Mechanistic insight into ochratoxin A adsorption onto the cell wall of *Lacticaseibacillus rhamnosus* Bm01 and its impact on grape juice quality

Jiang Li, Lu Gao, Zhirong Wang, Peiwen Huang, Tianzhu Guan, Xiangfeng Zheng*

College of Food Science and Engineering, Yangzhou University, Yangzhou, China

***Corresponding Author**: Xiangfeng Zheng, No.196 West Huayang Road, Yangzhou, Jiangsu 225009, China. Email: zxf@yzu.edu.cn

Received: 1 March 2024; Accepted: 3 June 2024; Published: 4 July 2023

OPEN ACCESS

RESEARCH ARTICLE

Abstract

The contamination of food products with ochratoxin A (OTA) is a significant and pervasive food safety concern. In this regard, the novel use of lactic acid bacteria (LAB) to eliminate OTA from food has shown strong potential. The adsorption of OTA to the Lacticaseibacillus rhamnosus Bm01 (Bm01) cell walls has been demonstrated to eliminate OTA from grape juice effectively. The present study investigated the specific components of the Bm01 cell wall on OTA adsorption and evaluated the effect of Bm01 on grape juice quality using high-performance liquid chromatography. The results showed that the treatment of methanol and formaldehyde caused cell membrane perforation and enhanced OTA adsorption of Bm01, which reduced 98.35% and 95.13% of OAT, respectively. The involvement of cell wall proteins in the adsorption of OTA was demonstrated because only 5.23% of OTA was removed by Bm01 without cell wall proteins. Lactic (from 0 to 1.69 mg/mL) and acetic acid levels (from 0.14 to 1.45 mg/mL) were increased, malic acid (from1.24 to 0.81 mg/mL), glucose (from 8.8 to 6.91 mg/mL), and fructose (from 12.73 to 7.47 mg/mL) levels were reduced after treatment with Bm01. The addition of Bm01 shows little negative impact on color and light transmission. Overall, the effect of the addition of Bm01 on the quality of grape juice was found to be minimal. These results indicate that Bm01 has the potential to be a viable biological solution for mitigating OTA contamination in beverages, thereby offering a practical and effective method for food safety.

Keywords: L. rhamnosus Bm01; mechanistic studies; practical applications; Ochratoxin A

Introduction

OTA is a mycotoxin primarily synthesized by various filamentous fungi, including *Aspergillus* and *Penicillium* (Azam *et al.*, 2019; Ringot & Chango, 2009). Numerous studies have demonstrated the diverse toxicological effects of OTA, including nephrotoxic, teratogenic, carcinogenic, and mutagenic effects (Garcia-Perez *et al.*, 2021; Patricia Casas-Junco *et al.*, 2019). It has been classified as a group 2B human carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 1993). Due to OTA's ability to resist both acid pH and high temperatures, it becomes difficult to eliminate OTA contamination from foods completely (Ben Taheur *et al.*, 2017; Delgado *et al.*, 2021; Wei *et al.*, 2021; L. Wu *et al.*, 2020). Therefore, OTA contamination represents a significant food safety problem and risk to human health.

OTA levels in food are a significant concern globally due to their high stability (Pittet, 1998). Grapes and grape products, such as raisins, grape juice, and wine, have been identified as one of the greatest sources of OTA intake by humans, accounting for 10–15% of the total daily intake of OTA (Stefanaki *et al.*, 2003). OTA

ISSN 1757-837X online, DOI 10.15586/qas.v16i2.1486

contamination in grapes and grape products has been reported in many countries, including Iran, Croatia, and Argentina (Heshmati & Nejad, 2015; Marino-Repizo *et al.*, 2017; Zurga *et al.*, 2019) as well as the USA, Italy, and other Western European countries (Di Stefano *et al.*, 2015; Palumbo *et al.*, 2015), with levels of contamination of individual samples ranging from 0.93% to 99.3% (Bejaoui *et al.*, 2006; Kumar *et al.*, 2020; Serra *et al.*, 2003). Due to its potential toxicity, the European Union (EU) has established maximum limits for OTA contamination in different food products. These limits include 5.0 μg/kg for raw cereals, 3.0 μg/kg for processed cereals (FAO, 2004), and 0.5 μg/kg for infant foods (Commission, 2005). The EU has also implemented OTA limits in grape products, including a maximum of 2.0 μg/kg in wine and grape juice (Commission, 2005) and 10.0 μg/kg in raisins (FAO, 2004). Therefore, there is a significant need to identify strategies for limiting OTA contamination, given its potential impact on human health and the need to adhere to strict regulatory requirements.

Biological methods have recently garnered increasing attention as a viable alternative to physical and chemical approaches for removing OTA from food. Various microorganisms, including yeasts, bacteria, and filamentous fungi, have been reported to effectively control OTA contamination (Zheng *et al.*, 2016; Chen *et al.*, 2018). Previous research has primarily focused on using yeasts to mitigate mycotoxins (Fiori *et al.*, 2014; Yang *et al.*, 2016). However, as a probiotic, LAB could be an ideal bio-preservative candidate for reducing mycotoxin by adsorption or degradation in the food industry (Smaoui *et al*., 2023; Punia *et al*., 2022). Piotrowska (2014) investigated the capacity of three active (living) or heat-inactivated (dead) LABs to remove OTA. Further studies of the underlying removal mechanism indicated that OTA adsorption was attributed to the surface binding of OTA on cell walls facilitated by hydrophobic and electrostatic interactions. Haskard *et al.* (2000) reported on the ability of the carbohydrate component of L. rhamnosus GG cell wall to be removed through hydrophobic interactions with aflatoxin B1 (AFB1) from an aqueous system. These findings were further supported in subsequent research conducted by Zoghi *et al.* (2014). However, the release of binding toxins, the factors affecting the adsorption capacity, and the adsorption sites need further study (Punia *et al*., 2022). Thus, regarding OTA removal, it is necessary to elucidate the precise mechanism by which LAB removes OTA from food products to facilitate their use for OTA decontamination.

The potential impact of microbial OTA removal on product safety and quality must be evaluated to gain regulatory approval for their commercial use. Enzymatic degradation and cell wall adsorption represent the two primary methods by which microorganisms remove OTA from food products. Several studies have reported that enzymes produced by microorganisms can degrade OTA and transform it into OTα, which is less harmful (Bejaoui *et al.*, 2014; Palmira *et al.*, 2015). However, despite the relatively lower toxicity of OTα, its potential effect on human health is uncertain, indicating the possibility that the elimination of degradation products may necessitate supplementary procedures and expenses. Consequently, cell wall adsorption of mycotoxins, such as OTA, for detoxification presents a distinct advantage as it does not deplete substrate components or generate secondary metabolites (Farbo *et al.*, 2016). Consequently, microorganisms whose adsorption is the primary mechanism of mycotoxin depletion have broader commercial applicability. Notably, however, the impact of biological mycotoxin removal on product quality should always be determined. Gumus and Demirci (2022) observed that introducing *L. acidophilus* DSM 20079 in grape juice reduced pH and viscosity, potentially compromising its overall quality. Wu *et al.* (2021) reported that incorporating LAB into grape juice altered the proportion of sugars to organic acids and increased the level of phenolic, enhancing its distinctive aroma and flavor. Leonardo *et al.* (2015) examined the impact of *S. cerevisiae* W13 and BM45 yeast strains on anthocyanin content in OTA-contaminated wines. Their results revealed that adding yeast cells significantly decreased anthocyanin content, resulting in a loss of color. Thus, while biological decontamination of mycotoxins in food products has provided favorable outcomes, it is not without potential problems, including a potential loss in product quality. Consequently, additional research on the impact of OTA removal from food products by microorganisms on product quality will provide valuable insights that can be utilized in the commercial implementation of biological approaches to mycotoxin removal.

Zheng *et al.* (2023) previously reported that *L. rhamnosus* strain Bm01 effectively removed OTA from grape juice, primarily through cell wall adsorption. Notably, Bm01 also successfully purified commercially available grape juice contaminated with OTA. However, the precise mechanism underlying Bm01cell wall adsorption of OTA and the impact of Bm01 on the quality of grape juice was still unknown. Therefore, the present study investigated the effect of cell chemical modification on the OTA adsorption capacity of Bm01. Furthermore, the main chemical components involved in OTA adsorption were studied by separating cell wall components. In addition, the influence of Bm01 on grape juice quality was also studied. The results would provide valuable insights that can be utilized in the potential commercial development of Bm01 for OTA removal in beverages.

