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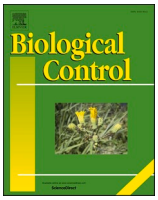

In vitro detoxification of aflatoxin B1 and ochratoxin A by lactic acid bacteria isolated from Algerian fermented foods

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HIGHLIGHTS

- New LAB isolated from Algerian foods showed *in vitro* AFB1 and OTA removal ability.
- Simultaneous removal of AFB1 and OTA by the new LAB was evidenced.
- The removal ability of Lab-L4/al and LP R1096 strains was pH dependent.
- Nonviable LAB cells showed higher AFB1 and OTA removal ability than viable cells.
- AFB1 and OTA reduction was due to binding mechanism by LAB strains.

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ABSTRACT

Wheat occupies the first place in Algerian's diet. However, because of the risk of mycotoxin contamination, its consumption can cause serious health concern to Algerian population. This study aimed to determine the *in vitro* detoxification properties of lactic acid bacteria (LAB) strains toward aflatoxin B1 (AFB1) and ochratoxin A (OTA), the most prevalent mycotoxins in wheat and derived products in Algeria. Eleven LAB strains isolated from Algerian fermented foods were characterized and identified using API 50 CHL and 16S rDNA sequencing methods. In order to study the ability of LAB strains to remove AFB1 and OTA, viable and heat inactivated cells of two LAB strains (Lab-L4/al and Lab-L1) selected among the eleven isolated and identified ones, as well as of the reference strain *Lactobacillus plantarum* (LP) R1096 were incubated individually in Citrate Phosphate Buffer (CPB 0.1 M, pH 6 or 5) containing AFB1 and/or OTA at a concentration each of 40 ng/mL for 24 h at 25 °C. Free mycotoxin concentrations were analyzed by HPLC-FLD. The isolated LAB strains were identified as *Lactococcus lactis* ssp l*actis* 1, *Lactococcus lactis* ssp *lactis* 2 and *Lactobacillus paracasei* ssp *paracasei* using API 50 CHL kit, whereas with the molecular method, the strains were identified as *Enterococcus faecium* and *Enterococcus durans*. Both viable and nonviable cells of Lab-L4/al and Lab-L1, as well as LP R1096, were able to remove AFB1 and OTA, with efficiency varying between the strains and higher for AFB1 with nonviable cells (>50 %). Removal ability of viable Lab-L4/al and LP R1096 cells increased with a decrease in pH from 6 to 5, while pH had no effect on the amounts of mycotoxins removed by viable Lab-L1 cells. In conclusion, the tested LAB strains were able to reduce the amounts of AFB1 and OTA *in vitro* conditions. This result suggests that these LAB strains could be used as additives or formative agents to reduce mycotoxin levels in fermented wheat foods such as sourdough bread.

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1. Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi mainly those belonging to *Aspergillus*, *Penicillium* and *Fusarium* genera (Paterson and Russell, 2006; Datsugwai et al., 2013). They can contaminate agricultural products during plant growth, through harvesting and/or during storage if the conditions are suitable for fungal activity (Ismaiel & Papenbrock, 2015; Tola & Kebede, 2016), as well as derived food products due to their thermal and chemical stability (Raters & Matissek, 2008; Kabak, 2009; Marcin et al., 2017). In addition, mycotoxins are often present as mixtures in food, therefore, human are exposed to more than one mycotoxin simultaneously (Battilani et al., 2020).

In Algeria, cereal production is estimated at 3.5 million tons in 2021. Wheat occupies the first position with a national production of 2.5 million tons in 2021 (FAO, 2022) and it is considered as the major staple food for Algerian people. This cereal is used to produce several traditional products in Algeria such as flat bread, pasta, couscous, frik and various types of traditional cakes (Chemache et al., 2018; Kezih et al., 2016). Numerous studies have reported the incidence of mycotoxins, especially OTA and AFB1, in Algerian wheat grains and derived products, at levels that may exceed the limits set out in the Commission Regulation (EC) No 1881/2006: 2 μg/kg for AFB1 in cereals and derived products; 5 μg/kg and 3 μg/kg for OTA respectively in unprocessed cereals and derived products (European Commission, 2006). The occurrence of OTA was reported for the first time by Riba et al. (2008), in 40 % of wheat samples (wheat, flour, semolina and bran) at a range varying between 0.21 and 41.55 μg/kg, with the highest concentration detected in flour samples. A survey conducted by Zebiri et al. (2019) showed that 69.2 % of wheat grain samples were positive for OTA (0.21–27.31 μg/kg). OTA was also detected in semolina and flour at concentration ranging from 0.16 to 34.75 μg/kg. The incidence of AFB1 in Algerian wheat was reported by Riba et al. (2010) who found that 56.6 % of wheat samples and derived products (flour, semolina and bran) were contaminated with AFB1 in the range of 0.13 to 37.42 μg/kg.

Aflatoxins (AFs) and OTA are considered the potent mycotoxins that can pose a serious health concerns to human. AFB1 is categorized in Group I as proven carcinogen in human by the International Agency for Research on Cancer (IARC). Dietary exposure to this toxin increases the risk of developing hepatocellular carcinoma (Hamid & Tesfamariam, 2013). OTA is considered the most toxic family member with nephrotoxic potential (Heussner & Bingle, 2015) and is classified by IARC as a possible human carcinogen (group 2B) (Ostry et al., 2017). Subsequently to the health hazardous impact associated with these natural contaminants, chemical, physical and biological strategies have been developed to prevent mycotoxigenic fungal growth and toxin production, and to detoxify contaminated food (Daou et al., 2021; Ismail et al., 2018). Biological strategies based on the use of LAB are considered as promising approach. This is claimed to be safe, non-pathogenic for human and to maintain the nutritional value of food (Muhialdin et al., 2020; Perczak et al., 2018). LAB have been used for centuries in food preservation because of their antimicrobial proprieties (Saranraj et al., 2017), but only in the recent years their effects on the mycotoxigenic fungal growth and mycotoxin production have been investigated. They can be isolated from several food products such as dairy products (Eddine et al., 2018; Karaduman et al., 2017; Tulini et al., 2016), vegetables (Aydin & ÇebĬ, 2019; Bamidele et al., 2019; Kafando & Dicko, 2019), fruits (Gajbhiye & Kapadnis, 2018; Taroub et al., 2019), sourdough and cereals (Alfonzo et al., 2017; Belkacem-Hanfi et al., 2014; Djaaboub et al., 2018; Ispirli et al., 2018; Kharazian et al., 2017; Tulini et al., 2016), meat (Martí--Quijal et al., 2020; Phong et al., 2016) and most of them constitute the microbiota of the gastrointestinal tract of human and animals (Dicks & Botes, 2010; Shea et al., 2009). Three main mechanisms can be considered in the reduction of mycotoxins by LAB: inhibition of mycotoxin producing fungi and mycotoxin production, mycotoxin degradation and mycotoxin adsorption (Sadiq et al., 2019; Petrova et al., 2022). LAB have been identified as biocontrol agents against a wide range of mycotoxigenic fungi. Generally their biocontrol activity was ascribed to the production of low molecular weight compounds, such as organic acids, phenolic compounds, hydroxy fatty acids, hydrogen peroxide, reuterin and proteinaceous compounds (Blagojev et al., 2012; Dalié et al., 2010; Sadiq et al., 2019). LAB strains are also able to produce proteolytic enzymes that can play an important role in the biodegradation and detoxification process of mycotoxins (Abrunhosa et al., 2014; Wang & Xie, 2020). The adsorption ability of LAB cell wall was suggested as another mechanism for mycotoxin removal (Luo et al., 2020). Several LAB strains belonging to the genera *Lactobacillus*, *Lactococcus*, *Weissella*, *Enterococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* and *Bifidobacterium* are reported to be able to bind mycotoxins *in vitro* or *in vivo* (Ahlberg et al., 2015; Kavitake et al., 2020; Topcu et al., 2010). In order to elucidate the binding mechanism, Haskard et al. (2000), Hernandez-Mendoza et al. (2009), Lahtinen et al. (2004) and Niderkorn et al. (2009), have studied the role of LAB cell wall in mycotoxin removal and they have suggested that polysaccharides, peptidoglycans and teichoic acids are the important components that can be involved in mycotoxin binding. The binding process has been related to several factors such as initial concentration of mycotoxins, LAB strains, LAB cell number, pH, temperature and incubation time (Liu et al., 2020). According to Haskard et al. (2001) and Piotrowska. (2014) nonviable LAB showed higher mycotoxin binding ability than viable LAB. The heat inactivation of LAB cells induced morphological changes in the bacterial cell wall (protein denaturation and pore generation) leading to the increase of the number of active sites responsible for mycotoxin adsorption.

