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Ochratoxin A removal in synthetic media by living and heat-inactivated cells of *Oenococcus oeni* isolated from wines

Eva M. Mateo[a], Ángel Medina[a], Francisco M. Valle-Algarra[b], Fernando Mateo[c], Isabel Pardo[a], Misericordia Jiménez[a*]

[a]Dpt. de Microbiologia y Ecologia. Universitat de València. Dr. Moliner 50, E-46100, Burjassot, Valencia, Spain.
[b]Dpt. de Química Analítica. Universitat de València. Dr. Moliner 50, E-46100, Burjassot, Valencia, Spain.
[c]Dpt. de Ingeniería Electrónica, Universitat Politécnica de València, Camino de Vera, 14, 46022, Valencia, Spain.

*Correspondence author
M. Jiménez,
Dpt. de Microbiologia i Ecologia. Universitat de València. Dr. Moliner 50, E-46100,
Burjassot, Valencia, Spain.
Phone: +34 963543144
FAX: +34 963543202
Email: misericordia.jimenez@uv.es

Abstract

This study evaluates the capacity of living and heat-inactivated cells of *Oenococcus oeni*, the main species responsible for malolactic fermentation in wine, to eliminate ochratoxin A (OTA) from synthetic media in different conditions. The dynamic of toxin removal was studied. Tests were done considering three factors: *O. oeni* strain, OTA level in the medium and incubation period. To verify whether bacteria viability and composition of the culture medium had some influence on toxin reduction levels, living and heat inactivated bacteria were used and tests were carried out in two culture media. The results indicate that all the tested *O. oeni* strains can eliminate OTA from the medium but with significant differences (*p*-values <0.01) in OTA removal dependent on the factor considered. OTA reduction in the culture medium ranged between 0.88 and 63.14% and between 1.75 and 57.53% in the media spiked with 2 and 5 µg OTA l^{-1}, respectively. Toxin removal was not related to bacterial viability and was independent on the culture medium composition. However, OTA removal was dependent on *the O. oeni* strain, OTA level in the medium and incubation period. This is the first study carried out to detect and study OTA removal dynamic by living and heat-inactivated cells of *O. oeni* isolated from wine. Selected *O. oeni* strains can be a very useful tool to control OTA levels and to decontaminate food and beverages and, very especially, the wine.

Keywords: ochratoxin A – mycotoxin reduction – lactic bacteria – wine – food safety

Ochratoxin A (OTA) is a naturally occurring secondary metabolite produced by some species of the genera *Aspergillus* and *Penicillium*. OTA affects agricultural products all over the world and causes harmful effects on human and animal health because of its highly toxic properties. It has been reported as having mutagenic, teratogenic and carcinogenic activities (*31*).

OTA has been detected in food and beverages (*33, 43, 44, 58, 59*) including grape juice and wine, where it was first reported by Zimmerli and Dick (1995). Since then, surveys conducted in different countries have revealed the presence of OTA in wine. The obtained data showed that both wine type and geographic region strongly influence contamination of wines with OTA. It has been observed that OTA concentrations usually follow the order white < rose < red < dessert wines. Several surveys carried out in different countries have reported OTA levels in grape products and wine ranging from 0.01 to 3.5 µg l^{-1}. These levels were higher in products from southern regions of Europe than in northern regions (*5, 10, 39-41, 46, 47, 49, 51*).

According to the Codex Alimentarius, 15% of the total OTA intake is due to wine, which is considered as the second major source of OTA intake after cereals (*16*). Grape juices can also show OTA contamination. It seems that they contain more OTA than some wines and thus contribute to OTA intake by children (*65*).

The Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives has discussed the imposition of a maximum tolerable weekly intake of 100 ng of toxin kg^{-1} of body weight (*31*) and a maximum level of 5 to 20 µg OTA kg^{-1} in processed or non-processed cereals (*32*). The Organization International de la Vigne et du Vin fixed 2 µg l^{-1} as a maximum level of OTA in wine (*45*). The same limit was regulated in the European Union, and a recommendation to keep OTA concentration to the lowest technologically feasible level has been made (*17*).