Materials and Methods

Chemical reagents

Chemical reagents used in the experiments included acetonitrile (Tedia, USA), methanol (Tedia, USA), and high-performance liquid chromatography (HPLC) grade acetic acid (Concord, China). One milligram of OTA standard sample (Pribolab Bio-logical Technical Company, Qingdao, China) was dissolved in 1 mL of methanol to produce a 1 mg/mL OTA stock solution. OTA was stored at –20°C and shielded from light to maintain stability. Phosphate buffered saline (PBS 0.1 M, pH 7.8, mixing 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 at a volume ratio of 3:47) was chosen as an excellent solvent and stabilizer for OTA degradation and protein treatment in the following experiments.

L. rhamnosus Bm01 culture conditions

The Bm01 strain utilized was obtained from previously preserved strains within our laboratory. The Bm01 was activated by inoculating the Bm01 to 5 mL de Man, Rogosa, and Medium (MRS) liquid media (Qingdao Hope Biological Co., Qingdao) and cultured at 37°C for 24 h. One hundred microliter of activated Bm01 (10^8 CFU/mL) was inoculated into 50 mL of fresh MRS liquid medium in a 250 mL flask and was cultured at 37°C for 24 h. The Bm01 cells in the culture were collected by centrifuge at 10,000 rpm and 20°C for 5 min. The cell pellets were washed three times with physiological saline (0.9% NaCl) prior to use in the experiments.

Bm01 cell wall modification

The Bm01 cell pellets were then inactivated at 100°C for 20 min. After that, a total of 5.0 g of inactivated Bm01 cells were placed in a 250 mL flask and mixed with 50 ml formaldehyde, 50 mL acetone, 50 mL of 0.1 M NaOH (alkaline solution), or 50 mL mixture of methanol and hydrochloric acid (v/v, 50/1), respectively. The mixtures were then stirred at room temperature for 6 h and collected by centrifuge at 10,000 rpm and 20°C for 5 min. The bacterial samples subsequently underwent a minimum of three washes with distilled water, followed by freeze-drying. The resulting samples of dried Bm01 cell walls were stored in desiccators until utilized.

Morphology of modified Bm01 cell

The physical structure and morphology of the different Bm01 cell wall samples obtained from the above step were observed under a GeminiSEM 300 scanning electron microscope (Carl Zeiss, Germany).

OTA adsorption by modified Bm01 cell wall

The efficacy of the heat-inactivated (B- Heat inactivated), formaldehyde (B- formaldehyde), acetone (B- acetone), alkaline (B- alkaline), and methanol (B- methanol) treated Bm01 cell wall in the removal of OTA was assessed as follows. Initially, 0.10 g of the modified cell wall sample was subjected to 5 mL of 0.1 M PBS (pH 7.8) containing 50 ng/mL OTA, respectively. The simple addition of heat-inactivated Bm01 cell walls was used as a control. Three replicates for each group were performed. All samples were incubated in a shaker incubator at 37°C for 12 h at 120 rpm. The samples were filtered through a 0.22 μm membrane (WondaDisc NY Organic Filter, Shimazu -GL Scientific, Shanghai, China). OTA content in the above sample was analyzed using an HPLC column ZORBAX SB-C18 (4.6 × 50 mm, 5μm) connected to the ShimadzuLC-10A system. The mobile phase (acetonitrile: 1% acetic acid, 6:4 [v/v]) with a 1 mL/min flow rate was used. OTA was detected using a fluorescence detector (λe_x = 333 nm; λe_m = 460 nm) after injecting 20 μL of the sample (Zheng *et al*., 2023).

X-ray diffractometer (XRD) analysis

XRD analysis evaluated the OTA binding effect of the modified (heat inactivation, formaldehyde, and acetone treated) Bm01 cell wall exhibiting the highest OTA adsorption level. Initially, 0.10 g of the cell wall sample was subjected to 5 mL of 0.1 M PBS (pH 7.8) containing 50 ng/mL OTA in a test tube. All samples were incubated in a shaker incubator at 37°C for 12 h at 120 rpm. The cell wall was collected by centrifuge at 10,000 rpm and 20°C for 5 min and used for XRD analysis on a D8ADVANCE (Bruker-AXS, Germany) x-ray diffractometer utilizing CuKA (radiation voltage 40 kV, current 30 mA). The cell wall sample without adding OTA was used as the control group.

Removal of Bm01 cell wall components on OTA adsorption

Various constituents of Bm01 cell walls were removed using the trichloroacetic acid (TCA) method (Kho & Meredith, 2018). Bm01 cells were first lysed by boiling them in a 4% sodium dodecyl sulfate (SDS) solution for 30 min, which dissolved the intracellular components. The solution was then cooled at 4°C for 5 min, after which the solution was subjected to centrifugation at

6000 rpm at room temperature for 10 min. The resulting precipitate of cell walls was collected and washed with deionized water until all traces of SDS were removed. The harvested cell walls were then subjected to 240 W sonication for 30 min using 4 sec of sonication and 2 sec of rest, allowing 300 repetitions. The resulting solution was again centrifuged, and three washes were done with deionized water. Phosphorylated acid was removed by suspending trypsin-treated precipitate in a 10% TCA solution and placing it in a water bath at 70 °C for 3 h, then cooling it to room temperature. Cell wall precipitates were collected using several cycles of centrifugation and washes with deionized water.

Degreasing: The pH of the 0.05 M sodium acetate solution was adjusted to 4.6 using 0.05 M acetic acid. The solution was combined with chloroform and methanol at 4:5:10 (v:v:v). Cell walls were subjected to degreasing by stirring cell wall precipitates with the sodium acetate solution (w/v = 1:20) for 24 h at room temperature. The solution was then centrifuged at 8000 rpm for 10 min, and the resulting precipitate was washed with methanol, followed by deionized water. The solution was centrifugated, and the final cell wall precipitate was collected.

Deproteinization: The obtained precipitate was added to 0.1 M PBS buffer solution (pH 7.8) containing 3 mg/mL trypsin at a ratio of 1:10 (w/v). The mixture was then incubated at 37 °C in a water bath for 12 h, followed by 5 – 6 deionized water washes and centrifugation to obtain a precipitate. The resulting precipitate was then boiled in 1% SDS for 10 min and then centrifuged and washed several times with deionized water to remove any traces of SDS. The final precipitate was then lyophilized and freezing stored until further use.

The efficacy of the above samples in the removal of OTA was assessed. Initially, 0.10 g of the cell wall sample was subjected to 5 mL of 0.1 M PBS (pH 7.8) containing 50 ng/mL OTA in a test tube. A test tube without adding Bm01 cell walls was used as a control. All samples were incubated in a shaker incubator at 37 °C for 12 h at 120 rpm. The samples were then centrifuged, and the OTA content in the remaining supernatant was measured by HPLC using the method described by Zheng *et al*. (2023).

Extraction of Bm01 surface protein and assessing its ability to bind OTA

Bm01 cells were cultured at 37 °C for 18 h using a 4% culture as starter inoculum each time. Cells after the third round of culture were retained for further processing. BM01 cells were harvested by centrifugation at 5000 rpm for 15 min at 4 °C, after which the supernatant was discarded, and the cells were rinsed three times with deionized water to remove any traces of the medium. Subsequently, a 5.0 M LiCl solution was added to the precipitated cells at a ratio of 1:10 (volume weight), and the mixture was shaken at 180 rpm for 60 min at 37°C and then centrifuged. The resulting supernatant was concentrated by dialysis through polyethylene glycol (PEG) applied to the dialysis bag containing the protein solution (Dialysis bag 27 mm, entrapped MW 3.5 kd, change the H_2O three times during 24 h under 4°C) and then freeze-drying. Proteins were then dissolved in 1 mL of 0.1 M PBS (pH 7.8). The protein content was then detected by SDS-polyacrylamide gel electrophoresis (SDS-PAGE electrophoresis) and Protein Assay Kit (PA115, Tiangen, Beijing, China). Subsequently, 1 mg of proteins were mixed with varying concentrations of OTA (0.2-1 mg/mL), followed by fluorescence polarization analysis using a fluorescence spectrophotometer with excitation wavelength (Ex) 333 nm and emission wavelength (Em) 460 nm. The level of polarization in the samples was calculated using the following formula: $P = (I_{VV} - GI_{VH})/(I_{VV} + GI_{VH})$, $G = I_{HV}/I_{HH}$. The polarization measurements are accomplished by taking vertical and horizontal fluorescence intensity readings with the excitation polarizer at both vertical (v) and horizontal (H) positions. These measurements yield four intensity readings, I_{VV}, I_{VH}, I_{HV}, and I_{HH}, where the order of letters v and h represents the positions of the excitation and emission polarizers. The I_{HV} and I_{HH} readings are used to correct for optical artifacts introduced by the instrument.