In Algeria, there are no available researches evaluating *in vitro* the efficiency of autochthonous LAB strains to remove mycotoxins. Therefore, this study aimed to characterize and identify LAB strains isolated from Algerian fermented foods and to evaluate their *in vitro* ability to reduce AFB1 and OTA using an experimental model that simulates bread fermentation conditions. The study focused on AFB1 and OTA because of their toxicity and their frequent occurrence in Algerian wheat and derived products at levels exceeding the European limits.

2. Material and methods

2.1. LAB strains

Eleven LAB strains, isolated from Algerian fermented foods, were used in this study. Five strains (Lab-L1/al, Lab-L2/al, Lab-L3/al, Lab-L4/al and Lab-L5/al) were isolated from fermented wheat “El Hammoum” according to the method described by Djaaboub et al. (2018). El-Hammoum was prepared from durum wheat (*Triticum durum*) stored and fermented for more than a year in an underground silo called “Matmour” located in Constantine (36° 17’ 00’’ Nord, 6° 37’ 00’’ Est. Algeria). Six strains (Lab-L1, Lab-L2, Lab-L3, Lab-L7; Lab-L8 and Lab-L9) were isolated from traditional fermented milk prepared from fresh milk that fermented spontaneously at room temperature during 24 to 72 h using the technique defined by Eddine et al. (2018). *Lactobacillus plantarum* (LP) R1096, Lallemand SAS, France, was used as reference strain. The LAB isolates and the reference strain were cultivated and stored at 4 °C on M17 agar (Biokar Diagnostics, France: tryptone 2.5 g/L, peptic digest of meat 2.5 g/L, papaic digest of soybean meal 5 g/L, yeast extract 2.5 g/L, meat extract 5 g/L, lactose 5 g/L, sodium glycerophosphate 19 g/L, magnesium sulfate 0.25 g/L, ascorbic acid 0.5 g/L and bacteriological agar 15 g/L, final pH 7.1 ± 0.2) slants and MRS agar (Biokar Diagnostics, France: enzymatic digest of casein 10 g/L, meat extract 10 g/L, yeast extract 4 g/L, glucose 20 g/L, tween 80 1.08 g/L, dipotassium phosphate 2 g/L, sodium acetate 5 g/L, ammonium citrate 2 g/L, magnesium sulfate 0.20 g/L, manganese sulfate 0.05 g/L and bacteriological agar 16 g/L, final pH 5.7 ± 0.1) slants, respectively. LAB strains were also preserved at −80 °C on microbeads (MAST Diagnostic, France) for long conservation.

2.2. Characterization and identification of LAB isolates

LAB strains were characterized through macroscopic and microscopic observation of colony and cell morphology, Gram staining and catalase activity. They were identified by evaluating their carbohydrate fermentation profile and gene sequencing.

2.2.1. Carbohydrate fermentation profile

The carbohydrate fermentation profile of LAB isolates was determined using API 50 CHL kit (Biomerieux, France). According to the manufacture's instructions, 10 colonies of fresh culture of each isolate on MRS agar at 30 °C for 48 h were immersed in 10 mL of API 50 CHL medium. Then 100 μL of each suspension were introduced in 49 wells containing different carbohydrate each, covered with paraffin oil and incubated at 30 °C for 48 h under humid atmosphere. Results were read after 24 h and 48 h of incubation, and LAB isolates were identified using the **apiweb™** identification software with database (V5.1).

2.2.2. Molecular identification of LAB

2.2.2.1. DNA extraction. Bacterial DNA was extracted using a cetyltrimethylammonium bromide (CTAB) method. LAB isolates were grown on MRS agar at 30 °C for 48 h. For each isolate, 10 colonies were introduced into 500 μL of sterile saline solution, homogenized by vortex for 2 min and centrifuged at 12,000 g for 10 min. The bacterial pellet was suspended in 100 μL of lysis buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8) and 100 μL of 25 mg/mL lysozyme, mixed with a micropipette and incubated at ambient temperature for 5 min. After adding 100 μL of 20 mg/mL proteinase K, the suspension was incubated at 42 °C for 20 min. Then 50 μL of 20 % sodium dodecyl sulfate (SDS) were added and the suspension was homogenized using micropipette and incubated for 5 min at ambient temperature then at 42 °C for 10 min. In order to remove cell wall debris, 400 μL of CTAB/NaCl solution (10 % Tris 1 M pH 8, 28 % NaCl 5 M, 4 % EDTA, 20 g CTAB) were added to the suspension, homogenized with micropipette and incubated at 65 °C for 10 min, then 700 μL of phenol–chloroform-isoamylalcohol (25:24:1,v/v/v) were added and the tubes were inverted quickly 10 times, then centrifuged at 12,000 g for 15 min. The aqueous supernatant was recovered in fresh micro-centrifuge tube and subjected to the previous step (x2). The recovered supernatant was mixed manually with 600 μL of chloroform-isoamylalcohol (24:1, v/v) and centrifuged at 12000*g* for 10 min, then 30 μL of sodium acetate (3 M, pH 5) and one volume of 100 % isopropanol were added in micro-centrifuge tubes. The solution was maintained 1 h at −20 °C and subsequently centrifuged at 12,000*g* for 30 min. The supernatant was eliminated and the DNA was washed with 500 μL of 70 % ethanol. After centrifugation at 12,000*g* for 5 min, the DNA pellet was dried in hood overnight, re-dissolved in 100 μL of sterile distilled water and stored at 4 °C. Bacterial DNA extraction was checked by visualizing DNA using an UV transilluminator (318 nm) after running on 0.8 % (w/v) agarose gel electrophoresis and staining with a GelRed® Nucleic Acid Gel Stain, 10,000X (Biotium, USA). Samples of 5 μL of extracted DNA and 3 μL of 1 Kb DNA ladder (Promega, USA) each mixed with 2 μL blue/orange loading dye 6X (Promega, USA) were run in Tris-Acetate-EDTA (TAE) 1X buffer pH 8.3 (Euromedex, France) at 100 V for 30 min.