In order to protect consumer health from the risk of exposure to this mycotoxin, reliable methods to reduce OTA level are highly desired.

Decontamination methodologies involving physical and chemical treatments have been proposed at laboratory scale. Although some of these methodologies such as the use of ozone (*42*), alkaline hydrogen peroxide (*25*) or gamma irradiation (*53*) have promising results, others such as hypochlorite treatment (*13*), ammoniation (*15*), or heat treatment (*9*) are not recommended for practical decontamination.

New opportunities involving removal of mycotoxins by microorganisms have been created. Some authors have studied this subject and the ability to eliminate OTA has been observed for some bacteria such as *Acinetobacter calcoaceticus* (*30*), *Phenylobacterium immobile* (*61*), and some *Lactobacillus* (*26, 50*). *Saccharomyces cerevisiae* (*6*) and some *Aspergillus* species such as *Aspergillus niger*, *A. carbonarius* and *A. japonicus* (*7, 60*) are also capable of performing OTA degradation.

Oenococcus oeni (formerly *Leuconostoc oenos*) (*35, 62*) can perform malolactic fermentation (MLF), converting L-malate to L-lactate and carbon dioxide in wines. As a consequence of the MLF, the wine quality improves due to acidity decrease, gaining of flavours and microbiological stability (*37*). This lactic acid bacterium is the most tolerant species to the unfavourable wine conditions (nutrient starvation, low pH, ethanol, fatty acids from yeasts, SO_2, etc.) and the main responsible of driving the MLF.

Knowledge about the capacity of *O. oeni* to remove OTA from the medium is very scarce. One only study (*18*) has been carried out to determine the ability of *O. oeni* in the exponential growth phase and of cell-free extracts (obtained by disrupting cells in a French pressure cell) to eliminate OTA in a medium spiked with 5 μg of toxin l^{-1}. The portion of OTA removed during bacterial growth ranged from 10.99 to 28.09% and OTA was no degraded by cell-free extracts.

Until now, the ability to remove OTA by *O. oeni* has been studied in a small number of strains. It is not known the influence of the strains, physiological states of the bacteria, incubation time, concentration of OTA in the medium or the medium itself on the dynamics of toxin elimination.

At the beginning of the malolactic fermentation levels usually not higher than 2 μg OTA l^{-1} are present in wine. During wine fermentation *O. oeni* resides in this medium for a long time during which different physiological states of the bacteria follow one to another. This period varies depending on the type of wine. Although different malolactic starters are employed, selected strains that are native of each wine are desirable to be used during wine maturation. A fastidious adaptation process of *O. oeni* represents the primary obstacle for an extensive use of malolactic starters in vinification.

The aim of this work was to assess the potential capability of wine strains of *O. oeni* to remove OTA from culture media under different conditions, paying especial attention to the influence of the strain, incubation period and concentration of the toxin in the medium. To perform this aim, ten selected *O. oeni* strains isolated from Spanish

wines of well-known origin (Utiel-Requena and La Rioja) were used. The dynamics of OTA elimination in culture media produced by living and heat-inactivated cells of *O. oeni* under different conditions was studied.

Materials and methods

Bacterial origin and growth conditions

Ten selected strains of *O. oeni* isolated from Spanish wines were used. Six strains (N171, N172, 6G, 124M, 228aM, 255M) from "Utiel-Requena" wines and three strains (S1, EB927-2, Mu1) from "La Rioja" wines were supplied by ENOLAB research group (Department of Microbiology and Ecology, University of Valencia). Another strain (4100 CECT) from "La Rioja" wine was supplied by the Spanish Collection of Type cultures (CECT, University of Valencia).

Before each experiment the bacterial strains were cultured in Petri dishes containing MLO (Medium for *Leuconostoc oenos*) (*12*) at 28ºC for 7 days. The pH was adjusted to 4.8 using a 10M sodium hydroxide water solution.

