field, but favorite conditions in storage can lead to an increase in mycotoxin levels.

Ochratoxins are a group of mycotoxins produced by some *Aspergillus* species (mainly *A.ochraceus*, but also by 33% of *A. niger* industrial strains) and some *Penicillium* species, especially *P. verrucosum* and *P. carbonarius* (6).

Black Aspergilli are widespread in vineyards and may not only cause ruin on berries but also are the main sources of ochratoxin A (OTA) production. Among black Aspergilli, *A. carbonarius* and *A. niger* are considered the most dangerous species, having the highest potential for OTA production in grape (7). OTA was usually found in food with the vegetable origin and its presence in raw ingredients may lead to severe contamination in processed beverages such as wine and grape juice (8).

Exposure to ochratoxin through diet can cause acute toxicity in mammalian kidneys. OTA has nephrotoxic, teratogenic, hepatotoxic, and carcinogenic effects in mammals (9, 10). Therefore, as a preventive measure, the European Union set the maximum permitted levels of OTA in wine and grape juice at 2 $\mu g \cdot kg^{-1}$ (11). Thus, the use of appropriate biological and chemical methods is needed to reduce the amount of production and harmful effects of OTA.

Too much use of chemical fungicides to control postharvest diseases cause the creation of drug-resistant fungi, which also causes crucial health concern in human populations (12-14). Studies show that biological control of postharvest fungal diseases is one of the best alternative methods of postharvest diseases (15, 16).

Biological control of plant pathogens has been conducted by various antagonistic microorganisms that are an efficient alternative method than using synthetic fungicides in reducing postharvest diseases and product loss (17). Also, biological control of pathogens by yeasts prevents the growth of pathogens by different mechanisms, such a challenge for nutrient and space, secretion of special lytic enzymes, and specific prohibitive secondary metabolites and many other mechanisms (15, 16).

In addition, yeasts are considered because of their role in winemaking processes, they may also represent an important tool in the biological removal of OTA from natural juice

(9, 16, 18). However, high concentrations of ethanol in must and juice leading to accelerating the fermentative process by yeast, which is a significant problem for their use as biocontrol agents because of reducing their efficacy as antagonists and their ability to remove OTA (19-21).

This stoppage may be overcome, at least in non -alcoholic grape juices, by using low-fermenting yeast isolates.

Islamic laws forbid Muslim populations from consuming alcoholic drinks and foods, even in a small amount (22). Nonetheless, alcohol is common in many biological systems: e.g. fresh fruits and/or their essences and juices can contain traces of alcohol (23, 24).

When alcohol is 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 (25, 26), although these limits may vary according to countries and religious groups. The objective of this study was to optimize and evaluate the biocontrol potential of five selected yeast isolates against *A. niger*, 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.

Materials and methods

Screening and identification of low- alcohol producing yeasts

Two native yeasts (A01 and G01) were isolated on PDA (potato dextrose agar) in 25°C for 5 days from apple and grape respectively, and three standard yeast strains (*Saccharomyces cerevisiae*, *Candida guilliermondii*, *Metschnikowia agaves*) and *Aspergillus niger* was purchased from Iranian biological resource center, Tehran (IBRC).

Two native isolates were identified by sequencing the D1 and D2 and ITS1 and ITS2 regions of the ribosomal DNA gene. In order to typing, yeast cell was inoculated in Sabouraud Dextrose Broth (SDB) overnight in 25°C and centrifuged in 6000 rpm for 10 min and pellet was re-suspended in 200 µl Breaking buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mMTris, pH 8.0, 1 mM EDTA, pH 8.0). After that, ~200 µl volume of glass beads and 200 µl of phenol/chloroform was added and mixed by vortex and ~400 µl of supernatant was transferred to another tube.