2.2.2.2. DNA amplification. LAB isolates were identified by 16S rRNA gene sequence analysis. Bacterial DNA was amplified by the polymerase chain reaction (PCR) in an Eppendorf™ Mastercycler X50 thermocycler (Germany) using different primer pairs:

- Two primer pairs specific for LAB: Lac1 (5′-AGCAGTAGGGAATCTTCCA-3′) or Lac3 (5′-AGCAGTAGGGAATCTTCGG-3′) and reverse primer Lac2 (5′-ATTYCACCGCTACACATG- 3′) (Santos et al., 2011). The PCR mixture (50 μL) contained 5 μL extracted DNA, 10 μL PCR buffer for Taq polymerase (Promega, USA), 0.25 μL Taq polymerase (Promega, USA), 10 μL of each primer (10 μL/mL), 1 μL dNTP (Promega, USA) and 13.75 μL sterile water, and the program was as follows: initial denaturation temperature at 94 °C for 2 min, then 36 cycles of 30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C, and an extension step at 72 °C for 5 min.
- The universal bacterial primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-TACGGTTACCTTGTTACGACTT-3′) (Hou et al., 2018). PCR was performed in a final volume of 20 μL containing 1 μL extracted DNA, 10 μL of 2X Phusion Flash PCR Master Mix (Thermo Scientific, France), 1 μL of each primer (10 μL/mL) and 7 μL sterile water. DNA amplification was achieved using the following PCR program: initial denaturation temperature at 98 °C for 1 min, then 30 cycles of 15 s at 98 °C, 15 s at 60 °C, 25 s at 72 °C, and an extension step at 72 °C for 1 min. Final PCR products were loaded in 2 % (w/v) agarose gel electrophoresis, under the same conditions described in 2.2.2.1, using a 100 bp DNA ladder (Promega, USA), and stored at 4 °C until DNA sequencing. The amplicon length was approximately 340 bp using Lac1/Lac2 or Lac3/Lac2 primer pairs, and about 1400 bp using the primers 27f/1492r (Dos Santos et al., 2019).

2.2.2.3. Bacterial DNA amplicon sequencing. Amplified DNA fragments were sent for Sanger sequencing at the platform GenSeq UM (Génotypage-Séquençage Université Montpellier). The sequences were submitted to the GenBank NCBI (National Centre of Biotechnology Information) database for identification of LAB isolates using Blast (Basic Local Alignment search tool).

2.3. Preparation of mycotoxin working solutions

Standard solutions of AFB1 (TSL-104, 25 μg/mL) and OTA (TSL-504–5, 10 μg/mL) were purchased from Trilogy Analytical Laboratory, and stock mycotoxin solutions of 1 μg/mL were prepared for each toxin in amber vials and stored at 4 °C. To prepare the mycotoxin working solutions, AFB1 and/or OTA stock solution were placed under nitrogen evaporator (Reacti-Vap™, Model 18780) for 10 to 15 min at 45 °C to remove solvents, acetonitrile (AFB1) and/or methanol (OTA). Mycotoxins were suspended in citrate phosphate buffer (CPB) 0.1 M, pH 6 or pH 5 to make a final concentration of 40 ng/mL for each toxin.

2.4. Determination of the mycotoxin removal ability of LAB strains

The two LAB strains isolated from Algerian fermented foods, Lab-L4/al (fermented wheat El-Hammoum) and Lab-L1 (traditional fermented milk), and the reference strain LP R1096 were tested to determine their ability to remove AFB1and OTA, alone or in mixture, in CPB 0.1 M, pH 6. The methods described by Niderkorn et al. (2006a,b) and Dawlal et al. (2017) were used in this study with a few modifications. For each LAB strain, two Erlenmeyer flasks containing 200 mL of culture broth, M17 broth (Biokar Diagnostics, France: composed of the same ingredients than the M17 Agar without the agar) for Lab-L4/al and Lab-L1 or MRS broth (Biokar Diagnostics, France: polypeptone 10 g/L, meat extract, 10 g/L yeast extract 5 g/L, glucose 20 g/L, tween 80 1.08 g/L, dipotassium phosphate 2 g/L, sodium acetate 5 g/L, ammonium citrate 2 g/L, magnesium sulfate 0.2 g/L and manganese sulfate 0.05 g/L, final pH 6.4 ± 0.2) for LP R1096, were inoculated with a fresh, single colony from 48 h culture on agar respective medium, and incubated at optimal growth temperature (30 °C for Lab-L4/al and Lab-L1; 37 °C for LP R1096). After 24 h of incubation, the two broth cultures were homogenized and an aliquot of 2 mL was used for quantifying the LAB concentration. A standardized concentration of LAB cells (10^{10} CFU/mL) was used for all strains across all tests.

Thereafter, the two broth cultures were each aseptically transferred to a sterile 250 mL centrifuge bottle and centrifuged at 3000*g* for 10 min

at 4 °C. The supernatant was removed and the LAB cell pellet was re-suspended and washed three times with 100 mL of phosphate buffer solution (PBS, 0.01 M pH 7) by mixing, then centrifuging under the same conditions. After the last wash step, 4 mL of PBS 0.01 M, pH 7 were added to each bacterial pellet in order to obtain a LAB concentration of 10^{10} CFU/mL, and homogenized by vortex. Each of the two bacterial suspensions of 4 mL was transferred to four sterile Eppendorf tubes (1 mL per tube) and centrifuged at 3000*g* for 10 min. After eliminating the supernatant, in duplicate, the bacterial pellet was re-suspended in 1 mL CPB 0.1 M, pH 6 containing mycotoxins (40 ng/mL of AFB1, 40 ng/mL of OTA or mixture of AFB1 and OTA at 40 ng /mL each) (test samples). Negative control was prepared by re-suspending bacterial pellet in 1 mL of CPB 0.1 M, pH 6, and 1 mL of each mycotoxin working solution in CPB 0.1 M, pH 6 was used as positive control. The test samples and controls were incubated at 25 °C for 1 h with shaking (480 rev/min) and 23 h without shaking. Then the samples were centrifuged at 3000*g* for 10 min, and supernatants were transferred to amber vials and stored at −20 °C until mycotoxin analysis by High Performance Liquid Chromatography coupled with fluorescence detection (HPLC-FLD). Experiment was repeated in triplicate.

2.5. Determination of LAB concentration

LAB concentration was determined by measuring optical density (OD) of LAB culture broth at the wavelength of 600 nm, then comparing to standard curves previously obtained for each LAB strain by relating colony forming units (CFU) per mL from plate counts and OD measurements over a range of LAB concentrations.

2.6. Effect of pH on mycotoxin removal ability of LAB strains

The pH value of unfermented bread dough was 6.14 ± 0.21 (Požrl et al., 2009; Alba et al., 2017) then decreased with fermentation time to reach pH 5.1 ± 0.2 (Hadaegh et al., 2017; Mert et al., 2014). For that reason, the ability of LAB strains to remove AFB1 and OTA, alone or in mixture, was investigated not only at pH 6 but also at pH 5 in CPB 0.1 M using the method detailed in 2.4.