OTA standard

A standard of OTA was purchased from Sigma (Sigma-Aldrich, Alcobendas, Spain). It was solved and diluted in acetonitrile-water-acetic acid (49.5:49.5:1 v/v/v) under sterile conditions to prepare a stock solution containing 100 µg l^{-1}. The proper amount of this solution was added to the different culture media to reach the desired concentrations.

Evolution of OTA level in O. oeni cultures

O. oeni strains were grown in liquid MLO at 28ºC without stirring until mid-log phase. At this time (time 0) the number of colony forming units (cfu) per millilitre of culture medium was $8x10^8$ (absorbance = 0.50, measured at 600 nm).

OTA stock solution was used to prepare MLO supplemented with 5 and 2 µg OTA l^{-1}. Nine millilitres of culture medium supplemented with OTA was poured in 10-

ml transparent plastic screw cap sterile tubes. Tubes were inoculated with 100 µl of each *O. oeni* culture ($8x10^7$ cfu). A control consisting of the same inoculum added to MLO without OTA addition was used and controls consisting in non-inoculated MLO spiked with OTA at the two levels were also run in parallel. Tubes were incubated at 28ºC and aliquots were removed at time 0 and 5, 10 and 14 days later to quantify OTA by LC. Assays were carried out in triplicate.

To evaluate if changes in OTA concentration of *O. oeni* cultures were caused by metabolic degradation of the toxin or by other kind of interactions, a supplementary assay involving two strains, 124M and 6G, was performed. The assay was made using cells in stationary growth phase in MLO. Ten 9-ml aliquots were taken from this culture and poured in 10 sterile tubes with screw caps. Five tubes were stored at 4ºC and the remaining 5 tubes were heated at 60ºC for 35 minutes to inactivate the bacteria. Once the heat treatment finished these tubes were also kept at 4ºC for 30 min.

Two tubes, with living bacteria were supplemented with 5 and 2 µg OTA l^{-1}, respectively, using the suitable volume of OTA standard solution, and 2 tubes with heat-treated bacteria were also supplemented with 5 and 2 µg OTA l^{-1}, respectively.

When acetate buffer medium was used, two MLO tubes with living bacteria were centrifuged at 6000 rpm and the supernatant was discarded to eliminate the nutrients. Bacterial pellet was washed with 9 ml of 0.5 mol l^{-1} acetate buffer (pH 4.8) and re-suspended again in 9 ml acetate buffer. A tube was fortified with 5 µg OTA l^{-1} and the other with 2 µg OTA l^{-1}. The same protocol was followed using the two tubes containing heat-inactivated bacteria.

The remaining two tubes, one containing living bacteria and the other containing heat inactivated bacteria, both without OTA, were used as controls.

Two additional controls consisting of acetate buffer without bacteria but supplemented with 5 and 2 µg OTA l^{-1} were also used.

All tubes were incubated at 28ºC and OTA was determined in the cultures at 0.5, 1.5, 3, 5 and 26 hours. All assays were carried out in triplicate.

Ochratoxin A determination

Tubes with bacterial cultures were shaken briefly and 0.6 ml sample of each tube was collected in sterile conditions and poured in Eppendorf centrifuge tubes, which

were centrifuged at 4000 rpm for 6 min. Then, 0.5 ml of supernatant was collected and used for OTA determination.

The suitable amount of 0.1 mol l^{-1} phosphoric acid solution was added to each sample so that the pH was in the range 2.5-3. Then, 2 ml of ethyl acetate was added. After shaking, the organic phase was loaded in an amber vial for LC analysis. This procedure was repeated once more time. After solvent evaporation under N_2 stream at 50 ºC, the residue was dissolved in 0.250 ml mobile phase. One hundred µl was injected into the LC system.

The LC system used consisted of a Waters 600E system controller, a Millipore Waters 717 Plus autosampler and a Waters 470 scanning fluorescence detector (Waters, Milford, Ma, USA). Excitation and emission wavelengths were 330 and 460 nm, respectively. The samples were separated using a C_{18} Phenomenex Gemini ® (150 x 4.6 mm, 5µm) (Phenomenex, Macclesfield, UK), with a guard column of the same material. Run time for samples was 20 min with OTA being detected at about 12 min. The flow rate of the mobile phase (acetonitrile-water-acetic acid; 40:58:2, v/v/v) was 1 ml min^{-1}.