Growth dynamics and OTA removing the effect of Bm01 in grape juice

One hundred µL of 9×10^8 CFU/mL washed Bm01 cells were added to 5 mL of commercial grape juice (CGJ), and 0.5 mL of samples of CGJ were subsequently collected at 12 h intervals (0, 12, 24, 36, 48, 60, 72 h). The collected samples were subjected to six rounds of dilution plating, after which 100 µL of the sample was plated on solid MRS media. The colonies were then counted after incubation at 37 °C for 24 h. Three replicates were used for each sample. A control group obtained from Bm01 cells cultured in 5 mL of grape juice without the addition of OTA was also included. Subsequently, 200 µL of the sample was collected at regular 12-hour intervals, and absorbance at OD_{600} nm was recorded for each sample using a spectrophotometer. OTA content in the grape juice at 0, 24, 48, and 72 h was measured by HPLC using the method described by Zheng *et al*. (2023).

Effect of Bm01 on pH, light transmission, and chromaticity of grape juice

The treatment group comprised 5 mL of CGJ and OTA to obtain a final 50 ng/mL concentration and 100 µL of

Bm01 cells (9×10^8 CFU/mL). Control groups comprised 5 mL of CGJ alone and 5 mL of CGJ amended with OTA (final concentration of 50 ng/mL). All samples were incubated at 37°C for 72 h. The pH of grape juice samples was determined using a PHS-2F pH meter (Yidian Co., Shanghai, China). Sugar content, comprising glucose and fructose, was determined by HPLC/RID utilizing a sugar column (250 mm × 4.6 mm, 4 μm) at 30°C. The mobile phase consisted of acetonitrile and water in a 75:25 (V/V) ratio, with a flow rate of 0.80 mL/min. Light transmission at 680 nm was determined in grape juice samples using a TU-1810 UV spectrophotometer (General Analytical Instrument, Beijing, China). A sample of commercial grape juice was utilized as a blank, and light transmission of sample supernatant was assessed after being mixed with saline at a volume ratio of 1:9. Light transmittance of the grape juice samples was calculated by converting the data using the formula A=lg1/T. The chromaticity of samples was assessed using a CR-400 colorimeter (Lighting: pulsed xenon lamp, standard observer: approximately 2° viewing angle, following the CIE1931 standard for inspectors, and aperture: Φ8 mm/Φ11 mm, Measurement time:1 second, Measurement interval: 3 seconds) (Ke Sheng Instrument Co., Ltd., Hangzhou, China), which provided values for L^* (0 = black, 100 = white), a^* (positive = red, negative = green) and b^* (positive = yellow, negative = blue).

Effect of Bm01 on organic acid content of grape juice

An HPLC protocol was established to detect organic acids, specifically tartaric, malic, citric, lactic, and acetic acids. The HPLC protocol utilized analysis column Shodex KC-811 (8.0 mm × 300 mm, 6 μm). The mobile phase consists of 3 mmol/L perchloric acid. The flow rate was set to 1 mL/min, and an injection volume of 10 μL was used. UV detection at 210 nm was assessed with the column temperature maintained at 50°C. Sample detection was carried out for 25 min. The retention time of standard organic acids was 5.5 min, 6.7 min, 8.5 min, 7.8 min, and 11.9 min for tartaric acid, malic acid, acetic acid, fumaric acid, and citric acid, respectively. A standard curve based on the correlation between organic acid content and peak area was built to quantify organic acid in the sample.

Effect of Bm01 on the sugar content of grape juice

An HPLC-differential refractometry protocol was established for the detection of sugar content. The HPLC protocol utilized analysis column Shodex KS-801 (8.0 mm×300 mm, 6 μm). The mobile phase consists of ultra-pure water. The flow rate was set to 0.7 mL/min, and an injection volume of 10 μL was used. The column temperature was maintained at 80 °C. Sample detection was carried out for 20 min. Standard glucose and fructose retention times were 14.2 min and 12 min, respectively. A standard curve based on the correlation between standard sugar content and sugar peak area was established to quantify sugar in the sample.

Statistical analysis

The experiments conducted in this study utilized three biological replicates, and the experiments were repeated twice. Data obtained in this study is presented as the mean ± standard deviation. A one- or two-way ANOVA was conducted to determine treatment effects using SPSS version 18.0.2. Significant differences between sample groups at P<0.05 were determined using the t-test or Duncan's Multiple Range Test (DMRT).

Results

OTA removal by chemically modifying Bm01 cell walls

Heat-inactivated Bm01 cells were treated with formaldehyde, acetone, sodium hydroxide, or methanol. It was found that heat-inactivated, sodium hydroxide or acetone-treated samples did not exhibit cell damage (Figure 1A, C, D). In contrast, cells treated with formaldehyde and methanol showed obvious damage and perforation (red arrows in Figure 1B and 1E). The methanol or formaldehyde treatments showed significant (p<0.05) increases in OTA adsorption when compared to heat-inactivated Bm01 cells, resulting in 98.35% and 95.13% reductions in OTA levels, respectively (Figure 1F). The alkaline treatment only reduced OTA content by 30.48%, which was significantly lower (p<0.05) compared to others (Figure 1F). X-ray analysis of the different cell wall preparations can determine if OTA crystals are present on the cell wall. Therefore, heat-inactivated, methanol-, and formaldehyde-treated Bm01 cell walls were analyzed using an X-ray diffractometer before and after OTA adsorption. Notably, the presence of new peaks at 2θ = 26.5°, 31.5°, 45.5°, 56.5°, and 75.5° (Figure 1H) was observed after OTA adsorption (Figure 1H) in comparison to x-ray spectra obtained prior to OTA adsorption (Figure 1G). The newly identified peaks were interpreted to represent characteristic OTA peaks, suggesting that the surface of heat-inactivated, methanol, and formaldehyde-treated Bm01 cells had adsorbed OTA. Based on these data, it can be inferred that the diffraction patterns of cells obtained after OTA adsorption exhibit the emergence of numerous novel OTA crystal peaks, providing evidence for changes in the interaction between OTA and modified Bm01 cells.

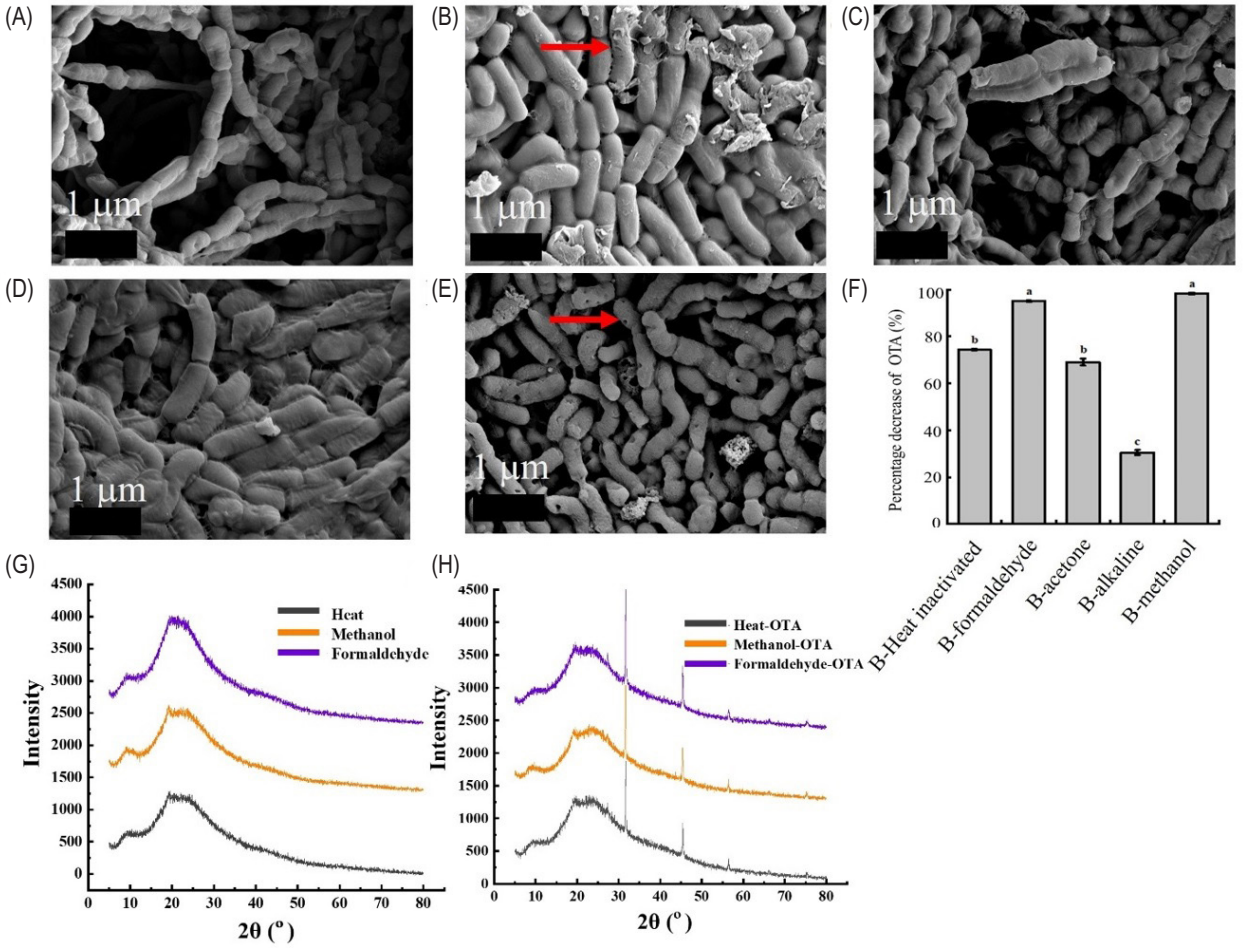

Figure 1. Morphology and OTA removal of chemically modified Bm01 cells. (A) Heat-inactivated. (B) Formaldehyde. (C) Acetone. (D) Sodium hydroxide. (E) Methanol. (F) Percentage decrease of OTA. Data represent the mean ± SD (n = 3). Lowercase letters above the bars indicate a significant difference (P<0.05). (G) x-ray energy spectra of treated cell walls before OTA adsorption. (H) X-ray energy spectra of treated cell walls after OTA adsorption.

