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Adsorption and Biodegradation of Ochratoxin A by *Lacticaseibacillus rhamnosus B1* Isolated from Algerian Dairy Products

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ABSTRACT

Background: This study evaluates the detoxification potential of *Lacticaseibacillus rhamnosus* B1, isolated from artisanal ewe milk butter in Laghouat, Algeria, against ochratoxin A (OTA) through both degradation and adsorption mechanisms.

Methods: API 50 CHL carbohydrate fermentation profiling and 16S rRNA gene sequencing were used to identify the strain studied in this work. HPLC with C18 reversed-phase column was used to analyze OTA.

Result: The isolate was confirmed as *Lacticaseibacillus rhamnosus* B1 (GenBank accession number PV577804). In degradation assays, *L. rhamnosus* B1 reduced OTA by 25% after 7 days at 37°C in MRS broth (1 µg/ml OTA), producing 0.250 µg/ml of the less toxic ochratoxin alpha (OTα), as confirmed by HPLC analysis (λexc = 333 nm, λem = 460 nm). Adsorption experiments revealed that dead cells adsorbed 80% of OTA (0.800 µg/ml in pellet) at pH 3.5 after 1 hour, while live cells achieved a 50% adsorption rate (0.500 µg/ml in pellet) under identical conditions. HPLC chromatograms highlighted OTA peaks at ~6.9 minutes, with minor peaks at ~2 minutes, suggesting the presence of impurities. The stability of OTA binding to bacterial biomass was found to be pH-dependent, with acidic conditions enhancing adsorption, possibly due to interactions with cell wall components such as peptidoglycans. These findings demonstrate the dual role of *L. rhamnosus* B1 in OTA detoxification and support its potential application in mycotoxin mitigation strategies for food safety.

Key words: Biological detoxification, Dairy products, HPLC analysis, *Lacticaseibacillus rhamnosus,* Ochratoxin A.

INTRODUCTION

Lactic acid bacteria (LAB) are widely recognised as beneficial microorganisms, extensively studied for their probiotic properties and their central role in the fermentation of dairy, meat, fish and vegetable products (Razni *et al.*, 2024 ; Dahou *et al.*, 2024 ; Radja *et al.*, 2022). Beyond their established contributions to food quality and safety, recent research has highlighted the capacity of certain LAB strains to mitigate the risks posed by mycotoxins, Dangerous secondary metabolites generated by fungi that often taint agricultural products (Zhang and Tang, 2024; Salminen *et al.*, 2010; Dalié *et al.*, 2010; Abrunhosa *et al.*, 2010; Shetty and Jespersen, 2006). Mycotoxins including aflatoxins, ochratoxin A (OTA) and trichothecenes are especially worrisome because they can cause cancer, genetic mutations, nerve damage and suppress the immune system (Bennett and Klich, 2003). Reducing mycotoxin levels in food and feed remains a global priority; however, conventional physical and chemical detoxification methods often have limitations in terms of efficacy and safety (EFSA, 2009). Consequently, biological approaches-especially those employing LAB-are gaining traction as sustainable alternatives. LAB can detoxify mycotoxins through adsorption to cell wall components such as exopoly saccharides and peptidoglycans, as well as through enzymatic degradation, resulting in less toxic metabolites (El-Nezami *et al.*, 1998; Fuchs *et al.*, 2008; Dalié *et al.*, 2010; Piotrowska, 2014). Several studies have demon strated that LAB strains, including *Lacticaseibacillus rhamnosus*, can achieve OTA reduction rates of up to 97% *in vitro*, with both adsorption and enzymatic hydrolysis to

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ochratoxin α (OTα) and phenylalanine contributing to detoxification (Luz *et al.*, 2018; Badji *et al.*, 2023). Ochratoxin A (OTA), which is produced by species of *Penicillium and Aspergillus*, is a widespread contaminant found in cereals, coffee, wine and various other foods and is considered a possible carcinogen for humans (Jørgensen, 2005; Bennett and Klich, 2003). Hydrolysis of OTA's amide bond, whether by microbial enzymes or during gastrointestinal digestion, yields OTα, a non-toxic metabolite with a much shorter half-life (Li *et al.*, 1997).