Calibration lines were constructed using culture medium spiked with the proper amount of OTA standard in the range 0.2-5 µg OTA l^{-1} of medium. The recovery rate was 85% from MLO medium with a limit of detection of <0.05 µg OTA/1 medium, based on a signal-to-noise ratio of 3:1. These determinations were made in triplicate.

Analysis of the results was carried out on a computer with Millennium® 4.0 software (Waters).

Statistical analysis

The statistical analysis of the data was performed using Statgraphics Centurion XV version 15.1.02 (StatPoint, Va, USA). For analysis of variance (ANOVA) significant differences in mean values were reported at *p*-value < 0.01. Tukey-honestly significant difference (Tukey-HSD) multiple range test at 99% confidence level was used to group the cases into homogeneous groups with regard to the different parameters used.

Results

Ten *O. oeni* isolates were tested for their ability to remove OTA from MLO medium supplemented with different OTA levels. The culture medium was inoculated with *O. oeni* cells in exponential growth phase. Cultures of the 10 strains were incubated for the period required (between 3 and 5 days depending on the strain) to achieve 0.50 unit of absorbance measured at 600 nm (≈$8x10^8$ cell ml^{-1}). This period was considered to be time 0 (Figure 1). OTA levels in the culture medium were measured at the end of the time 0 and at 5, 10 and 14 days later.

Figure 1 shows the percentage of toxin reduction in the culture medium for the different *O. oeni* strains throughout the incubation period. The figure shows that toxin level in the culture medium was reduced in all the cultures.

In order to discover which factors significantly influence OTA reduction in the culture medium, all data were studied applying multifactor ANOVA where the factors were, OTA level added to the culture medium (2 and 5 µg l^{-1}), *O. oeni* strain (10 *O. oeni* strains were assayed) and incubation time (time 0 and 5, 10 and 14 days after). The factors were also examined for any interaction between them. The statistical treatment of the data showed that the three factors in this study significantly affected OTA removal percentage (*p*-values <0.01). There is also one significant first-order interaction (strain x time).

Using Tukey-HSD multiple range test at 99% confidence level, the cases were grouped into homogeneous groups with regard to the different parameters used.

The factor OTA level split the cases into the two possible groups with no overlapping. This means that the amount of OTA present in the medium has a significant effect ($P < 0.01$) on OTA removal percentage. The reduction was significantly higher in the culture media spiked with 2 µg OTA l^{-1} (36.55%) in comparison to those spiked with 5 µg l^{-1} (30.08%).

The factor bacterial strain led to two homogeneous overlapping groups. The highest OTA removal percentages were found in MLO culture media inoculated with strains 255M, S1, Mu1, 4100T, N171, Eb927/2, 228AM, 124M and 6G, which were included in the same group. Average OTA reduction in culture media of these strains was 29.47%, 30.29%, 30.89%, 33.03%, 34.55%, 34.64%, 35.52%, 39.30%, and 40.73 %, respectively.

Incubation time split the cases into four homogenous groups with no overlapping. The groups corresponded to time 0, and the periods 0-5 days, 5-10 days and 10-14 days. The respective ranges of OTA reduction rate in cultures spiked with 2 µg OTA l^{-1} were 0.88% - 33.85%, 16.83% - 47.95%, 32.35% - 56.33% and 39.71% - 63.14%, while in cultures initially containing 5 µg OTA l^{-1} these ranges were 1.75% - 19.64%, 9.97% - 42.55%, 27.61% - 58.27%, and 34.2% - 56.87%. Variability was dependent on the strain. Shifts in OTA levels were observed in the culture medium of some strains over the studied incubation period (Figure 1).