Identifying Bm01 cell wall components for OTA adsorption

The bacterial cell wall primarily comprises teichoic acids, lipids, and proteins. Some have been investigated for their ability to bind to and remove OTA. The ability of Bm01 cell wall components to bind OTA was analyzed using a sequential removal method. Results indicated that Bm01 cell walls were gradually degraded after removing teichoic acids (Figure 2B), lipids (Figure 2C), and proteins (Figure 2D), compared to heat-inactivated cell walls (Figure 2A). Protein removal in the cell wall resulted in complete loss of cellular morphology (Figure 2D). Cells without teichoic acid remove 95.13% of OTA, whereas further lipid removal removes 97.78%. Conversely, 5.23% of OTA was removed by cells without teichoic acids, lipids, and proteins (Figure 2E). Therefore, Bm01 cell wall proteins were extracted (Figure 2F) and subjected to fluorescence polarization analysis with OTA. The S-shape illustrates the competitive binding patterns between proteins and OTA (Figure 2F). According to these results, the Bm01 cell wall protein is responsible for OTA absorption.

Change of organic acid and sugar content during OTA removing

The effect of the adsorption of OTA in grape juice by Bm01 was analyzed. OTA content was reduced from 50 ng/mL at 0 h to 8.78 ng/mL at 72 h after incubation with Bm01 (Figure 3A). The growth dynamics of Bm01 were monitored by measuring optical density (OD) values and dilution plating. Results indicated that the OD_{600} readings in grape juice consistently ranged from 0.8 to 0.95 over 72 h, while cell counts ranged between 9×10^8 and 1×10^9 CFU/mL (Figure 3B). The effect of Bm01 on organic acid and sugar contents was also analyzed. The concentration of tartaric, malic, citric, lactic, and acetic acids

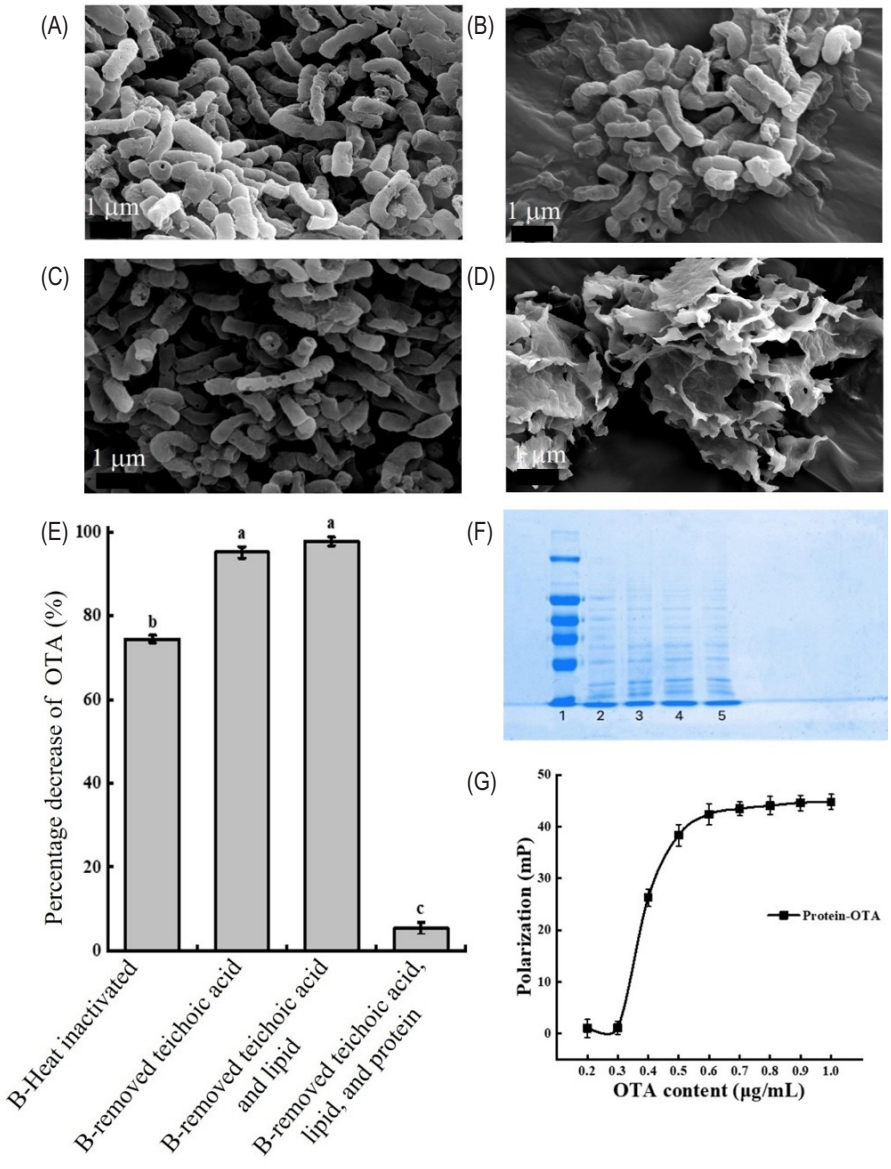

Figure 2. Cell morphology and OTA remove the effect of Bm01 cells lacking teichoic acids, lipids, and proteins. (A) Heat inactivated. (B) Removal of teichoic acid. (C) Removal of teichoic acid and lipids. (D) Removal of teichoic acid, lipids, and proteins. (E) OTA removing the effect of different Bm01 cells. (F) SDS-PAGE of proteins extracted from the Bm01 cell walls (Lane 1: size markers; Line 2-5: cell wall proteins). (G) Binding curve of Bm01 cell wall proteins with OTA. Data are the mean ± SD (n = 3). Lowercase letters above the bars indicate a significant difference (P<0.05).

was measured over a 72 h period in commercial grape juice (CGJ), CGJ + OTA (CGJ-OTA), and CGJ + OTA + living Bm01 cells (CGJ – OTA-Bm01). All acidic contents remained stable for 72 h in the CGJ and CGJ-OTA sample groups. The levels ranged from 0.1522 to 0.1663 mg/mL for tartaric acid (Figure 3C), 1.2106 to 1.2697 mg/mL for malic acid (Figure 3D), 2.4366 to 2.6275 mg/mL for citric acid (Figure 3E), 0.0036 to 0.0063 mg/mL for lactic acid (Figure 3F), and 0.1484 to 0.1668 mg/mL for acetic acid (Figure 3G). In contrast, lactic acid and acetic acid increased concentration in the CGJ-OTA + Bm01 sample group during the same time frame. Lactic acid increased from 0.005 to 1.69 mg/mL, while acetic acid content increased from 0.14 to 1.45 mg/mL (Figure 3F, G). Following treatment with Bm01, Malic acid in the CGJ-OTA-Bm01 sample group increased from an initial 1.24 mg/mL to 0.81 mg/mL (Figure 3D). Sugar is a major constituent of grape juice and is a determining factor in taste (along with organic acids) and quality. Glucose and fructose are the primary sugars present in grapes, while sucrose and other sugars are seldom detected. Therefore, the present study focused on the impact of Bm01 on the glucose and fructose levels in grape juice. The concentration of these sugars remained relatively stable over 72 h