This step was performed again and the aqueous layer was transferred to a new tube. 1 ml of 100% ethanol, was added and mixed well and spined at top speed for 3min. The pellet was re-suspended in 0.4 ml TE buffer and 30 µl of 1 mg/ml RNase A. was added and mixed well. Tubes were incubated at 37°C for 15min.10 µl of 4 M NH4OAc and 1 ml of 100% ethanol was added continuous and spin for 3min. At last 50 µl of TE buffer was added (27).

The extracted genomic DNA was amplified by PCR (28, 29).Amplification of ITS gene fragment from yeasts, genomic DNA was carried out by PCR using the following universal forward (5'-TCCGTAGGTGAACC TGCG-3') and reverse (5'-TCCTCCGCTTATTGATATGC-3')primers (30-32) with this volumes: PF: 0.25 µl, RF:0.25 µl, 10X: 2.5 µl, DNA: 0.1 µl, MgCl2: 0.75 µl,

taq:0.25 µl, dNTP: 0.5 µl, DDW: 19.5µl and under the following conditions: one cycle of initial denaturation (95°C, 15 min), followed by 35 cycles of 94°C (1 min), 55.5°C (2 min), 72°C (2 min) and terminated by one cycle of the final extension (72°C, 10 min).

Amplification of D1/D2 region of the large subunit of rRNA performed by following universal forward (5'- GCATATCAATAA-GCGGAGGAAAAG-3') and reverse (5'-GGTCCGTGTTTCAAGACGG-3') primers (30, 33) with this volumes: PF: 0.25 µl, RF:0.25 µl, 10X: 2.5 µl, DNA: 0.2 µl, $MgCl_2$: 1 µl, taq:0.5 µl, dNTP: 0.75 µl, DDW: 17.5µl and under the following conditions: one cycle of initial denaturation (94°C, 3 min), followed by 30 cycles of 94°C (1 min), 60°C (1 min), 72°C (1 min) and terminated by one cycle of final extension (72°C, 3 min). Agarose gel electrophoresis (1.5%) used for the survey of genomic DNA.

Evaluation of the fermenting activity of selected yeasts

Evaluation of alcohol production of native isolates and standard strains of yeasts was performed by the distillation test (ISIRI 2685). Selective yeasts were inoculated in Sabouraud Dextrose Broth (SDB) and incubated overnight at 25°C. Then yeast cells were recovered by centrifuge (12000×g, 20 min), washed and suspended in sterilized saline solution and cells were calculated by hemocytometer.

Fermentation reaction was performed in five sterile flasks containing 50 ml of natural pasteurized grape juice and five sterile flasks containing 50 ml of commercial pasteurized grape juice, 2 replicates per treatment and (final concentration of cell estimated 5×10^6 cells /mL) for 5 and 10 days. A flask containing un-inoculated grape juice was used as a control treatment. pH neutralization of grape juice was carried out by trace base and set ~7 by pH meter. Alcohol was separated by Distillation and driven to acetic acid by nitric acid and additive bichromate was measured with iodometry.

10 gr of the sample was transferred to a distillation balloon and 60ml of distilled water was added. Then 10-20mg phenolphthalein (Merck, Germany) and then sodium hydroxide 0.1 N was added until appears purple color. The boiling bead was added in a balloon attached to the distilled instrument on the electro mantle. 50ml of distilled water was poured in a balloon and was attached to the distilled instrument. The balloon was heated till boiling point and distillation start. When balloon volume accedes to 100ml, distillation was halted and to increase volume with distilled water. Then was poured 10 ml of distilled water as blank and 10ml of distilled liquid as a sample and was added 10ml Nitro-chromic acid without NO gas. All the flasks were corked with valves and stored in the dark for 30 minutes at 18-20°C. In the following, 50 ml of distilled water and 1gr potassium iodide was added to each Erlenmeyer and tittered after 1min with sodium thiosulfate 0.1 N (Iranian fruit juice standard experiment: ISIRI2685) (34, 35).

pH and temperature conditions optimization of biocontrol activity of yeasts in OTA contaminated grape juice

In order to evaluation of biocontrol activity, the

first yeasts were as inoculated in Sabouraud Dextrose Broth (SDB) and incubated overnight at 25°C and counted. A part of the overnight grown cells was separated and filtered through 0.4 µm filters (Biofil, China) while another part was both filtered and autoclaved. Petri dishes (diameter of 110mm) contained 2% agar discussion (Quelab, USA) were inoculated with selected yeasts follows: (I) with 1 ml of each yeast suspension (106 CFU/ml), (II) with 300 µL of filtered cultivated broth medium, (III) with 300 µL of both filtered and sterilized cultivated broth medium.