2.7. Effect of heat treatment of LAB cells on mycotoxin removal ability

In order to test the mycotoxin removal ability of nonviable LAB cells, after the washing step of LAB cells with PBS 0.01 M pH 7 (see method detailed in 2.4), the pellet of each strain was re-suspended in 4 mL of PBS 0.01 M, pH 7, homogenized by vortex and transferred to 2 mL Eppendorf tubes (1 mL per tube), than LAB cells were inactivated at 80 °C in water bath for 15 min. Subsequently, all samples were centrifuged at 3000*g* for 10 min and the supernatants were removed. Toxin solutions of AFB1 (40 ng/mL), OTA (40 ng/mL) and a mixture of AFB1 and OTA (40 ng/mL for each toxin) were prepared in CPB 0.1 M, pH 6. In duplicate, each pellet of nonviable bacteria (10^{10} CFU) was re-suspended in 1 mL of mycotoxin-buffered solution. Negative control was prepared by re-suspending nonviable bacterial pellet in 1 mL of CPB 0.1 M, pH 6 and 1 mL of each mycotoxin-buffered solution was used as positive control. Test samples and controls were incubated at 25 °C for 1 h under shaking (480 rev/min) and 23 h without shaking. After incubation time, tubes were centrifuged at 3000*g* for 10 min. Supernatants were transferred to amber vials and stored at −20 °C until mycotoxin analysis using HPLC-FLD. Experiment was repeated in triplicate.

2.8. Mycotoxin quantification

2.8.1. Afb1

AFB1 was quantified using HPLC-FLD (Shimadzu RF 20A, Japan) with post-column electrochemical derivatization (KobraCell™, R- Biopharm Rhône ltd., Glasgow, UK). Separation was performed using a C18 reverse-phase column (Uptisphere type, ODB, 5 µm particle size, 250x4.6 mm) with identical pre-column, thermostatically controlled at 40 °C in which sample volumes of 100 µL were injected, at a flow rate of 0.8 mL/min of the mobile phase which consisted of a mixture of water/methanol 55:45 (v/v) with 119 mg KBr 0.001 M and 350 µL of nitric acid 4 M. AFB1 detection was set at 360 nm excitation and 450 nm emission, and the retention time was approximately 22 min. Detection and quantification limits were established at 0.05 and 0.2 ng/mL, respectively. AFB1 concentrations were calculated from a calibration curve established from an AFB1 standard (TSL-104, 25 µg/mL, Trilogy Analytical Laboratory) and the percentage of AFB1 removed by the different LAB strains was calculated using the following formula:

$$AFB1\,(\%) = 100 \times \left(1 - \frac{\textbf{Concentration of AFB1 in the supernatant}\left(\frac{ng}{ml}\right)}{\textbf{Concentration of AFB1 in positive control}\left(\frac{ng}{ml}\right)}\right)$$

2.8.2. Ota

OTA quantification was carried out using HPLC-FLD (Shimadzu RF 20A, Japan) with a C18 reverse-phase column (Uptisphere type, ODB, 5 µm particle size, 250 × 4.6 mm), with identical pre-column, thermostatically controlled at 35 °C, in which sample volumes of 100 µL were injected. Chromatographic separation was performed under isocratic flow rate of 1 mL/min of mobile phase: water-acetonitrile-acetic acid, 69:30:1, v/v/v. OTA detection was set at 333 nm excitation and 460 nm emission, and the retention time was approximately 9.5 min. Detection and quantification limits were established at 0.085 and 0.25 ng/mL, respectively. OTA concentrations were calculated from a calibration curve established from an OTA standard (TSL-503, 10 µg/mL, Trilogy Analytical Laboratory) and the percentage of OTA removed by the different LAB strains was calculated using the following formula:

$$\textbf{OTA}(\%) = 100 \times \left(1 - \frac{\textbf{Concentration of OTA in the supernatant}\left(\frac{ng}{ml}\right)}{\textbf{Concentration of OTA in positive control}\left(\frac{ng}{ml}\right)}\right)$$

2.8.3. AFB1 and OTA in a mixture

Separation and quantification of AFB1 and OTA in a mixture were performed using HPLC-FLD (Shimadzu RF 20A, Japan) after post-column electrochemical derivatisation (KobraCell™, R-Biopharm Rhône ltd., Glasgow, UK), under the same operating conditions used for AFB1 quantification. The mobile phase consisted of water-methanol (20:80, v/v) with 119 mg KBr 0.001 M and 350 µL of nitric acid 4 M and the flow detection was 0.8 mL/min. The injection volume was 100 µL and the wavelengths of excitation and emission were 360 nm and 450 nm (0 min to 31 min) for AFB1 and 333 nm and 460 nm (31 min to 55 min) for OTA. The retention times were approximately 22 and 43 min for AFB1 and OTA, respectively. Detection and quantification limits were established at 0.05 and 0.2 ng/mL, respectively. Mycotoxin concentrations were calculated from a calibration curve established from OTA standard (TSL-503, 10 µg/mL, Trilogy Analytical Laboratory) and AFB1 standard (TSL-104, 25 µg/mL, Trilogy Analytical Laboratory), and the percentage of OTA and AFB1 removed by the different LAB strains was calculated using the formulas mentioned above for each mycotoxin.

2.9. Statistical analysis

All experiments were conducted in triplicate and mycotoxin analysis were performed in duplicate for each test. Results were expressed as means ± standard deviation (SD), and analyzed using a two-way analysis of variance (ANOVA) to determine whether the tested parameters (mycotoxin, viable LAB strain) at pH 6 affected the removal ability between the viable LAB strains and the mycotoxins (AFB1 and OTA, alone or in mixture). A three-way ANOVA was used to test the parameters mycotoxin, LAB strain and pH or LAB strain viability. Where significant differences were found on the ANOVA test, the Tukey's Honest Significance Difference (HSD) Test based on the studentized range distribution was used to determine for which parameters (mycotoxin, LAB strain, pH,

LAB strain viability) there were significant differences.

3. Results

3.1. Characterization and identification of LAB isolates

Colony and cell feature are presented in Table 1. All strains were catalase negative, Gram-positive, and grew on M17 agar at 30 °C under aerobic and anaerobic conditions. They are coccus grouped in pairs, short chains of varying length or in clusters.

The eleven strains were identified using API 50 CHL system and by sequencing 16S rDNA region (Table 2). The API 50 CHL system identified isolates as *Lactococcus lactis* spp *lactis* 1 (54.5 %), *Lactococcus lactis* spp *lactis* 2 (27.3 %) and *Lactobacillus paracasei* spp *paracasei* 3 (18.2 %). The percent identity was 83.2 % for Lab-L1, Lab-L2 and Lab-L7, and 87.3 % for Lab-L1/al, Lab-L2/al and Lab-L4/al, identified as *Lactococcus lactis* spp *lactis* 1 and *Lactococcus lactis* spp *lactis* 2, respectively. It was 53.4 % for Lab-L3, Lab-L8 and Lab-L9, and 39.8 % for Lab-L3/al and Lab-L5/al, identified as *Lactococcus lactis* spp *lactis* 1 and *Lactobacillus paracasei* spp *paracasei*, respectively. With the use of Lac1/Lac2 or Lac3/Lac2 primer pairs, LAB isolates could be identified only until genus as *Enterococcus*, but not at the species level because the NCBI BLAST parameters (score, query cover, expect value, percent identity) were identical for the different species suggested for each isolate (*Enterococcus faecium*, *E. feacalis*, *E. durans*, *E. hirae*, *E. thailandicus*, *E. ratti* or *E. villorum*). These results showed that each LAB strain shared the same 16S rDNA sequence with approximatively 20 BLAST hits. In this respect, the amplicon obtained using the Lac1/Lac2 or Lac3/Lac2 primer pairs is too short for distinguishing the different LAB species. In contrast, with the use of 27f/1492r primer pair, LAB strains could be identified at species level as *E. faecium* and *E. durans* with percent identity of 95.5 % and varying from 99.7 % to 100 %, respectively.