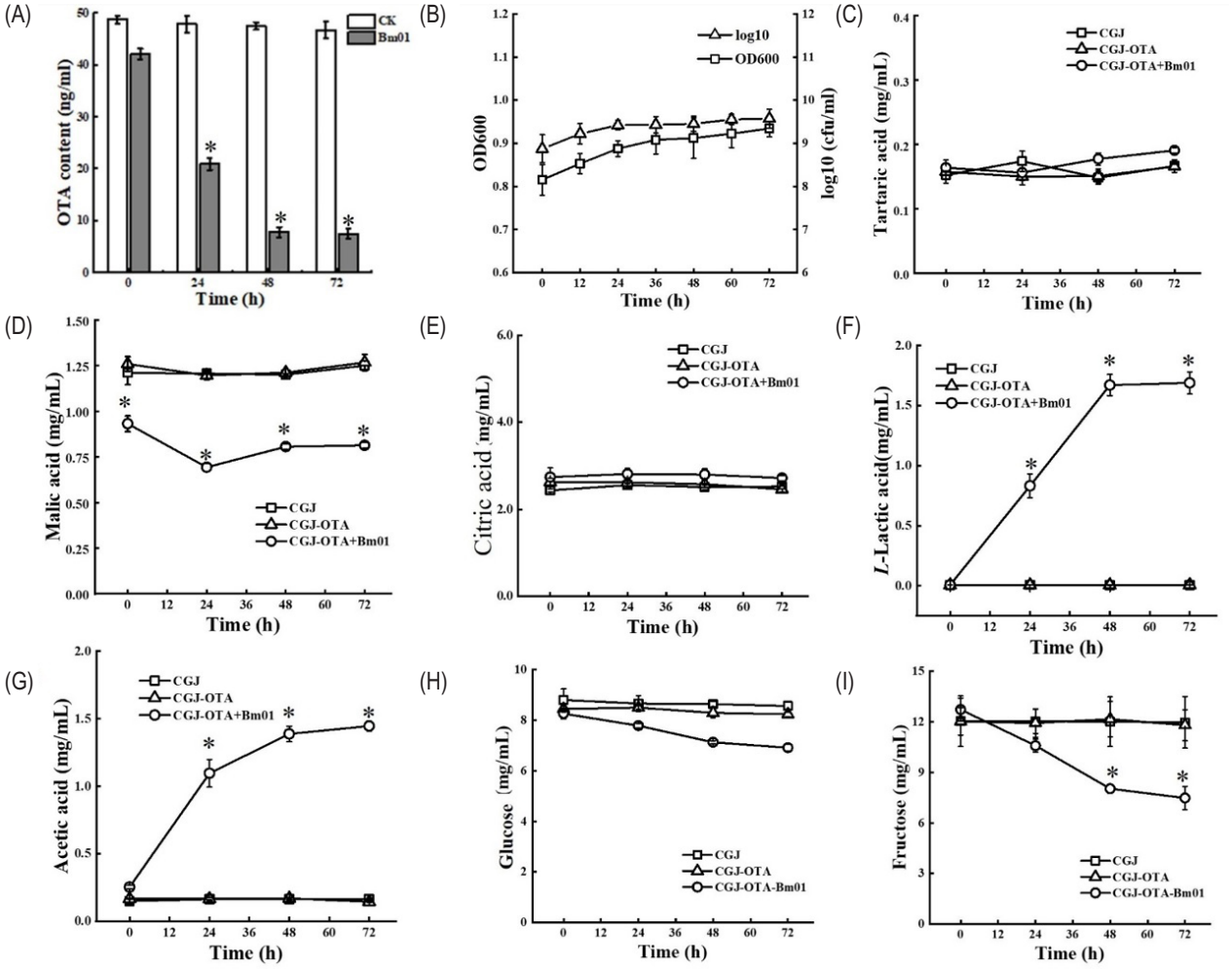

Figure 3. The organic acid and sugar content of grape juice were removed during OTA. (A) OTA content in grape juice with (Bm01) or without Bm01 (CK). (B) Growth dynamic of Bm01. (C) Tartaric acid. (D) Malic acid. (E) Citric acid. (F) Lactic acid. (G) Acetic acid. (H) Glucose. (I) Fructose. CGJ is commercial grape juice; CGJ-OTA is commercial grape juice + OTA; CGJ-OTA + Bm01 is commercial grape juice + OTA + Bm01. Data are the mean ± SD (n = 3). * Represents a significant difference compared with 0 h (P<0.05).

in the CGJ and CGJ-OTA sample groups (Figure 3H, I). The levels ranged from 8.3029 to 8.8021 mg/mL for glucose and 11.9667~12.0387 mg/mL for fructose over 72 h. In contrast, a gradual decrease in both glucose and fructose content was observed in the CGJ-OTA + Bm01 sample group over 72 h. Glucose content decreased from 8.80 to 6.91 mg/mL (Figure 3H), and fructose decreased from 12.73 to 7.47 mg/mL (Figure 3I).

pH and color changes of grape juice during OTA removing

The impact of living Bm01 cells on the pH and color changes of grape juice during OTA removal are presented in Figure 4. pH value exhibited a slight rise from 2.73/2.78 to 2.92 in the groups of CGJ and CGJ-OTA. pH value was decreased from 2.79 to 2.63 and then stabilized at pH 2.64 in the group adding Bm01 (Figure 4A). These results indicated that the presence of living Bm01 cells caused a minor increase in acidity. The impact of Bm01 on light transmission was also detected because the growth of Bm01 may affect the turbidity of the grape juice. Light transmission in the CGJ and CGJ-OTA groups exhibited minimal variation throughout measurement (Figure 4B). A significant decline was observed in light transmission in the CGJ-OTA + Bm01 sample, which decreased to 73.91%. Moreover, the effect of Bm01 cells on the color of commercial grape juice was investigated. Results are presented in Figure 4C, D, and E, where L^* values represent brightness, a^* values indicate red and green values, and b^* values reflect yellow and blue values. Results indicated that L^* values in the CGJ sample group exhibited a slight