The next step was to assess if OTA reduction in the medium is influenced by the bacterial viability, and if the matrix has some influence on this reduction. An experiment was designed to keep the number of cells in the culture constant and two media were used, MLO and acetate buffer. To maintain the number of bacteria constant during the test, stationary phase cultures of isolates 6G and 124M were used for their ability to remove OTA from these media. These two strains were chosen because in previous experiment they showed greater toxin removal capability. Cultures with living bacteria in the stationary phase (LB) and cultures with heat treated dead bacteria (HTB) were used. The number of bacteria in LB and HTB cultures was the same and remained constant throughout the test period (26 hours), which was checked by measuring absorbance in the cultures and additionally to absorbance measure, CFU were tested in the non-heated samples.

Figures 2 shows the percentage of OTA reduction in MLO medium spiked with different toxin levels over 26 hours after OTA addition. Figure 3 shows this percentage when using acetate buffer. Both figures show the results obtained with living bacteria (LB) and inactivated bacteria (HTB).

In the culture of strain 6G in MLO spiked with 2 µg OTA l^{-1}, the toxin amount decreased dramatically during the first 30 minutes, with a reduction in this period of 44.6% and 51.1% in LB and HTB cultures, respectively (Figure 2). At 3 hours of incubation 68% of the initial OTA content had been eliminated in both cultures and from 3 to 26 hours a slight release of toxin to the medium was observed. When 5 µg toxin l^{-1} was added to the culture medium, OTA reductions during the first 30 minutes in LB and HTB cultures were 30.23% and 33.75%, respectively. After that time, OTA level stayed more or less stable with few reductions or releases over the incubation

period. Finally, the toxin reduction percentages in LB and HTB cultures were 37.56% and 33.41%, respectively.

In MLO with the strain 124M a similar OTA reduction dynamics was observed (Figure 2). In the first 3 hour, reductions about 58% in LB and HTB cultures spiked with 2 µg toxin l^{-1} were observed. Toxin reduction rates in LB and HTB cultures of this strain at the end of the incubation period were 67.52% and 66.98%, respectively. In the cultures spiked with 5 µg OTA l^{-1} there was also a reduction of toxin with slight variations. At the end of the assay (26 hours) OTA level in LB cultures decreased by 40.70% and in HTB cultures by 34.39%.

The results obtained in this experiment were treated by multifactor ANOVA. Factors analysed in order to elucidate their effect on OTA reduction were bacterial viability (living or dead bacteria), incubation time and initial OTA level in the culture medium. No significant differences were found in OTA reduction levels between LB and HTB cultures. These results were obtained in the cultures of the two strains and for the two OTA concentrations tested. This therefore suggests that OTA reduction by *O. oeni* is not dependent on bacterial viability.

However, time and initial OTA level in the culture medium do significantly affect OTA removal in the medium. In the first stage of the incubation period (0-3 hours) toxin reduction was high. In the second stage (between 3-26 hours) there were slight variations in toxin levels in the medium. Toxin reduction was greater in tests with 2 µg OTA l^{-1} than in tests with 5 µg l^{-1}.

In acetate buffer (Figure 3), the two *O. oeni* strains reduced OTA levels following the same pattern shown in MLO medium. The amount of OTA decreased continuously in the culture of strain 6G spiked with 2 µg toxin l^{-1} and no release of toxin was observed over the cultivation period. The final reduction rates were 65.45% in LB cultures and 57.47% in HTB cultures. When 5 µg OTA l^{-1} was added, toxin level in the medium decreased continuously but in HTB cultures the toxin level in the medium increased after 3 hours of incubation and at the end of the incubation period the OTA reduction percentage was 47.37% and 29.67% in LB and HTB cultures, respectively.

Strain 124M showed similar behaviour to 6G. OTA reduction was continuous up to 3 hours of incubation and changes in toxin levels in the medium were observed between 3 and 26 hours (Figure 3).

The data obtained in acetate buffer were also treated by multifactor ANOVA. Factors analysed were viability of bacteria (living or dead), incubation time and initial OTA level. As with MLO medium, there were no significant differences in OTA removal between LB and HTB cultures. However, as with MLO medium, time and initial OTA level in acetate buffer did have a significant effect on OTA removal in the medium.