Within this framework, the current study examines the capacity of *Lacticaseibacillus rhamnosus*, isolated from Algerian ewe milk butter, to bind and biodegrade OTA. Thus, it contributes to the development of effective biological strategies for mycotoxin detoxification in food systems.

MATERIALS AND METHODS

All experiments were carried out at the Laboratory of Experimental Toxicology, BioDePollution and Phyto Remediation, SNV Faculty, Oran 1 University (Algeria), during the period from December 2023 to April 2025.

Bacterial strain

Isolation and adsorption potential of *Lacticaseibacillus rhamnosus* B1

Lacticaseibacillus rhamnosus B1 was isolated from artisanal butter made from ewe milk sourced from local producers in Laghouat, Algeria (33°48′N, 2°52′E). The butter was traditionally produced through spontaneous fermentation at ambient temperature for 48-96 hours, following the method described by Wouters *et al.* (2002). The strain was isolated using the protocol of Piotrowska *et al.* (2014) and was selected based on its superior OTA adsorption performance.

Characterisation and Identification of *Lacticaseibacillus rhamnosus* B1

Lacticaseibacillus rhamnosus B1 was characterised through macroscopic and microscopic examinations of colony morphology and cellular structure, as well as Gram staining and catalase activity tests. Its identity was confirmed by assessing its carbohydrate fermentation profile and genetic sequencing.

Carbohydrate fermentation profile

The carbohydrate fermentation capability of *Lacticaseibacillus rhamnosus* B1 was analysed using the API 50 CHL system (Biomerieux, France). Following the manufacturer's guidelines, ten colonies from a 48-hour fresh culture grown on MRS agar at 37°C were suspended in 10 ml of API 50 CHL medium. Subsequently, 100 µL of the suspension was dispensed into 49 wells containing a unique carbohydrate substrate, sealed with paraffin oil and incubated at 37°C for 48 hours in a humid environment. Fermentation outcomes were recorded after 24 and 48 hours and the strain's identity was confirmed using the APIwebTM software with the V5.1 database.

Molecular identification

Genomic DNA extraction

Genomic DNA was isolated with the Promega Genomic DNA Extraction kit, adhering to the manufacturer's instructions to achieve effective cell lysis and optimal DNA quality. The extracted DNA's concentration and purity were measured with a NanoDrop spectrophotometer (Thermo Scientific, USA), which measured the absorbance at 260/280 nm to confirm suitability for downstream applications. The purified DNA served as a template for amplification of the bacterial 16S rRNA gene using universal primers 27F and 1492R, which are widely used for nearly full-length amplification of the 16S rRNA gene in lactic acid bacteria, providing high specificity and coverage (Lane, 1991).

PCR amplification

PCR amplification was performed in a Biorad thermocycler (Biorad, USA) under the following cycling conditions: an initial denaturation at 94°C for 5 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds and extension at 72°C for 90 seconds; followed by a final elongation at 72°C for 10 minutes (Altschul *et al.*, 1997). The resulting PCR products were sequenced and the obtained sequences were compared to reference 16S rRNA gene sequences in the GenBank database (NCBI) using BLAST analysis for species identification. This method, recognised for its accuracy in characterising lactic acid bacteria at the genus and species levels (Altschul *et al.*, 1997), was employed.

Chemicals

Standard OTA and OTα (purity>98%) were obtained from Sigma (St. Louis, MO) and a stock solution was prepared in toluene/acetic acid (99:1, v/v). All additional chemicals used were of analytical grade. Solutions were freshly prepared with sterile distilled water and kept at 4°C until needed.

Biodegradation of ochratoxin A by *Lacticaseibacillus rhamnosus* B1

To assess the biodegradation capacity of lactic acid bacteria (LAB) for ochratoxin A (OTA), an MRS broth medium supplemented with 1.0 µg/ml OTA was prepared. A stock solution of OTA (25 µg/ml) was prepared using a commercial standard (O1877, Sigma) dissolved in a toluene/acetic acid mixture (99:1, v/v) and stored at -20°C until use. The required volume of this stock solution was mixed with MRS broth (Oxoid) in flasks, followed by autoclaving at 121°C for 15 minutes and subsequent cooling. Aliquots of 5 ml were then aseptically transferred into sterile 15 ml conical tubes. Each tube containing MRS-OTA was inoculated in triplicate with 0.1 ml of a bacterial inoculum of *Lacticaseibacillus rhamnosus* B1 at a concentration of 10^9 CFU/ml. Negative controls were established in triplicate by adding 0.1 ml of sterile MRS broth instead of the inoculum. The tubes were incubated at 37°C for 7 days. After incubation, the culture medium and