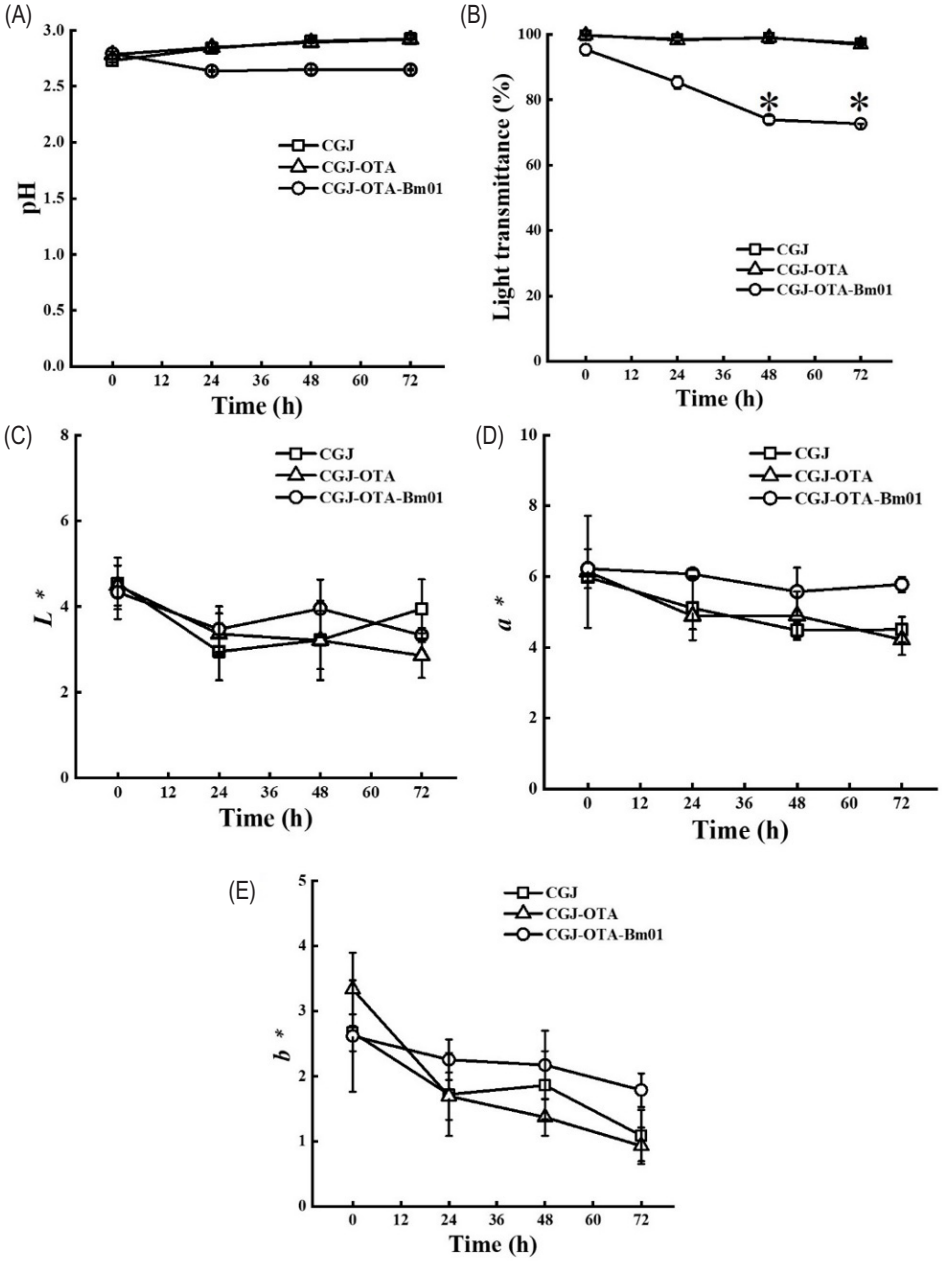

Figure 4. Changes in pH and color of grape juice during OTA removal by living Bm01 cells. (A) Effect of Bm01 on the pH. (B) Effect of Bm01 on the light transmittance. C-E, Effect of Bm01 on the brightness values *L (C), red-green values *a** (D), and yellow-blue values *b** (E). CGJ is commercial grape juice; CGJ-OTA is commercial grape juice + OTA; CGJ-OTA + Bm01 is commercial grape juice + OTA + *L. rhamnosus* Bm01). Data are the mean ± SD (*n* = 3). * Represents a significant difference compared with 0 h (*P*<0.05).**

decrease from 0 to 24 h, followed by an increase from 24 to 72 h. In comparison, L^* values in the CGJ-OTA sample group exhibited a decrease from 4.49 to 2.85 over 72 h (Figure 4C). Conversely, L^* values in the CGJ-OTA + Bm01 sample group fluctuated, ranging from 3.33 to 4.33 over 72 h. The CGJ and CGJ-OTA sample groups exhibited a gradual decline in a^* (red-green values) over 72 h, decreasing from 5.97/6.13 to 4.5/4.21, respectively. In contrast, the CGJ-OTA+Bm01 sample group maintained consistent a^* values over 72 h. These results suggest that the presence of Bm01 in grape juice helped to stabilize red and green values (Figure 4D). Lastly, b^* values in all three sample groups are presented in Figure 4E. All three sample groups exhibited a gradual decline in b^* values over 72 h. Notably, the b^* value in the CGJ-OTA + Bm01 sample group (1.78) at 72 h was higher than in the CGJ and CGJ-OTA sample groups (1.08 and 0.93, respectively).

Discussion

Ochratoxin A and its adsorption mechanism by Bm01 cell wall

OTA (a carcinogen) contamination of food products is a global problem, particularly in grapes and products derived from grapes (Huff *et al.*, 1992; Mayura *et al.*, 1984). Therefore, much research has been performed on preventing and eliminating OTA contamination in grape products. For OTA detoxification, biological methods offer a safer, more efficient, and more environmentally sustainable than physical and chemical approaches, which may compromise food quality. The utilization of LAB for OTA removal in food products is still preliminary in China, necessitating further comprehensive studies. It is necessary to identify LAB strains that can remove OTA from food products and comprehensively understand the OTA-removing mechanisms. According to our previous study (Zheng *et al.*, 2023), the LAB strain (*L. rhamnosus* Bm01) can efficiently remove OTA from grape juice by adsorbing it onto its cell walls. However, for Bm01 to be commercially viable, it is crucial to understand how this process works and impacts grape juice quality.

Based on this study's findings, treating Bm01 cells with formaldehyde or methanol resulted in cell wall perforation. Treated formaldehyde or methanol shows higher OTA absorption capacity than heat-inactivated, possibly due to perforation resulting in greater exposure of the active sites responsible for adsorbing this toxin (Haskard *et al.*, 2001). Wang *et al.* (2015) also reported that chemical treatments resulting in the modification of cell wall structure can significantly enhance the surface area of the cell wall, leading to greater OTA adsorption capacity. Furthermore, the primary reaction that occurs in cell walls treated with formaldehyde or methanol is the conversion of $RCH_2NH_2 \xrightarrow{HCHO+HCOOHR} CH_2N(CH_3)+CO_2$ and $RCOOH + CH_3OH \xrightarrow{H+} RCOOCH_3 + H_2O$, respectively (Debdatta, 2022). As a result of these reactions, the amino and the carboxyl groups of the proteins are transformed into CH_2N (CH_3) and $RCOOCH_3$, respectively. Such an adsorption mechanism may involve hydrophobic and electrostatic interactions, as demonstrated for LAB's cell wall by Piotrowska *et al.* (2014). The OTA adsorption capacity of alkaline treatment Bm01 was lower than heat-inactivated, possibly because alkaline hydrolyzes the amide bond, resulting in the loss of OTA adsorption sites in the proteins. The results agree with previous research conducted by Haskard *et al.* (2000), who demonstrated the significant involvement of protein components of LAB cell walls in the adsorption of mycotoxin. Similar results in the adsorption of toxins by yeast were reported by Guo *et al.* (2012). Further research is required to elucidate the precise structural and compositional attributes of the cell wall responsible for OTA adsorption and their operational basis.

Peptidoglycan and polysaccharides in cell walls play a vital role in the binding of mycotoxins (Niderkorn *et al.*, 2009). However, OTA adsorption capacity was increased after the removal of teichoic acid and teichoic acid-lipids from Bm01 cell walls (>97%) but was decreased after the removal of proteins (5.33%). This phenomenon can be attributed to alterations in cell wall structure that increase the exposure of binding sites on cell wall proteins. Fluorescence polarization analysis showed that the OTA was competitively bound to Bm01 surface protein because the S-type polarization curve reached saturation at an OTA concentration of 0.7 g/mL. Based on the above, Bm01 cell wall-bound OTA is mainly carried out via cell wall proteins; however, the kind of proteins is unknown and needs further investigation. The conclusion that electrostatic interactions may also be associated with OTA adsorption capacity must be thoroughly verified to make it convincing. These results demonstrated that cell wall proteins were involved in OTA adsorption by Bm01.

Grape juice quality assessment

A previous study by Zheng *et al.* (2023) found that adding 50 ng/mL OTA to grape juice was reduced by 84.43% within 72 h by Bm01. Additionally, the presence of 20 ng/mL OTA in grape juice was completely removed by Bm01 within 48 h. This indicates that Bm01 exhibits a significant OTA removal capacity in grape juice and thus has commercial potential. However, the utilization of active Bm01 for OTA removal in various food products can be challenging due to the potential impact of the fermentation capacity of living LAB on the sensory characteristics of foods, as highlighted by Sheng *et al.* (2022). Hence, the impact of Bm01 on the composition and quality attributes of grape juice was evaluated.

As indicated by the Food and Agriculture Organization (FAO) and World Health Organization (WHO), the ingestion of appropriate quantities of probiotics has numerous health advantages, including enhanced populations of beneficial gut microbiota and greater intestinal functionality (Malviya *et al.*, 2021). Numerous studies have investigated the feasibility of probiotics in fruit juices as non-dairy probiotic alternatives (Rivera-Espinoza & Gallardo-Navarro, 2010). In this regard, Sheehan *et al.* (2007) assessed the growth of LAB in orange, pineapple, and cranberry juices. They found that *L. rhamnosus* GG, *L. casei* DN-114001, and *L. paracasei* NBFC43338 exhibited robust growth in these juices,

with orange juice reaching a viable cell count of 10^7 CFU/mL and pineapple juice reaching 10^6 CFU/mL after 12 weeks of refrigeration. Champagne and Gardner (2008) reported that *L. rhamnosus* displayed superior growth to *L. acidophilus* when grown in a mixture of juices from various fruits. In the present, Bm01 exhibited favorable growth in grape juice, and quality parameters after 72 h of OTA removal by Bm01 were assessed, including the effect of living Bm01 cells on the content of organic acids, sugars content, pH, light transmission, and color. Among the various organic acids in grapes, tartaric, malic, and citric acids are typically the most prevalent. Lactic and acetic acids are organic acids commonly present in juice, typically due to microbial metabolism (Robles *et al.*, 2019). Results of our study indicated that lactic acid increased from 0.005 to 1.69 mg/mL over 72 h, while the level of acetic acid increased from 0.14 to 1.45 mg/mL. These findings can be attributed to the metabolic activity of *L. rhamnosus* Bm01. Notably, humans can only synthesize L-lactate dehydrogenase, so only L-lactate can be metabolized. As a result, WHO does not recommend the inclusion of D-type and DL-type lactic acid in the diet of infants under three months of age. Notably, *L. rhamnosus* LGG exclusively generates L-lactic acid during the fermentation process without producing any other acids that may compromise the safety and palatability of a food product. Consequently, consuming fermented foods containing *L. rhamnosus* LGG should not adversely affect individuals, particularly infants and children. The decrease in malic acid content may be caused by Bm01 using it as a substrate to produce lactic acid or synthesizing other substances as an intermediate product of the TCA cycle. Only a minimal alteration was observed in the concentration of tartaric and citric acid over 72 h in the CGJ-OTA-Bm01 sample group. Lai *et al*. (2022) reported that the fermentation of *L. rhamnosus* only changed the pH value of the apple juice with 0.02 pH units. In the present study, the pH of the juice also did not significantly change with the change in acid content, which only decreased by approximately 0.15 pH units. A decrease in glucose (8.80 mg/mL - 6.91 mg/mL) and fructose (12.73 mg/mL-7.47 mg/mL) content was observed due to the presence and growth of Bm01. The results were similar to those reported by Lai *et al*. (2022), that *L. rhamnosus* reduced the total sugar content in apple juice from 6.07 to 5.62 mg/mL. Pineli *et al.* (2016) assessed the impact of reduced sugar content on the sensory properties of orange juice and identified the optimal sweetness level of orange juice. Their findings indicated that a 15–45% reduction in sugar had a minimal impact on orange juice's acceptability and sensory properties.