Discussion

Different authors have studied and reviewed the elimination of mycotoxins by microorganisms (*1, 4, 6-8, 11, 14, 34, 54*). The interest in OTA degradation in different matrices has increased over recent years, especially since it has been reported that the toxin is frequently present in food and beverages common in the diet such as cereals, coffee, wine and beer. Recent studies stress OTA reduction during the winemaking process (*2, 27, 28, 51, 55*). Some studies report the effect of some bacteria (*18, 26*), fungi (*1, 7*) and yeasts (*6, 11, 14*) on OTA reduction, but until now there has been no study on the dynamics of OTA removal by living and dead *O. oeni*, the main species involved in malolactic fermentation in wine.

The results of this study show that the 10 tested strains of *O. oeni* from Spanish wines are capable of eliminating OTA from liquid media. These results agree with previous results published by other authors describing the ability of different bacteria to remove OTA from different matrices (*30, 50, 56, 61*), although in those studies higher concentrations of the toxin, which are uncommon in naturally contaminated matrices, were used. Two levels of mycotoxin (2 and 5 $\mu g\ l^{-1}$) are used in this study. The first one was chosen by its oenological interest (it is the maximum permitted OTA level by the European regulation) (*17*) and the second one was chose with comparative aims, to study whether toxin content affects OTA reduction rate by the selected strains of *O. oeni* from Spanish wines used in this study. Both concentrations were tested using two matrices and our study extended longer time than previous. That is important because *O. oeni* is the main species responsible for malolactic fermentation, which lasts between 7 and 30 days. The reductions detected in this study at the end of the incubation period varied between 40% and 63% depending on the strain, which represents a very important removal of the toxin. Moreover, it is remarkable that significant differences depending on the toxin concentration in the medium were found. OTA reduction was

significantly higher in the media spiked with 2 µg OTA l^{-1} than in the media spiked with 5 µg l^{-1}. This result is interesting in wine technology because wine usually show low OTA levels (0.01 to 3.5 µg l^{-1}) (*5, 10, 39, 40, 41, 46, 47, 49, 51*).

Del Prete et al. (2007) have reported much shorter reductions of OTA content by *O. oeni* (10.99-28.09%). On the basis of the results here reported, disagreement can be due, among other factors, to the fact that those authors studied only the most unfavourable OTA level (5 µg l^{-1}), a very short incubation period (only one control for OTA was performed in the late exponential growth phase) and other strains.

The dynamics of OTA reduction studied in the present paper for the first time show that reduction goes on increasing after cultures reach the exponential growth phase and as the incubation period proceeds. OTA reduction levels are dependent on the strain of *O. oeni*, time spent by bacteria in the medium and level of OTA in that medium. These are very relevant findings because in oenology the use of selected *O. oeni* strains for MLF is common practice and hence careful selection of the strain is undoubtedly fundamental for determining final OTA levels in the wine. Grazioli et al. (2006) described OTA reduction during MLF in red wine winemaking using naturally contaminated grapes. In their studies, OTA concentration was reduced during MLF in a range of 38% - 82.2% in two different type of wines. Our results explain that *O. oeni* is directly involved in this high OTA reduction rate and support the decontaminating effect of these lactic acid bacteria during winemaking.

In order to study more deeply the mechanism by which *O. oeni* eliminates OTA from the medium, experiments were done with living and dead bacteria to compare the results. The number of cells in the media remained constant and two very different media were used: MLO, a nutrient-rich medium (pH 4.8) , and 0.5 mol l^{-1} acetate buffer, adjusted at pH 4.8, a nutrient-poor medium where there is no population increase and cell integrity is maintained by avoiding cell lysis during the test period. Two strains (6G and 124M) that had shown greater capacity for toxin reduction were used. The results for all the cultures showed very significant toxin reduction especially in the first stages of the incubation period (first 3 hours) and no differences were found in OTA removal between the cultures inoculated with living or dead, heat treated, bacteria. Nor were any medium-related differences found. Some of these results agree with previous report that described toxin elimination by *O. oeni* as a cell–binding phenomenon (*18*) but our results show that this binding is unrelated to bacterial viability. These authors report that

cell-free extracts of *O. oeni* have not ability to degrade OTA. Therefore, it seems that toxin removal by *O. oeni* depends on the cellular integrity but not on its viability.