bacterial cells were extracted by adding 5 ml of acetonitrile/acetic acid (99:1, v/v) to each tube, followed by vortexing for 1 minute. A 2 ml sample from each tube was filtered using microfilters with 0.45 µm pores (Merck) and stored at 4°C until analysed by high-performance liquid chromatography (HPLC) (Abrunhosa and Venâncio, 2007).

Adsorption of ochratoxin a by inactivated cells of *Lacticaseibacillus rhamnosus* B1

To investigate the potential adsorption of Ochratoxin A onto the cell walls of *Lacticaseibacillus rhamnosus* B1, inactivated (dead) cells were utilised. The bacterial inoculum was prepared as described previously and then autoclaved at 121°C for 15 minutes to inactivate the cells. In triplicate, 5 ml of MRS-OTA (1.0 µg/ml OTA) was combined with the inactivated cells to achieve a final concentration of 10^9 CFU/ml, followed by vortexing for 30 seconds. The mixture was vortexed intermittently every 15 minutes for 1 hour. Subsequently, the tubes were centrifuged at 9000 × g for 20 minutes and the supernatant was collected and filtered through syringe-fitted polypropylene (PP) filters with 0.2 µm pores (Merck). OTA was extracted from the supernatant using the method described earlier. OTA extraction was performed on the cell pellets by adding 2 ml of acetonitrile/water/acetic acid (99:1:0.1, v/v/v), vortexing for 1 minute and then filtering the extract into clean glass vials. The samples were stored at 4°C until HPLC analysis (Abrunhosa *et al.*, 2014; Abrunhosa and Venâncio, 2007).

Stability assessment of OTA binding to *Lacticaseibacillus rhamnosus* B1 biomass

The stability of OTA binding to *Lacticaseibacillus rhamnosus* B1 biomass was evaluated under varying ph conditions (3, 5, 7 and 8.5) and a temperature of 37°C. After the initial OTA binding phase, the bacterial pellet was resuspended in 5 mL of phosphate-buffered saline (PBS) adjusted to the specified pH values and incubated at 37°C for 1 hour. Following incubation, the mixture was centrifuged and the supernatant was collected for analysis using chloroform as solvent. The OTA concentration in the supernatant was quantified using HPLC, following established protocols (El-Nezami *et al.*, 1998; Piotrowska and Zakowska, 2005; Piotrowska *et al.*, 2014).

High-performance liquid chromatography (HPLC) analysis

The samples were analysed using HPLC with fluorescence detection, adapted from the method described by Abrunhosa and Venâncio (2007). The HPLC system was equipped with a Varian Prostar 210 pump, a Varian Prostar 410 autosampler and a Jasco FP-920 fluorescence detector, which was set to excitation and emission wavelengths of 333 nm and 460 nm, respectively, with a gain of 100. Chromatographic data were processed using a Varian 850-MIB data system interface and a Galaxie chromatography data system. Separation was achieved on a C18 reversed-phase YMC-Pack ODS-AQ column (250 × 4.6 mm I.D., 5 µm), equipped with a matching pre-column. An isocratic elution was performed at a flow rate of 0.8 ml/min for 21 minutes, with an injection volume of 50 µl. The mobile phase, composed of acetonitrile, water and acetic acid (99:1:0.2, v/v/v), was filtered and degassed using a 0.2 µm membrane filter (GHP, Gelman). OTA and OTα were identified by comparing their retention times to standards, with approximate retention times of 19.8 minutes for OTA and 7.5 minutes for OTα. Calibration standards (0.5, 1.0, 2.5, 5.0 and 7.5 µg/mL) were prepared by diluting a 25 µg/mL OTA stock solution and a calibration curve was constructed and routinely verified for accuracy. OTA was quantified by measuring peak areas and comparing them to the calibration curve, while OTα was quantified in OTA equivalents (Abrunhosa and Venâncio, 2007).