We also examined the light transmission of grape juice containing Bm01 cells and found that it decreased throughout 72 h, while no negative impact was observed on color. Malganji *et al.* (2016) conducted a study in which three LABs (*L. deuterium*, *L. plantarum*, and *L. rhamnosus*) were individually inoculated into grape juice and under non-fermentation conditions and which was then placed under refrigeration. They subsequently conducted an assessment of microbial activity and a sensory evaluation. Results of the sensory evaluation indicated that grape juice inoculated with *L. rhamnosus* exhibited a higher level of acceptability after a storage period of 4 weeks than grape juice inoculated with the other two LABs. The above finding suggests that using Bm01 to detoxify OTA in grape juice can effectively reduce its toxin concentration with a limited effect on grape juice quality. Evaluating grape juice quality based on only a few key indicators may be one-sided, and it is necessary to make a comprehensive assessment of physicochemical properties and organoleptic qualities.

Conclusions

The precise mechanism underlying OTA adsorption by Bm01 and Bm01 impacts grape juice quality is unclear. In this study, the OTA adsorption capacity of the Bm01 cell wall was improved by chemical modification, which can be used to enhance the OTA adsorption effect of LAB. After removing cell wall proteins, the OTA adsorption capacity of Bm01 decreased significantly, indicating that protein is the main component of OTA adsorption. The adsorption of proteins to OTA was further confirmed by fluorescence polarization analysis. Analysis of the effect of Bm01 on the critical quality indexes of grape juice after OTA removal indicated that Bm01 significantly decreased malic and fructose content but increased the lactic acid and acetic acid content. However, the pH value of grape juice has changed little. However, the effect of adding Bm01 on the taste and quality of grape juice must be evaluated more fully through blind tasting, electronic tongue analysis, or further determination of volatile aroma components, soluble solids content, and other indicators. Bm01 has practical and commercial potential in reducing OTA pollution in beverages.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

This work was financially supported by the Postdoctoral Research Foundation of China (2022M712693), the National Natural Science Foundation of China (grant number 31901801), and the National Key Research and Development Project of China (grant number 2016YFC1300200).

Author contributions

J.L.: data curation, writing- original draft preparation; L.G.: data curation, writing - review and editing; Z.W.P.H.: data curation; T.G.: writing - review and editing; X.Z.: writing- original draft preparation, funding acquisition; writing- reviewing and editing;

References

Abrunhosa, L., Inês, A., Rodrigues, A.I., Guimarães, A., Pereira, V.L., Parpot, P., et al. (2014). Biodegradation of ochratoxin A by *Pediococcus parvulus* isolated from Douro wines. International Journal of Food Microbiology, 188, 45–52. https://doi.org/10.1016/j.ijfoodmicro.2014.07.019

Azam, M.S., Yu, D., Liu, N. & Wu, A. (2019). Degrading ochratoxin A and zearalenone mycotoxins using a multifunctional recombinant enzyme. Toxins, 11(5), 301–307. https://doi.org/10.3390/toxins11050301

Bejaoui, H., Mathieu, F., Taillandier, P. & Lebrihi, A. (2006). Biodegradation of ochratoxin A by *Aspergillus* section *Nigri* species isolated from French grapes: a potential means of ochratoxin A decontamination in grape juices and musts. FEMS Microbiology Letters, 255(2), 203–208. https://doi.org/10.1111/j.1574-6968.2005.00073.x

Ben Taheur, F., Fedhila, K., Chaieb, K., Kouidhi, B., Bakhrouf, A. & Abrunhosa, L. (2017). Adsorption of aflatoxin B1, zearalenone and ochratoxin A by microorganisms isolated from Kefir grains. International Journal of Food Microbiology, 251, 1–7. https://doi.org/10.1016/j.ijfoodmicro.2017.03.021

Champagne, C.P. & Gardner, N.J. (2008). Effect of storage in a fruit drink on subsequent survival of probiotic lactobacilli to gastro-intestinal stresses. Food Research International, 41(5), 539–543. https://doi.org/10.1016/j.foodres.2008.03.003

Chen, W., Li, C., Zhang, B., Zhou, Z., Shen, Y., Liao, X., et al. (2018) Advances in biodetoxification of Ochratoxin A-a review of the past five decades. Frontiers in Microbiology, 26, 1386–1391. https://doi.org/10.3389/fmicb.2018.01386

Commission, E. (2005). Commission Regulation (EC) No 123/2005 of 26 January 2005 amending Regulation (EC) No 466/2001 as regards ochratoxin A. European Communication, 25, 3–5.

Delgado, J., Rondan, J. J., Nunez, F. & Rodriguez, A. (2021). Influence of an industrial dry-fermented sausage processing on ochratoxin A production by *Penicillium nordicum*. International Journal of Food Microbiology, 339, 109016. https://doi.org/10.1016/j.ijfoodmicro.2020.109016

Di Stefano, V., Pitonzo, R., Avellone, G., Di Fiore, A., Monte, L., & Ogorka, A. Z. T. (2015). Determination of aflatoxins and ochratoxins in sicilian sweet wines by high-performance liquid chromatography with fluorometric detection and immunoaffinity cleanup. Food Analytical Methods, 8(3), 569–577. https://doi.org/10.1007/s12161-014-9934-3

Farbo, M. G., Urgeghe, P. P., Fiori, S., Marceddu, S., Jaoua, S. and Migheli, Q. (2016). Adsorption of ochratoxin A from grape juice by yeast cells immobilised in calcium alginate beads. International Journal of Food Microbiology, 217, 29–34. https://doi.org/10.1016/j.ijfoodmicro.2015.10.012

Fiori, S., Urgeghe, P. P., Hammami, W., Razzu, S., Jaoua, S. & Migheli, Q. (2014). Biocontrol activity of four non- and low-fermenting yeast strains against *Aspergillus carbonarius* and their ability to remove ochratoxin A from grape juice. International Journal of Food Microbiology, 189, 45–50. https://doi.org/10.1016/j.ijfoodmicro.2014.07.020

Garcia-Perez, E., Ryu, D., Lee, C. & Lee, H. J. (2021). Ochratoxin A induces oxidative stress in HepG2 cells by impairing the gene expression of antioxidant enzymes. Toxins, 13(4), 271. https://doi.org/10.3390/toxins13040271

Gumus, S. and Demirci, A. S. (2022). Survivability of probiotic strains, *Lactobacillus fermentum* CECT 5716 and *Lactobacillus acidophilus* DSM 20079 in grape juice and physico-chemical properties of the juice during refrigerated storage. Food Science and Technology, 42, e08122. https://doi.org/10.1590/fst.08122

Haskard, C., Binnion, C. & Ahokas, J. (2000). Factors affecting the sequestration of aflatoxin by *Lactobacillus rhamnosus* strain GG. Chemico-Biological Interactions, 128(1), 39–49. https://doi.org/10.1016/s0009-2797(00)00186-1

Haskard, C.A., El-Nezami, H. S., Kankaanpaa, P. E., Salminen, S., & Ahokas, J. T. (2001) Surface binding of aflatoxin B1 by lactic acid bacteria. Applied and Environmental Microbiology, 67, 3086–3091. https://doi.org/10.1128/AEM.67.7.3086-3091.2001.

Heshmati, A. & Nejad, A. S. M. (2015). Ochratoxin A in dried grapes in Hamadan province, Iran. Food Additives and Contaminants: Part B Surveillance, 8, 255–259. https://doi.org/10.1080/19393210.2015.1074945

International Agency for Research on Cancer (IARC); World Health Organization (WHO). (1993). Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* (pp. 56). IARC, Lyon, France.