The two LAB strains, Lab-L4/al and Lab-L1, isolated from two different matrices (fermented wheat El-Hammoum and traditional fermented milk, respectively) and identified as *E. faecium* and *E. durans*, respectively, were selected to evaluate *in vitro* their detoxification ability against AFB1 and OTA.

Table 1
Macroscopic and microscopic morphology of LAB isolates cultivated on M17 agar at 30 °C for 48 h.

LAB isolate code	Macroscopic characteristics			Microscopic characteristics	
	Color	Form	Size	Cell's form	Association type
Lab-L1/al	Whitish	Spherical	Small (2 mm)	Cocci	In pair or in short chain
Lab-L2/al	Whitish	Spherical	Small (2 mm)	Cocci	In clusters
Lab-L3/al	Whitish	Spherical	Small (2 mm)	Cocci	Isolated diplococcus
Lab-L4/al	Whitish	Spherical	Small (2 mm)	Cocci	In pair or in short chain
Lab-L5/al	Whitish	Spherical	Very small (1 mm)	Cocci	Isolated diplococcus
Lab-L1	Whitish	Spherical	Small (2 mm)	Cocci	In short or long chain
Lab-L2	Whitish	Spherical	Small (2 mm)	Cocci	In long chain
Lab-L3	Whitish	Spherical	Small (2 mm)	Cocci	Isolated diplococcus
Lab-L7	Clear	Spherical	Very small (1 mm)	Cocci	In short chain
Lab-L8	Whitish	Spherical	Small (2 mm)	Cocci	In pair or in short chain
Lab-L9	Whitish	Spherical	Small (2 mm)	Cocci	In pair or in short chain

Table 2
Biochemical and molecular identification of LAB isolates using API 50 CHL system and sequencing of 16S rRNA gene.

LAB isolate code	LAB species identified using API 50 CHL (percent identity)	LAB species identified using sequencing of 16S rRNA gene (percent identity)	
		Lac1/Lac2, Lac3/Lac2	27f/1492r
Lab-L1/al	*Lactococcus lactis* ssp *lactis* 2 (87.2 %)	*Enterococcus* spp (100 %)	*Enterococcus faecium* (95.5 %)
Lab-L2/al	*Lactococcus lactis* ssp *lactis* 2 (87.3 %)	*Enterococcus* spp (99.65 %)	*Enterococcus faecium* (95.5 %)
Lab-L3/al	*Lactobacillus paracasei* ssp *paracasei* 3 (39.8 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (99.7 %)
Lab-L4/al	*Lactococcus lactis* ssp *lactis* 2 (87.2 %)	*Enterococcus* spp (100 %)	*Enterococcus faecium* (95.5 %)
Lab-L5/al	*Lactobacillus paracasei* ssp *paracasei* 3 (39.8 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (99.7 %)
Lab-L1	*Lactococcus lactis* ssp *lactis* 1 (83.2 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)
Lab-L2	*Lactococcus lactis* ssp *lactis* 1 (83.2 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)
Lab-L3	*Lactococcus lactis* ssp *lactis* 1 (53.4 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)
Lab-L7	*Lactococcus lactis* ssp *lactis* 1 (83.2 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)
Lab-L8	*Lactococcus lactis* ssp *lactis* 1 (53.4 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)
Lab-L9	*Lactococcus lactis* ssp *lactis* 1 (53.4 %)	*Enterococcus* spp (100 %)	*Enterococcus durans* (100 %)

3.2. Mycotoxin removal ability of viable LAB strains under simulated bread fermentation conditions

The selected LAB isolates, Lab-L4/al and Lab-L1, and the reference strain LP R1096, were tested for their ability to remove AFB1 and OTA (alone or in mixture) from CPB 0.1 M, containing 40 ng/mL of each toxin, over 24 h in conditions simulating bread fermentation parameters (25 °C with pH decreasing from 6 to 5). The results are presented as means ± SD in Table 3. The ANOVA carried out indicated that the tested parameters mycotoxin, viable LAB strain and pH had significant effects on the mycotoxin removal percentages (*P*-values below the significance level of 0.05).

All three tested viable LAB strains were able to remove AFB1 and OTA under pH 6 (start of bread fermentation), with no significant difference when these toxins were incubated alone or in mixture. In addition, the percentages of AFB1 removed were significantly higher than the OTA ones (Fig. 1) ranging from 25 % to 55 % for AFB1 (16 % to 38 % for OTA), and from 27 % to 53 % for AFB1 in mixture (14 % to 39 % for OTA) (Table 3). Lab-L4/al had the lowest mycotoxin removal ability compared to LP R1096 and Lab-L1. For these two LAB strains, no significant difference was observed except for the percentage of OTA removal when the toxin was incubated alone which was higher (38 %) for LP R1096 (Fig. 2).

The mycotoxin removal ability of Lab-L4/al and LP R1096 (exempt for AFB1 incubated in mixture) was significantly higher (two to three times) at pH 5 than at pH 6. In contrast, removal of mycotoxins by Lab-L1 (34 % OTA, 54 % AFB1, and 35 % OTA and 49 % AFB1 in mixture) and by LP R1096 (76 % AFB1 in mixture) was not influenced by pH medium (Fig. 3). As for the results obtained at pH 6, the amounts of OTA and AFB1 removed, when incubated alone or in mixture, were similar for each strain.

Table 3
Effect of pH and bacterial viability on mycotoxin removal ability of LAB strains.

Mycotoxin removal (%) ± SD									
	Lab-L4/al			Lab-L1			*Lactobacillus plantarum* (LP) R1096		
Mycotoxins	pH5 (viable bacteria)	pH6 (viable bacteria)	pH6 (nonviable bacteria)	pH5 (viable bacteria)	pH6 (viable bacteria)	pH6 (nonviable bacteria)	pH5 (viable bacteria)	pH6 (viable bacteria)	pH6 (nonviable bacteria)
OTA	49 ± 6.7	16 ± 2.2	54 ± 3	34 ± 4.8	24 ± 3.8	66 ± 1.6	64 ± 1.1	38 ± 2.3	70 ± 1.7
AFB1	73 ± 7	25 ± 1.7	63 ± 5.1	54 ± 3.5	52 ± 3.3	80 ± 7	70 ± 3.4	55 ± 5.1	76 ± 1
OTA + AFB1									
OTA	44 ± 2	14 ± 2	52 ± 2	35 ± 3.6	34 ± 4.8	68 ± 3.8	68 ± 2.5	39 ± 5.3	68 ± 4
AFB1	56 ± 1.3	27 ± 3.2	63 ± 1.8	49 ± 2.8	46 ± 4.6	74 ± 3.1	76 ± 4.3	53 ± 4.4	76 ± 3.2

Data shown are the mean ± SD of triplicates. Bacteria, 10^{10} CFU/mL^{-1} were incubated in the presence of toxins at 25 °C for 24 h (1 h with agitation and 23 h without agitation). AFB1, 40 ng/mL; OTA, 40 ng/mL and AFB1 + OTA 40 ng/mL each.