The conidial suspension (105 conidia/ml) of *A. niger* grown on potato dextrose agar (PDA) media (Titan Biotec) at 28 °C for 7 days, was prepared in distilled and sterile water containing 0.1% Tween 20 (Merck, Germany) to impede conidia clumping. Three 10 µL aliquots of the suspension were marked apart in each plate. This trail was carried out in triplicate and all plates were sealed, stored at 25 °C, and the mean diameter of grown fungal colonies was assessed after 4 days of growth and compared with control colonies in the lack of living yeast, filtered, or filtered and autoclaved culture broth (11).

Biocontrol study on detached grape berries

For every yeast *Invivo* assay, four grapes (Vitisvinifera L. fakhri. Malayer, Iran) bunches which containing five mature berries were selected, were prepared, disinfected with 1% sodium hypochlorite for 15 min and washed twice with sterile distilled water. All berries were injured with a sterile needle (1 wound for each berry, 2 mm diameter) and entire bunches were immersed into100 mL suspension of each yeast (10^7 CFU/ml. Then Bunches were dried in air (25°C and 70% humidity) and afterward sprayed with an *A. niger* conidia suspension (10^7 conidia/ml) by using a handy sprayer until runoff (1 ml for each bunch).

Following inoculation, grape bunches were put in plastic boxes (21×21×10 cm) for a week and incubated at 28 °C in the dark under high relative humidity (90 ± 5%). After a week berries were examined and a 0-100 infection index was appointed to them as the percentage (0, 25, 50, 75 and 100%) of the berry surface covered by *A. niger* (11, 36).

OTA Reduction of grape juice

To evaluate the potential of the native yeast isolates and standard yeast strains to eliminate OTA from grape juice, *A. niger* was inoculated in yeast extract sucrose (YES) broth culture media which adjusted pH on 5, 5.5, 5.8 and 6 at 25° to 28°C under constant shaking (120 rpm) for 7 days. Myceliums were separated with passing cultured media from Gauze and No:1 Whatman filter papers and their dry weight was measured. 200ml of natural grape juice was poured into flasks and 10ml of inoculated and filtered YES and 1ml of each yeast suspension (10^7 CFU/ml) was added. In order to the examination of OTA redaction in absence of yeast and capacity of yeast isolates to OTA production, negative control treatments with 200ml grape juice and 10ml of inoculated and filtered YES and a positive control treatment with 200ml grape juice and 1ml of all yeast isolates was set. The experiment was carried out in triplicate (11).

OTA purification and HPLC analysis

OTA was extracted from grape juice by adding 50ml chloroform and shaking (200 rpm) for 20 min. following centrifugation (12000×g, 20 min), the supernatant was discarded and condensed below the nitrogen atmosphere, then re-suspended in the HPLC mobile phase t o endure HPLC analysis. The extraction of OTA was executed in triplicate and the experiments were reiterated at least twice.

OTA determination was performed on an HPLC system (Waters Alliance 2695 and 2475 fluorescence (flr) detector) according to Iran national standard: KHA- S0016872 (Marjaan khatam laboratory- Tehran).

Statistical analyses

Statistical analyses were carried out on the results obtained from optimum conditions for biocontrol activity. Statistical significance was determined by one-way analysis of variance (ANOVA One-way) and pairwise comparisons using Tukey's method. Significance was determined at α value of $p<0.05$. Statistical analysis was performed using the software Minitab V16.2.