RESULTS AND DISCUSSION

Identification

Lacticaseibacillus rhamnosus B1 exhibited a distinctive carbohydrate fermentation profile using the API 50 CHL system (Biomerieux), showing positive acidification for glucose, galactose, lactose, maltose, mannose, saccharose, trehalose, esculine, amygdaline and rhamnose, while remaining negative for arabinose, xylose, sorbitol, inulin, melibiose, raffinose and glycerol. This homofermentative strain, isolated from traditional ewe milk butter in Laghouat, Algeria, was selected for its mycotoxin-binding capabilities. Taxonomic identification was confirmed through Gram-positive staining, catalase negativity and growth optimisation at 37°C and pH 5.5. Molecular analysis *via* 16S rRNA sequencing (GenBank accession number PV577804) aligned with the APIwebTM database (V5.1) profiles for *L. rhamnosus*, resolving ambiguities inherent to phenotypic methods in the closely related *L. casei* group (Altschul *et al.*, 1997; Lane, 1991). The strain demonstrated environmental robustness, tolerating 6.5% NaCl and exhibiting acidogenic metabolism, with rapid pH reduction in dairy matrices.

Degradation of OTA by *Lacticaseibacillus rhamnosus* B1

The HPLC analysis revealed two distinct peaks in the chromatogram corresponding to the degradation products of ochratoxin A (OTA) by *Lacticaseibacillus rhamnosus B1* after a 7-day incubation at 37°C in MRS medium supplemented with one µg/ml OTA (Table 1). The concentration of OTA decreased from the initial one µg/ml to 0.750 µg/ml. At the same time, a new peak corresponding to Ochratoxin alpha (OTα) appeared at 0.250 µg/ml,

Table 1: The degradation of OTA by *Lacticaseibacillus rhamnosus* cultivated in MRS media supplemented with 1 µg of OTA/ml for 7 days at 37°C.

Strain	OTA µg/ml	OTα µg/ml	Elimination rate (%)
Lacticaseibacillus rhamnosus	0.750	0.250	25 (%)

indicating a 25% elimination of OTA. The larger peak observed at approximately 3 minutes retention time corresponds to the residual OTA (0.750 µg/ml), whereas the smaller peak at around 2 minutes is attributed to OTα (0.250 µg/ml) (Fig 1). This 3:1 peak area ratio confirms the enzymatic degradation of Ochratoxin A into the less toxic metabolite OTα by *L. rhamnosus*.

Adsorption of OTA by *Lacticaseibacillus rhamnosus B1* dead cells

The HPLC results demonstrated that dead cells of *Lacticaseibacillus rhamnosus B1* incubated at pH 3.5 for 1 hour with 1 µg/ml ochratoxin A (OTA) achieved a high adsorption rate, with OTA in the supernatant reduced to 0.200 µg/ml and 0.800 µg/ml detected in the pellet, corresponding to 80% adsorption (Table 2). The chromatogram displayed a significant peak at approximately 6.9 minutes, representing the OTA remaining in the supernatant and a minor peak at around 2 minutes, likely due to a small impurity or degradation product (Fig 2).

Stability of toxin binding to the bacterial biomass

The complex formed between OTA and *Lacticaseibacillus rhamnosus* B1 was significantly stable, after five washes with water, 87% and 96% of the OTA remained bound and could be subsequently recovered by chloroform extraction. A wider range of pH (3, 5, 7 and 8.5) extraction solutions was used to study the stability of the *via*ble *Lacticaseibacillus rhamnosus* B1-OTA complex. Only 4 to 13% of bound OTA was released by chloroform from these complexes in water at pH 3, 5 and temperatures of 37°C (Fig 3).

The HPLC chromatogram revealed the adsorption of ochratoxin A (OTA) by live *Lacticaseibacillus rhamnosus* B1 cells incubated for 1 hour at 37°C in a ph 3.5 buffer containing 1 µg/ml OTA (Table 3). The OTA concentration in the supernatant was reduced to 0.500 µg/ml, with an equal amount (0.500 µg/ml) detected in the bacterial pellet, indicating a 50% adsorption rate. The chromatogram showed a prominent peak at approximately 6.9 minutes, corresponding to OTA remaining in the supernatant (peak area 590.642) and a smaller peak near 2 minutes, which may represent a minor impurity or trace degradation product (Fig 4). However, no significant production of ochratoxin alpha (OTá) was expected, as the focus was on adsorption by live cells rather than enzymatic degradation.