Kho, K. & Meredith, T. C. (2018). Extraction and analysis of bacterial teichoic acids. Bio-protocol, 8(21): e3078. https://doi.org/10.21769/BioProtoc.3078

Kumar, P., Mahato, D. K., Sharma, B., Borah, R., Haque, S., Mahmud, M. M. C., et al. (2020). Ochratoxins in food and feed: Occurrence and its impact on human health and management strategies. Toxicon, 187, 151–162. https://doi.org/10.1016/j.toxicon.2020.08.031

Leonardo, Petruzzi, Antonietta, Baiano, Antonio, De, et al. (2015). Differential adsorption of ochratoxin a and anthocyanins by inactivated yeasts and yeast cell walls during simulation of wine aging. Toxins, 26, 4350–4365. https://doi.org/10.3390/toxins7104350.

Malganji, S., Sohrabvandi, S., Jahadi, M., Nematollahi, A. & Sarmadi, B. (2016). Effect of refrigerated storage on sensory properties and viability of probiotic in grape drink. Applied Food Biotechnology, 3, 59–62. https://doi.org/10.22037/afb.v3i1.10544

Marino-Repizo, L., Gargantini, R., Manzano, H., Raba, J. & Cerutti, S. (2017). Assessment of ochratoxin A occurrence in Argentine red wines using a novel sensitive quechers-solid phase extraction approach prior to ultra high performance

liquid chromatography-tandem mass spectrometry methodology. Journal of the Science of Food and Agriculture, 97(8), 2487–2497. https://doi.org/10.1002/jsfa.8065

Niderkorn, V., Morgavi, D. P., Aboab, B., Lemaire, M., & Boudra, H. (2009). Cell wall component and mycotoxin moieties involved in the binding of fumonisin B1 and B2 by lactic acid bacteria. Journal of Applied Microbiology, 106, 977–985.

Palmira, Bellis, D., Mariana, Tristezza, Miriam, & Haidukowski, et al. (2015). Biodegradation of Ochratoxin A by bacterial strains isolated from vineyard soils. Toxins, 7, 5079–5093. https://doi.org/10.3390/toxins7124864.

Palumbo, J. D., O'Keeffe, T. L., Ho, Y. S. & Santillan, C. J. (2015). Occurrence of Ochratoxin A contamination and detection of ochratoxigenic *Aspergillus* species in retail samples of dried fruits and nuts. Journal of Food Protection, 78(4), 836–842. https://doi.org/10.4315/0362-028X.JFP-14-471

Patricia Casas-Junco, P., Raymundo Solis-Pacheco, J., Arturo Ragazzo-Sanchez, J., Rosa Aguilar-Uscanga, B., Ulises Bautista-Rosales, P. & Calderon-Santoyo, M. (2019). Cold plasma treatment as an alternative for Ochratoxin A detoxification and inhibition of mycotoxigenic fungi in roasted coffee. Toxins, 11, 337–345. https://doi.org/10.3390/toxins11060337

Pineli, L. d. L. d. O., Aguiar, L. A. D., Fiusa, A., Botelho, R. B. D. A. & Melo, L. (2016). Sensory impact of lowering sugar content in orange nectars to design healthier, low-sugar industrialized beverages. Appetite, 96, 239–244. https://doi.org/10.1016/j.appet.2015.09.028

Piotrowska, M. (2014). The adsorption of ochratoxin A by Lactobacillus species. Toxins, 6(9), 2826–2839. https://doi.org/10.3390/toxins6092826

Pittet, A. (1998). Natural occurrence of mycotoxins in foods and feeds - an updated review. Revue de Medecine Veterinaire, 149, 479–492.

Ratna, D. (2022). Chemistry and general applications of thermoset resins (Chapter 1). In Ratna, D. (Ed.), *Recent Advances and Applications of Thermoset Resins* (2nd ed.) (pp. 1–172), Elsevier.

Ringot, D. & Chango, A. (2009). Risk assessment of ochratoxin A (OTA). In: Rai, M., Varma, A. (eds) *Mycotoxins in Food, Feed and Bioweapons*. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-00725-5_18

Robles, A., Fabjanowicz, M., Chmiel, T. & Potka-Wasylka, J. (2018). Determination and identification of organic acids in wine samples. Problems and challenges. TrAC - Trends in Analytical Chemistry, 103, 21–33. https://doi.org/10.1016/j.trac.2018.03.006

Serra, R., Abrunhosa, L., Kozakiewicz, Z. & Venancio, A. (2003). Black *Aspergillus* species as ochratoxin A producers in Portuguese wine grapes. International Journal of Food Microbiology, 88(1), 63–68. https://doi.org/10.1016/s0168-1605(03)00085-0

Sheng, J., Shan, C., Liu, Y., Zhang, P., Li, J., Cai, W., et al. (2022). Comparative evaluation of the quality of red globe grape juice fermented by *Lactobacillus acidophilus* and *Lactobacillus plantarum*. International Journal of Food Science and Technology, 57(4), 2235–2248. https://doi.org/10.1111/ijfs.15568

Smaoui, S., Agriopoulou, S., D'Amore, T., Tavares, L., & Mousavi Khaneghah, A. (2023). The control of *Fusarium* growth and decontamination of produced mycotoxins by lactic acid bacteria. Critical Reviews in Food Science and Nutrition, 63(32), 11125–11152. https://doi.org/10.1080/10408398.2022.2087594

Punia Bangar, S., Sharma, N., Bhardwaj, A., & Phimolsiripol, Y. (2022) Lactic acid bacteria: A bio-green preservative against mycotoxins for food safety and shelf-life extension. Quality Assurance and Safety of Crops & Foods, 14, 13–31. https://doi.org/10.15586/qas.v14i2.1014

Stefanaki, I., Foufa, E., Tsatsou-Dritsa, A. & Dais, P. (2003). Ochratoxin A concentrations in Greek domestic wines and dried vine fruits. Food Additives and Contaminants, 20(1), 74–83. https://doi.org/10.1080/0265203021000031537

Tahmassebi, J. F. & BaniHani, A. (2020). Impact of soft drinks to health and economy: a critical review. European Journal of Paediatric Dentistry, 21, 109–117. https://doi.org/10.1007/s40368-019-00458-0

Wang, L., Yue, T., Yuan, Y., Wang, Z., Ye, M. & Cal, R. (2015). A new insight into the adsorption mechanism of patulin by the heat-inactive lactic acid bacteria cells. Food Control, 50, 104–110. https://doi.org/10.1016/j.foodcont.2014.08.041

Wei, W., Liu, C., Ke, P., Chen, X., Zhou, T., Xu, J., et al. (2021). Toxicological and physiological effects of successive exposure to ochratoxin A at food regulatory limits. Food and Chemical Toxicology, 151, 112128. https://doi.org/10.1016/j.fct.2021.112128

Wu, B., Liu, J., Yang, W., Zhang, Q. & Jiao, Z. (2021). Nutritional and flavor properties of grape juice as affected by fermentation with lactic acid bacteria. International Journal of Food Properties, 24, 906–922. https://doi.org/10.1080/10942912.2021.1942041

Wu, L., Zhang, H., Hu, X., Zhang, Y., Sun, L., Li, W., et al. (2020). Deacetylation of 3-acetyl-deoxynivalenol in wheat flour is mediated by water-soluble proteins during the making of Chinese steamed bread. Food Chemistry, 303, 125341. https://doi.org/10.1016/j.foodchem.2019.125341

Yang, Q., Wang, J., Zhang, H., Li, C. & Zhang, X. (2016). Ochratoxin A is degraded by Yarrowia lipolytica and generates non-toxic degradation products. World Mycotoxin Journal, 9, 269–278. https://doi.org/10.3920/WMJ2015.1911

Zhang, H. Y., Apaliya, M. T., Mahunu, G. K., Chen, L., & Li, W. (2016). Control of ochratoxin A-producing fungi in grape berry by microbial antagonists: a review. Trends in Food Science and Technology, 51, 88–97. https://doi.org/10.1016/j.tifs.2016.03.012

Zheng, X., Xia, F., Li, J., Zheng, L., Rao, S., & Gao, L., et al. (2023). Reduction of ochratoxin A from contaminated food by *Lactobacillus rhamnosus* Bm01. Food Control, 143, 109315. https://doi.org/10.1016/j.foodcont.2022.109315

Zoghi, A., Khosravi-Darani, K. & Sohrabvandi, S. (2014). Surface Binding of Toxins and Heavy Metals by Probiotics. Mini-Reviews in Medicinal Chemistry, 14(1), 84–98. https://doi.org/10.2174/1389557513666131211105554

Zurga, P., Vahcic, N., Paskovic, I., Banovic, M. & Staver, M. M. (2019). Occurence of Ochratoxin A and biogenic amines in croatian commercial red wines. Foods, 8, 348–353. https://doi.org/10.3390/foods8080348