Results

Molecular identification of yeasts

Two native isolates were identified by sequencing the D1 and D2 and ITS1 and ITS4regions of ribosomal DNA as belonging to *Saccharomyces* (Accession numbers: MH307755, MH304332, MH304420, and MH304418). The PCR results were shown in figures 1 and 2.

Evaluation of the fermenting activity

In this experiment all isolated yeasts have trace alcohol production, thus low fermenting activity either in natural and commercial grape juice after 10 days. The alcohol production value of isolates was shown in tables1 and 2.

According to the results, 30004 has more

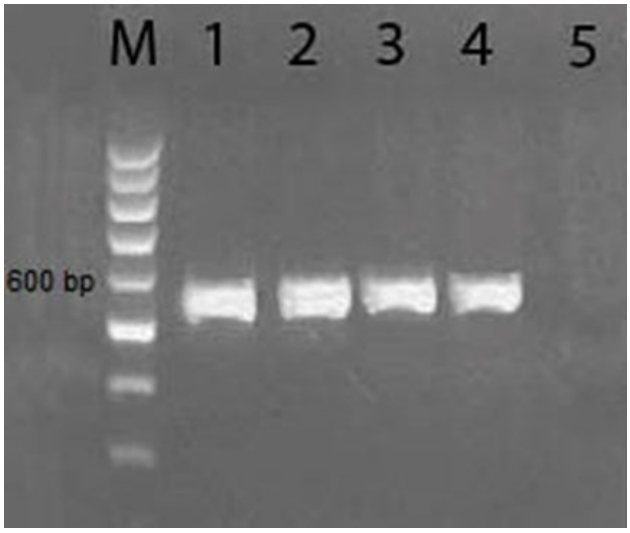

Figure 1. Large subunit ribosomal RNA gene PCR. M: 1kb marker, 1: 304332 (28S rRNA), 2: 304332 (28S rRNA), 3: 307755(28S rRNA), 4: 307755 (28S rRNA), 5: negative control. All of 1, 2, 3, 4 lanes: 590bp sequence .

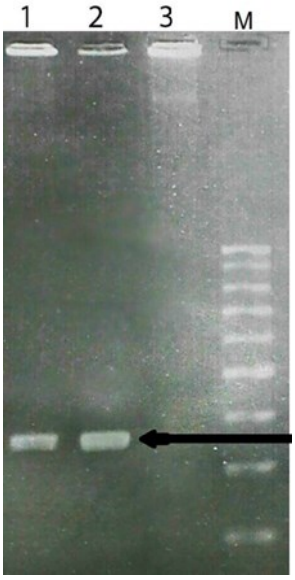

Figure 2. Internal transcribed spacer, 5.8S ribosomal RNA gene PCR. M: 1kb marker, 1: 304420(5.8S rRNA), 2: 304418(5.8S rRNA), 3: negative control number 1, 2, lanes: 255bp sequence.

Table 1. Yeasts alcohol production after 5 days in natural and commercial juice.

Yeast	Natural grape juice (g/100ml)	Commercial grape juice (g/100ml)
A01	1.31	1.50
G01	0.93	1.06
30004	1.37	1.82
30071	0.28	0.30
30107	0.37	0.41

Table 2. Yeasts alcohol production after 10 days in natural and commercial juice.

Yeast	Natural grape juice (g/100ml)	commercial grape juice (g/100ml)
A01	0.60	0.58
G01	0.28	0.36
30004	1.20	1.31
30071	0.29	0.33
30107	0.21	0.22

alcohol production than others and fermenting activity of A01 and G01 in alcohol production by 0.05 P-value is more efficient than versus other yeasts.

In vitro biological control experiments

On YPD topped with filtered or filtered and autoclaved culture broth, *A. niger* was not prevented and *A. niger* colony growth was significantly higher compared to the control plates (Table 4).