The strain isolated and genetically identified in this study as *Lacticaseibacillus rhamnosus* B1, exhibited physiological characteristics that demonstrate environmental robustness, combined with its adsorption efficiency for ochratoxin A, this makes it a promising candidate for the production of a probiotic ferment, especially in acidic and osmotically challenging food systems. These findings are consistent with the results reported by Ait Abdeslam *et al.* (2019) and Kouadri Boudjelthia *et al.* (2023).

The observed 25% degradation rate is consistent with previous findings by Piotrowska (2014), who reported a 30% reduction of OTA by *L. rhamnosus* under similar conditions. However, it is lower than the 40% OTA degradation reported by Fuchs *et al.* (2008) for *Lacticaseibacillus acidophilus* after 5 days, which may be explained by differences in enzymatic activity or the lower initial OTA concentration used (0.5 µg/ml). More studies provide additional context.

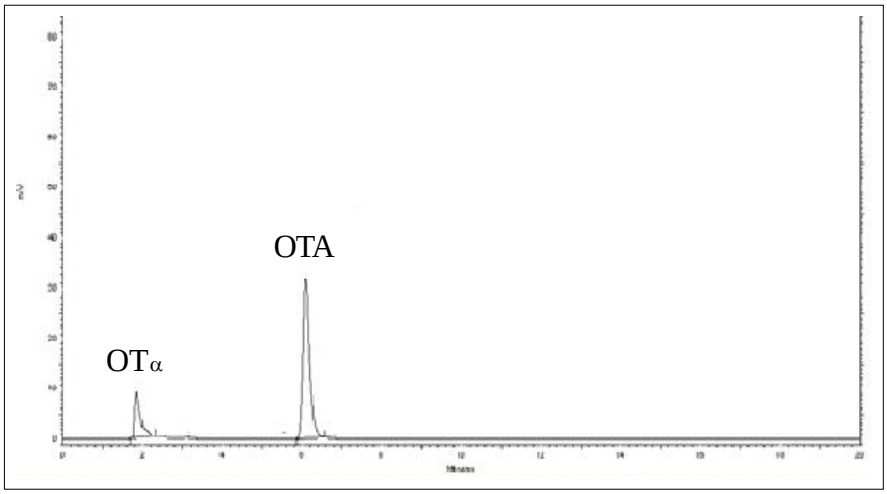

Fig 1: Degradation of OTA by *Lacticaseibacillus rhamnosus* B1 Strain: HPLC Chromatogram of Metabolite Profile at 254 nm.

Table 2: The adsorption of OTA by dead cells of *Lacticaseibacillus rhamnosus* B1 incubated in pH 3.5 buffer supplemented with 1 µg of OTA/mL for 1 hour at 37°C.

Strain	OTA in supernatant	OTA in pellet	Rate of adsorption (%)
Lacticaseibacillus rhamnosus	0.200 µg/ml	0.800 µg/ml	80%

In vitro studies demonstrated that *Lactobacillus rhamnosus GG* (10^{10} CFU/mL) achieved 35% ochratoxin A adsorption after 7 days under optimized acidic conditions (pH 5.0-5.5). This efficiency was attributed to enhanced bacterial cell wall binding at lower pH (*Abrunhosa et al., 2010*). Similarly, Mazurkiewicz (2011) reported a 45% OTA reduction by *Lactiplantibacillus plantarum*, which increased to 60% when co-cultured with other strains, suggesting that factors such as pH adjustment, inoculum size, or co-culturing could enhance OTA biodegradation by *L. rhamnosus.* The authors Zheng *et al.*, (2023), examined a LAB strain, namely *L. rhamnosus* Bm01, which showed an ability to eliminate 83.58% of OTA (50 ng/mL) in 48 h.