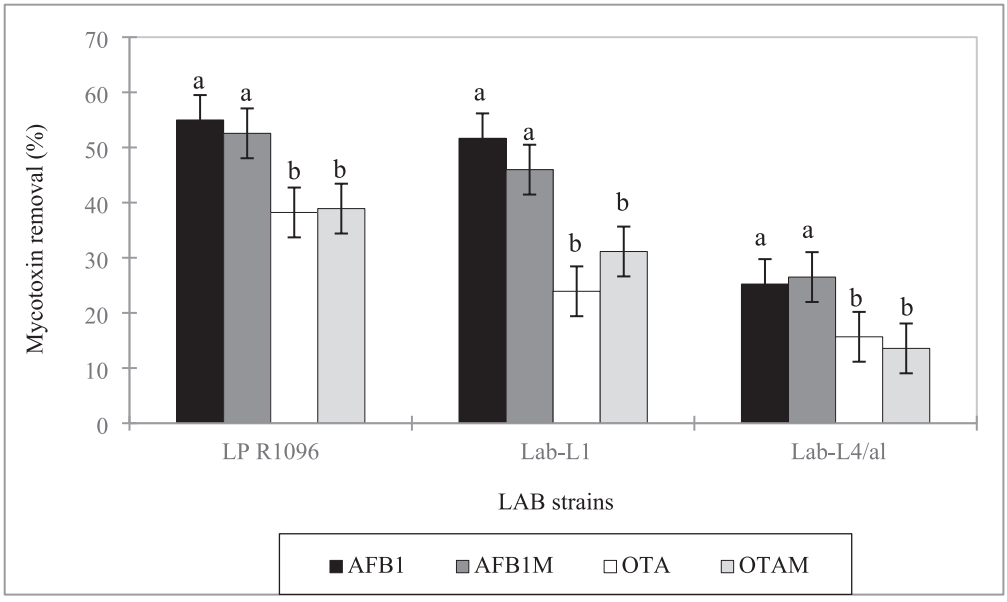

Fig.1. Effect of mycotoxins incubated alone (OTA and AFB1) or in mixture (OTAM and AFB1M) on the percentage of mycotoxin removal by viable LAB strains in CPB 0.1 M, pH 6 at 25 °C for 24 h. Data are presented as the means ± SD of triplicate assays. Mycotoxins found to be not different at 95 % significance level are labeled with the same letter according to the results of the Tukey's HSD test.

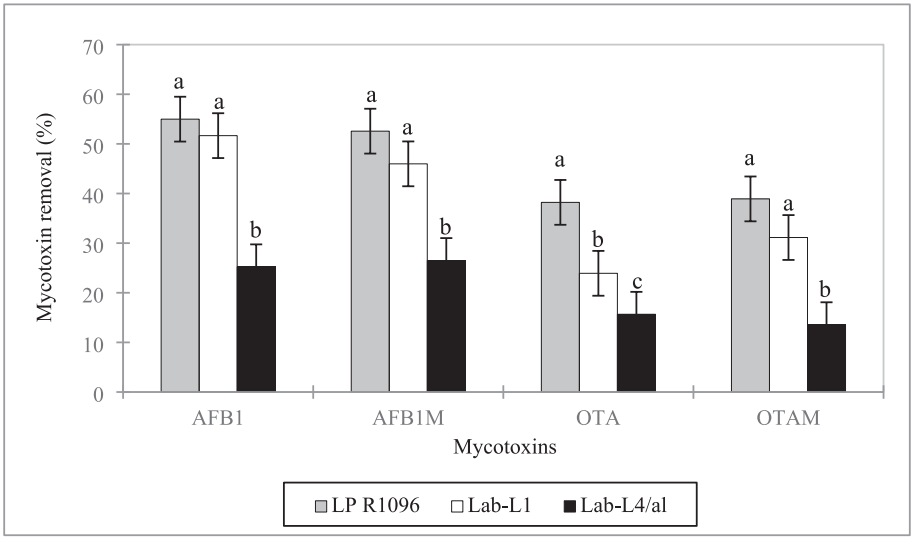

Fig.2. Effect of viable LAB strains on the percentage of mycotoxin removal when the toxins were incubated alone (OTA, AFB1) or in mixture (OTAM, AFB1M) in CPB 0.1 M, pH 6 at 25 °C for 24 h. Data are presented as the means ± SD of triplicate assays. Strains found to be not different at 95 % significance level are labeled with the same letter according to the results of the Tukey's HSD test.

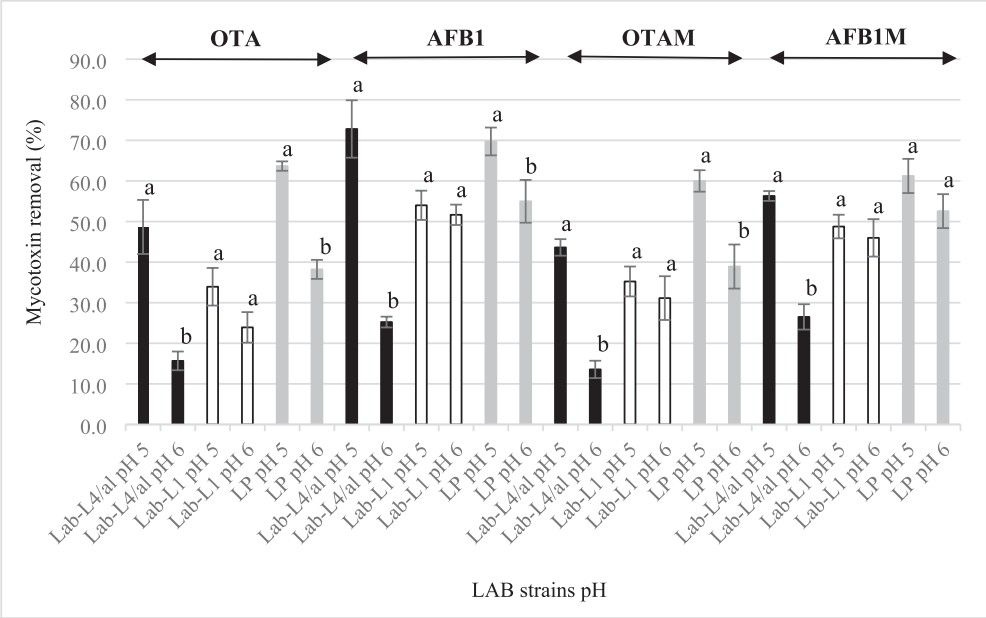

Fig.3. Effect of pH (5 or 6) on the percentage of mycotoxin removal by viable LAB cells when the toxins were incubated alone (OTA and AFB1) or in mixture (OTAM AFB1M) in CPB 0.1 M at 25 °C for 24 h. Data are presented as the means ± SD of triplicate assays. pH found to be not different at 95 % significance level are labeled with the same letter according to the results of the Tukey's HSD test.

3.3. Effect of bacterial viability on mycotoxin removal ability of LAB strains

For all tested LAB strains and mycotoxins, alone or in mixture, mycotoxin removal percentages at pH 6 were significantly higher for nonviable LAB cells than for viable ones, with similar removed amounts for toxins alone or in mixture for each strain (Fig. 4). The maximum mycotoxin removal occurred with nonviable cells of Lab-L1 and LP R1096: respectively 80 % and 76 % for AFB1 alone, 74 % and 76 % for AFB1 in mixture, 66 % and 70 % for OTA alone, and for both strains 68 % for OTA in mixture. With nonviable cells of Lab-L4/al, the mycotoxin removal percentages were 54 % for OTA, 63 % for AFB1, and 52 % for OTA and 63 % for AFB1 in mixture.