The effect of living yeast cells on grape decay caused by A. niger

All yeasts, namely A01, G01, *Saccharomyces cerevisiae*, *Candida guilliermondii,* and *Metschnikowia agaves*, reduced significantly (p-value <0.05) the incidence of contamination by *A. carbonarius* on detached grape berries (Table 5). Different English letters represent the statistical difference between each factor and control (p< 0.05).

OTA adsorption by antagonistic yeast in grape juice

When yeast cells were incubated in containing *Aspergillus niger* grape juice for 8 days, *Saccharomyces cerevisiae, Candida guilliermondii, Metschnikowia agaves*, A01 and G01 were able to significantly reduce OTA content in containing *Aspergillus niger* grape juice by 68, 62, 78, 44 and 60%, respectively, (Table 6).

Discussion

Biocontrol reduction of *Aspergillus* pollutions in grapes and its products is an important trend in agricultural studies. Our goal was to improve a new biocontrol approach to prevent OTA contamination that could encounter Islamic laws regarding the presence of alcohol in halal beverages. In the current study, we

Table 3. Analysis of variance (mean squares) by Dankan test.

		Mean squares			
S.O.V	df	commercial grape juice (g/100ml)-5 days	Natural grape juice (g/100ml)- 5 days	commercial grape juice (g/100ml)- 10 days	Natural grape juice (g/100ml)- 10 days
A01	1	172.421**	195.368**	245.689*	356.387*
G01	1	325.241*	144.8101*	365.275*	321.025*
30004	1	120.1423**	112.3521**	102.5413*	113.5204*
30071	1	442.1542*	451.3682*	471.02**	444.6206**
30107	1	470.2102*	389.289*	358.4891*	402.3256*
Error		1.19	1.65	1.42	1.35

**: significant at 5% and 1% probability levels, respectively.

Table 4. *Aspergillus niger* colony diameter versus yeasts (mm).

Yeast	Living yeast	*P*	Filtrate	*P*	Autoclaved filtrate	*P*
A01	0	0.0002	40 ± 0.6	0.04	45 ± 0.6	0.03
G01	3 ± 0.3	0.004	30± 0.5	0.03	31 ±0.8	0.02
30004	0	0.0001	34± 0.5	0.03	35± 0.5	0.04
30071	4 ± 0.2	0.003	22± 0.8	0.02	15± 0.8	0.01
30107	0	0.0001	42± 0.6	0.03	48± 0.6	0.04

Table 5. *Aspergillus niger* infectious grape berry surface (%).

Yeast	1st grape cluster		2nd grape cluster		3rd grape cluster		4th grape cluster	
A01	0	c	0	c	0	c	25	b
G01	0	c	0	c	0	c	0	c
30004	0	c	0	c	0	c	0	c
30071	0	c	20	b	0	c	0	c
30107	0	c	0	c	30	b	0	c
control	85	a	90	a	85	a	85	a

a: meaningful difference b: average c: weak difference

have selected five antagonistic low-fermenting yeast isolates, that are capable to control the fungus and OTA-producer *A. niger* in grape. Low-fermenting yeast isolates showed significant antagonistic activity against *A. niger* both in in-vitro experiments and on grape berry's surface. Since their filtrate and autoclaved filtrate culture broth were not capable to prevent firmly the *A. niger* growth, it is likely to suppose that the chief mechanism of the biocontrol ability of these isolates consists of the competition for nutrients and space rather than with the release of the distributable antifungal substance.

The collection of CO_2 and declined levels of oxygen may also play a role in declining sporulation or fungal growth (37). Since these parameters were not appraised in our research, it is not might be to control the role of challenge for oxygen in the yeast-*A. carbonarius* interaction.

Among the tested yeast, *Saccharomyces cerevisiae*, *Candida guilliermondii*, and *Metschnikowia agaves* presented a significant capacity to adsorption of the OTA on grape juice amended with this mycotoxin.

From the past, a lot of physical or chemical efforts were performed on grape juice to decrease its toxicity. In 2000 Crous and their coworkers showed the effect of hot-water treatment on fungi occurring in apparently healthy grapevine cuttings (38).