These findings are consistent with other studies reporting substantial OTA adsorption by lactic acid bacteria, particularly in the presence of dead or inactivated cells. For example, the GG strain of *L. rhamnosus* has been shown to adsorb more than 60% of OTA under similar *in vitro* conditions, with dead bacterial cells generally exhibiting greater adsorption efficiency than live cells due to enhanced exposure of cell wall binding sites (Bejaoui *et al.*, 2004; Damoon Ghofrani *et al.*, 2018; Chen *et al.*, 2018;).

Table 3: The adsorption of OTA by live cells of *Lacticaseibacillus rhamnosus* incubated in ph 3.5 buffer supplemented with 1 µg of OTA/ml for 1 hour at 37°C.

Strain	OTA in supernatant	OTA in pellet	Rate of adsorption (%)
Lacticaseibacillus rhamnosus B1	0.500 µg/ml	0.500 µg/ml	50%

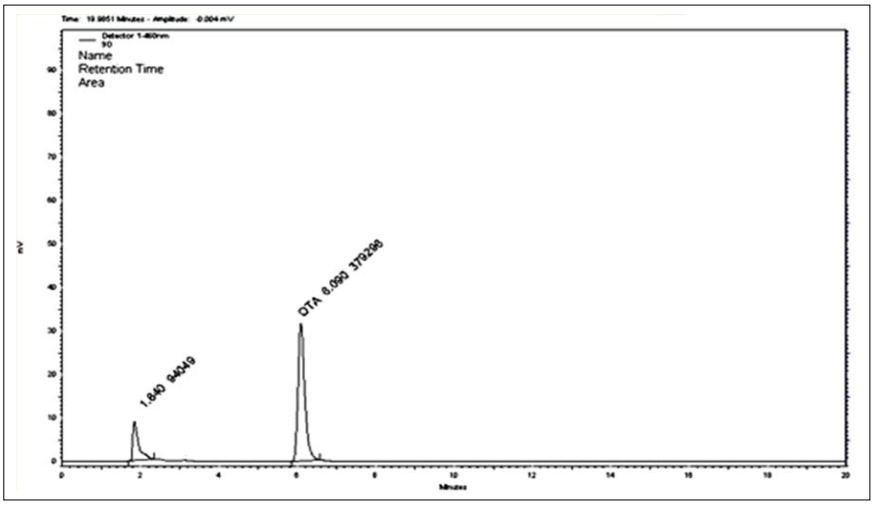

Fig 2: HPLC Chromatogram of OTA Degradation Product by *Lacticaseibacillus rhamnosus* B1 Strain at 140 nm.

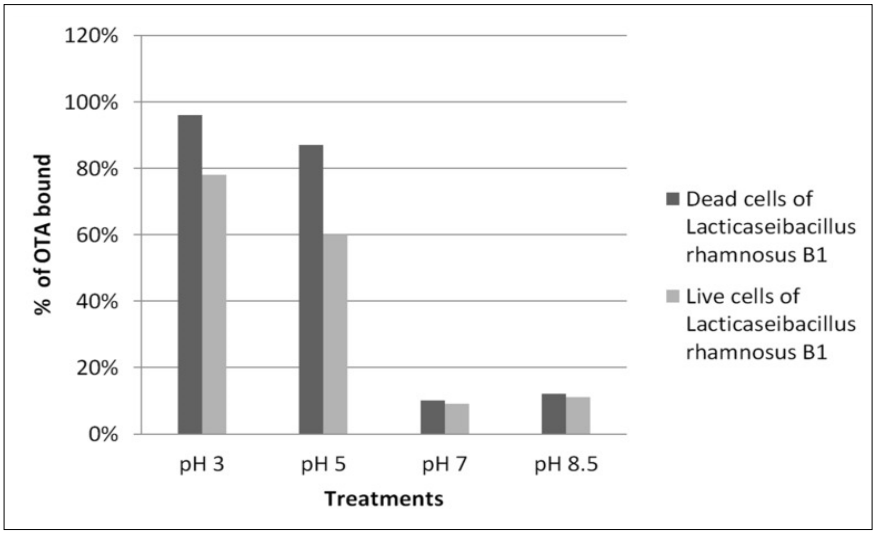

Fig 3: Ochratoxin A Percentage bound to *L. rhamnosus* B1 released on incubation at a range of pHs and temperature 37 with a chloroform extraction.