Some authors have described that heat inactivated bacteria, different to *O. oeni*, remove aflatoxin B1 and zearalenone from the culture medium (*21, 23, 24, 29, 36, 48*). However, the chemistry and the molecular basis of mycotoxin binding remain unknown. In the present study, heat-treated bacteria removed OTA equally well or even more effectively than viable cells, although significant differences were not found. That may be related to the fluidity state of the lipid double layer and the variation in the lipid/protein ratio in the membranes of *O. oeni* produced by heating (*57*).

It has been reported that reduction of OTA and other mycotoxins by yeasts is due to mycotoxin binding with parietal yeast mannoproteins (*3, 19, 54, 63*). *O. oeni* may act in a similar way.

The data in this study show that in some of the cultures the removal process is partly reversible and upon culture prolongation a very small part of the toxin is released back into the medium (Figures 2 and 3). These results are in agreement with the results for aflatoxin B_1 reduction using *Lactobacillus rhamnosus* GG published by El-Nezami et al. (*20-22*). These authors described the ability of this *Lactobacillus* strain to eliminate 80% of the toxin from the medium exclusively through physical binding by the cell wall components and that part of the toxin could be released back into the medium.

To encourage the development of *O. oeni*, the pH of the culture media (MLO and acetate buffer) was set to 4.8. Although the aim of our study was not to assess the influence of the pH on OTA removal rate by the used *O. Oeni* strains, it has been recently described in lactic bacteria different to *O. Oeni* (*26*) that optimal removal of OTA takes place at low pH values (pH 5). These authors report a 50% reduction in OTA removal ratio by *Lactobacillus acidophillus* at pH values between 7 and 8. Likely, pH differences could have a great influence on the OTA binding ability of *O. oeni* cells.

The ability of *O. oeni* to eliminate OTA from the medium and the fact that this elimination is independent on bacteria viability and dependent on the strain opens up an important route for the possible use of this species as a tool for OTA removal from food and beverages and, very especially, from wine.

Conclusion

Oenococcus oeni, the most important bacterium involved in the conduction of malolactic fermentation of wine, has the ability to remove OTA from the MLO medium and acetate buffer. This reduction is dependent on the strain, incubation time, and OTA concentration in the medium (p-value < 0.01) but no significant differences were observed in OTA removal with regard to the type of culture medium and strain viability. On the basis of our results and those from other authors it may be concluded that the reduction mechanism seems to be the physical binding of the toxin to the cell wall by adsorption, although bacterial integrity appears to be necessary to this physical binding. Therefore, it is possible to conclude that application of selected strains of *O. oeni* as starters of malolactic fermentation or the use of heat-treated *O. oeni* cells in the winemaking and others processes might be a promising tool to reduce OTA contamination in some wines and others beverages and food.

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FIGURE CAPTIONS

Figure 1. Evolution of OTA reduction rate in MLO culture medium inoculated with ten strains of *O. oeni* isolated form grape and incubated at 28ºC. A) Medium spiked with 2 µg OTA l^{-1}. B) Medium spiked with 5 µg OTA l^{-1}.

Figure 2. Evolution of OTA level in MLO culture medium spiked with 2 or 5 µg OTA l^{-1} and inoculated with living or death cells of two strains of *O. oeni* (6G, 124M) in stationary phase. Incubation temperature: 28ºC.

Figure 3. Evolution of OTA level in acetate buffer (pH 4.8) spiked with 2 or 5 µg OTA l^{-1} and inoculated with living or death cells of two strains of *O. oeni* (6G, 124M) in stationary phase. Incubation temperature: 28ºC.