4. Discussion

LAB occur naturally as indigenous microbiota of fermented foods and beverages (Rezac et al., 2018), and due to their potential to remove mycotoxins, they can be used as biocontrol agents to reduce mycotoxin levels in contaminated food (Muhialdin et al., 2020). In this study, eleven LAB isolated from Algerian fermented foods (fermented wheat El-Hammoum and traditional fermented milk) were characterized by phenotypic properties and identified using API 50 CHL kit and 16S rDNA sequencing. Based on the biochemical identification (API 50 CHL), LAB strains were classified in two groups. The first group was identified as *Lactococcus lactis* ssp. *lactis* (Lab-L1/al, Lab-L2/al and Lab-L4/al isolated from fermented wheat El-Hammoum, and Lab-L1, Lab-L2, Lab-L3, Lab-

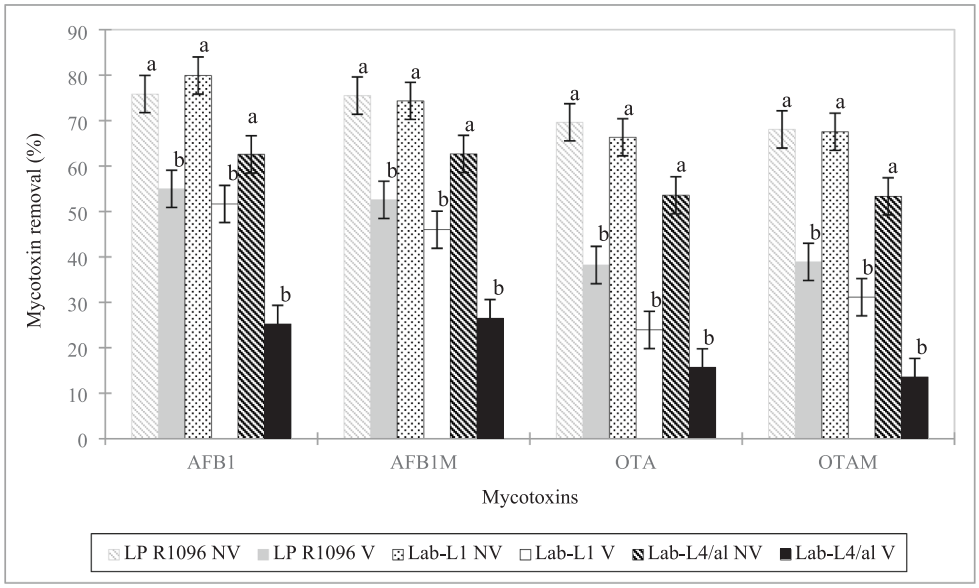

Fig.4. Effect of LAB strain viability on the percentage of mycotoxin removal when the toxins were incubated alone (OTA and AFB1) or in mixture (OTAM and AFB1M) in CPB 0.1 M, pH 6 at 25 °C for 24 h. Data are presented as the means ± SD of triplicate assays. LAB strain viability found to be not different at 95 % significance level are labeled with the same letter according to the results of the Tukey's HSD test.

L7, Lab-L8 and Lab-L9 isolated from traditional fermented milk), and the second group was identified as *Lactobacillus paracasei* ssp. *paracasei* (Lab-L3/al and Lab-L5/al isolated from fermented wheat El-Hammoum). These results are in agreement with those of previous studies. It was reported that *Lactococcus lactis* ssp *lactis* strains were present in fermented dairy products (Garmasheva, 2016; Li et al., 2020). *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus* and *Streptococcus* were the most dominant LAB genera identified in the fermented wheat El-Hammoum (Mokhtari et al., 2016). Benakriche et al. (2016) isolated *Lactococcus lactis* ssp. *lactis* and *Lactobacillus paracasei* ssp. *paracasei* from fermented wheat El-Hammoum collected in west Algeria. LAB isolates were subsequently subjected to 16S rDNA sequencing in order to confirm or infirm their identification. The LAB isolates were not identified until species using the Lac1/Lac2 or Lac3/Lac2 primer pairs specific for LAB, whereas with the use of the universal bacterial primers, 27f and1492r, they were identified as *E. faecium* and *E. durans* with percent identity of 95.5 % and varying from 99.7 % to 100 %, respectively.

The biochemical identification did not coincide with the molecular one, the two methods providing different patterns of genera and species identification for the LAB isolates. Using the API 50 CHL kit, the LAB isolates were identified as *Lactococcus* and *Lactobacillus* with lower identity percentage ranging from 39.8 % to 53.4 % for Lab-L3/al; Lab-L5/al; Lab-L3; Lab-L8 and Lab-L9, and from 83.2 % to 87.3 % for Lab-L1/al; Lab-L2/al; Lab-L4/al; Lab-L1; Lab-L2 and Lab-L7. These results were similar to findings of Fguiri et al. (2015) and Moraes et al. (2013) reporting differences between the molecular and biochemical tests, and their limitations in LAB identification. Indeed, the most frequent genera observed by these authors was *Enterococcus* spp. using 16S rDNA sequencing, whereas *Lactococcus lactis* ssp *lactis*, *Lactobacillus* spp. and *Pediococcus* spp were mostly identified using API 50 CHL kit.

Enterococci represent a large proportion of the autochthonous microflora of gastrointestinal tract of mammals. They can be found in soil, food, water and plants. The presence of these microorganisms in food products has long been considered as an indicator of fecal contamination (Giraffa, 2002). In contrast, many authors suggested that *Enterococcus* strains constitute an important part of the microbiota involved in the fermentation activity and the development of the organoleptic properties of fermented foods (Braiek and Smaoui, 2019). In addition, enterococci have been reported to produce antimicrobial compounds including bacteriocins, organic acids, activated oxygen metabolites, exopolysaccharides (Valyshev, 2014). Moreover, certain members of enterococci are used as probiotics and starter cultures (Moreno et al., 2006; Marcinakova et al., 2008; Nami et al., 2019; Cavalheiro et al., 2021). Some strains of *E. faecium* and *E. feacalis* are used as probiotics to treat diarrhoea or antibiotic-associated diarrhoea, to lower cholesterol levels or to improve host immunity (Franz et al., 2011). Enterococci species occur in several dairy products. Their predominance, especially *E. durans* and *E. faecium*, in milk and artisanal cheeses was reported by Akhmetsadykova et al. (2015) and Terzić-Vidojević et al. (2021). Corsetti et al. (2007) reported that *E. casselivaflavus*, *E. durans*, *E. feacalis*, *E. faecium* and *E. mundtti* were the most prevalent enterococci species in Italian wheat grain and non-conventional flour. Studies on the microbiota of Algerian fermented wheat *Lemzeiet* or El-Hammoum revealed that three isolates were identified as *E. faecium* (Merabti et al., 2019).