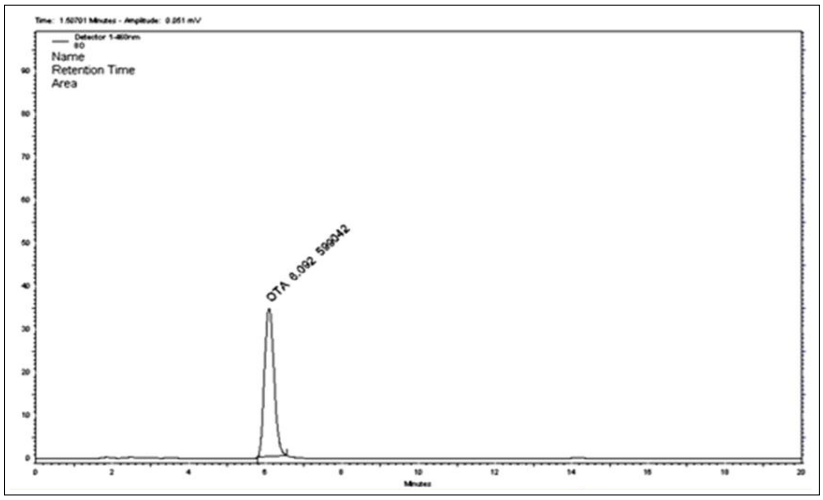

Fig 4: HPLC Chromatogram of OTA adsorption by live cells of *Lacticaseibacillus rhamnosus* B1 Strain.

The primary mechanism for OTA removal is physical adsorption onto cell wall components such as peptidoglycans and polysaccharides, especially at acidic pH, where OTA's protonated form increases binding affinity (Ding *et al.*, 2023). These results confirm that dead *L. rhamnosus* cells are effective bioadsorbents for OTA, supporting their potential application to detoxify acidic food matrices.

This moderate adsorption capacity suggests that live *L. rhamnosus* cells bind OTA primarily through interactions with cell wall components such as peptidoglycans and exopolysaccharides. The acidic pH of 3.5 likely enhances this binding by increasing the proportion of OTA in its protonated form, which has a higher affinity for the negatively charged bacterial surface.

These findings are consistent with previous reports. Damoon Ghofrani *et al.* (2018) observed a 40.55% OTA adsorption rate by live *L. rhamnosus* GG at pH 3.0, with dead cells showing higher efficiency (61.43%) due to enhanced exposure of cell wall components. Similarly, Luz *et al.* (2018) reported that *Lactiplantibacillus plantarum* CECT 749 reduced OTA level by 95% under comparable acidic conditions (pH 3.5), highlighting strain-specific differences in cell wall composition and binding mechanisms. In contrast earlier work by Piotrowska (2014) documented lower adsorption rates (30-35%) by live *L. Rhamnosus* under neutral pH environments (pH ~6.5), where enzymatic degradation pathways tend to dominate over physical adsorption.

The HPLC method with detection at 460 nm proved effective for OTA quantification, however, the minor peak observed at approximately 2 minutes requires further analysis to rule out the presence of degradation products such as ochratoxin alpha (OTα), which would indicate metabolic activity beyond adsorption.

CONCLUSION

This study demonstrates the efficacy of *Lacticaseibacillus rhamnosus* B1, isolated from Algerian artisanal ewe milk butter, in detoxifying ochratoxin A (OTA) through biodegradation and adsorption mechanisms. The strain exhibited a 25% OTA degradation rate after 7 days at 37°C, converting OTA into the less toxic ochratoxin alpha (OTα), as confirmed by HPLC analysis. Additionally, adsorption experiments revealed that dead cells of *L. rhamnosus* B1 achieved an impressive 80% OTA removal rate at pH 3.5 within 1 hour. In comparison, live cells adsorbed 50% under identical conditions, highlighting the role of cell wall components, such as peptidoglycans, in OTA binding, particularly under acidic conditions. These findings underscore the dual detoxification potential of *L. rhamnosus* B1, making it a promising candidate for mitigating OTA contamination in dairy products and other acidic food matrices. Further research is needed to optimise degradation efficiency by investigating factors such as pH, inoculum density and co-culturing, as well as to explore the practical application of this strain in food safety protocols.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data

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