In 2010 Raspor teams studied biocontrol of grey mold on the grape with Autochthonous Wine Yeasts as *Aureobasidium pullulans*, *Metschnikowia pulcherrima*, *Pichiaguillier-mondiiand Saccharomyces cerevisiae* and indicated its inhibition (39).

Migheli in 2012indicated that Saccharomyces cerevisiae wine strain Inhibits of fungus growth and decreases Ochratoxin A biosynthesis by *Aspergillus carbonarius* and *Aspergillus ochraceus* (19). In 2012 Gholampour-Azizi studied on the evaluation of the presence of Ochratoxin A in grape juices and raisins and proposed hygienic and predictive in the production process. But they didn't mention reduction methods of OTA (40).

In 2014 Migheli showed the potential of some yeast strains against *Aspergillus carbonarius* and their ability to remove ochratoxin A from grape juice (11). In that year Türkel studied the same study on the biocontrol activity of the

Table 6. OTA reduction on grape juice by living yeast cells.

Treatment	OTA content (ng/mL ± SE)			
	Grape juice		Yeast cells	
Control	20.6±1.1	d	0	a
Saccharomyces cerevisiae	7.0±0.7	b	8.9±0.2	d
Candida guilliermondii	8.2±1.0	b	6.7±0.9	c
Metschnikowia agaves	5.1±1.0	a	9.5±0.8	d
A01	11.8±0.8	c	4.2±0.6	b
G01	8.6±0.4	b	5.3±1.2	c

a: meaningful difference b: average c: weak difference d: very weak difference

local strain of *Metschnikowia pulcherrima* on different postharvest fungus of grapes like *Penicillium roqueforti*, *P. italicum*, *P. expansum* and *Aspergillus oryzae* (4).

The Islamic laws indicate that foods are regarded as "lawful" (i.e., permissible) for Muslims. These laws are found in the Sunna and the Quran (22). Alcohol is one of the main concerns, as alcoholic foods are forbidden in Islam (25).

The Islamic Food and Nutrition Council of America (IFANCA) has defined a standard of 0.5% alcohol content in the ingredients (41).

Therefore, any effort to decrease mycotoxin contamination in food by yeasts should promise that no small amounts of ethanol are produced during the process.

Grape contamination by *Aspergillus* spp. Happens as soon as the previous throughout harvesting, transit, and storage of grape bunches (42). During the winemaking process, OTA is not completely released (8), while OTA tends to reduce throughout the yeast and malolactic fermentations, apparently due to adsorption on the yeast surface, or to breakdown by lactic bacteria (43). On the opposed, in grape juice production OTA contamination may arrive at meaningful levels since the lactic bacteria and activity of fermenting yeast are absent.

The biocontrol approach represents a more effective avoiding strategy to decrease OTA contamination, since selected antagonists may be applied both before harvesting to control grape contamination by OTA-producing fungi and also during treating to act as OTA detoxifiers. Field experiments are being performed to evaluate the competitive capability of low fermenting yeasts in the direction of resident microflora, their ability, and resistance to multiple environmental stresses (44).

Conclusion

Our results show that selected low-fermenting yeast isolates may be efficiently developed as a biological control factor in postharvest disease management of grape as well as processing aids in the production of grape juice, to reduce mycotoxin contamination if OTA levels pass the limits fixed by food regulation.

Processing aids are commonly used in the food industry as an assistant in food processing and do not need to be reported on the label by law in many countries. 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. The halal food industry is among the biggest and fastest extending niches in the food market, representing one-fifth of global food trade. Research and development of microorganisms to be adopted as biocontrol agents in fruit post-harvest or as a biological adsorbent to remove mycotoxins from fruit juices should fulfill the request imposed by the halal food market to guarantee that products meet religious standards. In this perspective, it is recommended to use low-fermenting yeast that releases only.

Ethical Consideration

Authors of all ethics including non-plagiarism, Dual publishing has complied with data distortions and data making in this article.

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