Because of their different genotypes and origin, Lab-L4/al and Lab-L1, isolated from fermented wheat El-Hammoum and traditional fermented milk respectively, were selected to study their ability to remove AFB1 and OTA, using an experimental model that simulated wheat bread fermentation conditions (25 °C with pH decreasing from 6 to 5). LP R1096 was also used in this study as reference strain because of its highest binding ability of fumonisins B1 (FB1) and B2 (FB2) reported by Dawlal et al. (2017) and Niderkorn et al. (2006a). The results demonstrated that the reference strain LP R1096, and the two strains (Lab-L4/al and Lab-L1) belonging to *Enterococcus* genus were capable of removing AFB1 and OTA from contaminated CPB in tested conditions with highest efficiency for AFB1. Many reports have demonstrated the potential of *Lactobacillus* strains to remove mycotoxins from contaminated liquid medium (Chlebicz & Śliżewska, 2020; Freire et al., 2021; Piotrowska, 2014). Mohammad & Hashemi. (2019) reported high OTA removal (32–58 %) by *Lactobacillus plantarum* strains in cream after 24 h of fermentation. The ability of *Lactobacillus plantarum* to decrease AFB1 concentration (69.11 %) was also found by Damayanti et al. (2017). Several studies have reported the mycotoxin removal ability of *Enterococcus* strains. Two *E. faecium* strains were evaluated for their ability to remove AFB1 and patulin from PBS solution under different pH and incubation time conditions. The results showed that the tested strains remove 19.3 to 37.5 % and 15.8 to 45.3 % of AFB1 and patulin, respectively (Topcu et al., 2010). Hatab et al. (2012) reported that *E. faecium* was able to remove 64.5 % of patulin from apple juice. In addition, It has been shown that *E. faecium* strains cause removal of AFB1 from aqueous solution by up to 42 %. (Juri Fernandez et al., 2014). Authors suggested that these strains could be used in the manufacturing of fermented foods to reduce the bioavailability of mycotoxins in human diet and animal feed. Niderkorn et al. (2007) showed that in conditions simulating corn silage, *Enterococcus* and *Streptococcus* were the most effective genera capable of binding zearalenone, deoxynivalenol, fumonisins B1 and B2, with an average fraction bound of 35 %, 22 %, 14 % and 43 %, respectively, for the 4 tested strains of *Enterococcus*, compared to *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus and Propionibacterium* genera. Therefore, enterococci can potentially be utilized to detoxify corn silage contaminated by *Fusarium* toxins and to decrease their toxicity in animals.

According to literature data, removal of mycotoxins from contaminated medium occurs through binding them to LAB cell wall (Dawlal et al., 2019; El-Nezami et al., 1998; Franco et al., 2011; Haskard et al., 2000; Juri Fernandez et al., 2014; Piotrowska, 2014; Zhao et al., 2015). To confirm this hypothesis, heat inactivated cells (nonviable cells) and viable cells of the three LAB strains (Lab-L4/al, Lab-L1 and LP R1096) were investigated for their ability to bind AFB1 and OTA in CPB 0.1 M, pH 6 at 25 °C over 24 h of incubation time. Nonviable cells had the highest mycotoxin removal capacity compared to viable cells, confirming a binding mechanism to the LAB cell wall to explain AFB1 and OTA reduction, and not mycotoxin biodegradation. This result is similar to those presented by Franco et al. (2011); Piotrowska (2014) and Zhao et al. (2015). It was reported that peptidoglycans, polysaccharides and teichoic acids were the main components of LAB cell wall implicated in the binding mechanism of mycotoxins (Hernandez-Mendoza et al., 2009; Lahtinen et al., 2004; Zhao et al., 2016). These components were affected by heat inactivation causing changes in the bacterial cell wall (protein denaturation and pore generation) that led to the appearance of new binding sites (Haskard et al., 2000; Haskard et al., 2001). This can explain the highest binding efficiency obtained in our study with inactivated LAB cells.

Numerous investigations have looked into factors affecting mycotoxin binding ability of LAB and showed that it depends on different parameters such as type of LAB strains (Fazeli et al., 2009), LAB cell density (El-Nezami et al., 1998; Fuchs et al., 2008; Piotrowska, 2014), LAB viability (Franco et al., 2011; Zhao et al., 2015), mycotoxin concentration (Fuchs et al., 2008; Zhao et al., 2015), pH medium (Taheur et al., 2017; Zhao et al., 2016), temperature (El-Nezami et al., 1998; Zhao et al., 2016; Zhao et al., 2015) and incubation time (Chlebicz & Śliżewska, 2020; Zhao et al., 2016). In the present experiment, it was observed that AFB1 and OTA amounts removed by viable LP R1096 (exempt for AFB1 incubated in mixture) and Lab-L4/al cells depended on the pH medium (CPB). Reduction in pH from 6 to 5 caused higher mycotoxin removal. Similar observation was made by Dawlal et al. (2017) and Fuchs et al. (2008) who found that optimal removal of OTA, patulin and fumonisins occurred at low pH. Hydrogen ions (H^+) concentration is higher in an acidic medium. Thus, they may affect the surface charge of the bacterial cell wall that causes interaction between protonated binding sites and negatively charged mycotoxin molecules,

leading to better adsorption (Haskard et al., 2000). At this point, it was postulated that hydrogen bonds were formed in the binding of mycotoxins (OTA and AFB1) by the LAB strains. In contrast, there was no significative difference in AFB1 and OTA reduction by viable Lab-L1 cells at different pH (5 and 6). Similarly, experimental data obtained by Juri Fernandez et al., 2014; Niderkorn et al. (2006a,b) and Topcu et al. (2010) demonstrated that pH had no effect on mycotoxin detoxification by LAB strains, suggesting that a cation-exchanging mechanism was not operating. The removal of AFB1 and OTA was also strain dependent. Difference in AFB1 and OTA binding potential was observed between Lab-L4/al, Lab-L1 and LP R1096, which could be due to the genetic differences between strains, the shape, size and surface area of each strain or differences in bacterial cell wall components (Dawlal et al. 2017; Fazeli et al. 2009). Except the amounts of AFB1 bound by Lab-L4/al strain at pH 5, the percentages of AFB1 and OTA bound by the three tested LAB strains were identical when the mycotoxins were incubated alone or in mixture. This result coincides with the findings of Niderkorn et al. (2006a,b) who observed similar percentages of deoxynivalenol (DON) and nivalenol (NIV) bound by LAB strains when the toxins were incubated alone or in mixture. There are two explanations for such a result: the first one is that the binding sites were not the same for AFB1 and OTA, the second one is that the concentration of mycotoxins used in this study was insufficient to saturate all binding sites.

5. Conclusion

The present study is the first attempts to evaluate the extent to which autochthonous LAB strains isolated in Algeria can reduce the levels of AFB1 and OTA under simulating wheat bread fermentation conditions. The results showed that LAB, Lab-L4/al (*E. faecium*) and Lab-L1 (*E. durans*), and the reference strain LP R1096, have the ability to decrease mycotoxin content *in vitro* conditions with efficiency varying between strains and higher for AFB1, the reference strain and nonviable LAB cells. The reduction of mycotoxin contents was due to binding mechanism by LAB strains. These results suggest that the detoxification ability of the two autochthonous tested strains has the potential to reduce AFB1 and OTA levels in contaminated foods derived from wheat and provide a new approach to reduce mycotoxin contamination.

CRediT authorship contribution statement

Tiziri Badji: Methodology, Writing – original draft. **Noël Durand:** Data curation. **Farida Bendali:** Methodology. **Isabelle Piro-Metayer:** Methodology. **Abdellah Zinedine:** . **Jalila Ben Salah-Abbès:** . **Samir Abbès:** . **Didier Montet:** . **Amar Riba:** Supervision. **Catherine Brabet:** Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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