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The mechanism of *Lactobacillus* strains for their ability to remove fumonisins B1 and B2

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ABSTRACT

Two *Lactobacillus* strains, *L. plantarum* B7 and *L. pentosus* X8, exhibited high efficiency in removing fumonisins (FB1 and FB2) from aqueous medium. 52.9% FB1 and 85.2% FB2 were bound by *L. plantarum* B7, and 58.0% FB1 and 86.5% FB2 by *L. pentosus* X8, respectively. Temperature, incubation time, and pH affected the binding ability of two strains. Cell viability was not necessary for the binding ability. The various components of cell wall were determined for their ability to absorb FBS. The results revealed that the intact peptidoglycans exhibited the greatest capacity in binding FBs. Especially the better structural integrity of the peptidoglycans the more FBs was bound. Thus, the absorption of two bacterial cells to FBs is proposed to be a physical process, and peptidoglycans should be the main binding site. Additionally, Caco-2 cell lines were used to evaluate the ability of the two strains to reduce the damage of FBs *in vitro*. Caco-2 cell's death was reduced after the cell lines were subjected to both viable and non-viable *L. pentosus* X8, respectively. The two *Lactobacillus* strains might be used as a biological detoxification for the removal of FBs from diet and feed in the future.

1. Introduction

Fumonisins, belong to mycotoxin, are fungal secondary metabolites that primarily produced by *Fusarium verticillioides* and *Fusarium proliferatum*. Altogether 28 fumonisins can be classified into four groups, A, B, C and P groups (Maurício et al., 2015). Among them, Fumonisin B1 (FB1) and Fumonisin B2 (FB2) are the most abundant and toxic (Shephard et al., 1996; Maurício et al., 2015; Li et al., 2015; Niderkorn et al., 2009), and they are reported wide in maize and maize-based product, especially accumulated during growing process (Arino et al., 2007; Berthiller et al., 2013; Wei et al., 2013). Fumonisins is available in daily life, and long-term exposure could increase the risk of cancer. For the reason FB1 has been classified as a group 2B carcinogen by the International Agency for Research on Cancer (IARC, 2002).

Microorganisms including lactic acid bacteria (LAB) have been studied for their potential to either degrade mycotoxins or reduce their bioavailability (El-Nezami et al., 1998; Fuchs et al., 2008; Haskard et al., 2000). LAB, which are widely used in food industry, are beneficial to health, especially in regulating and improving the intestinal flora of human and animals (Ouwehand et al., 2002). They have been reported to have an ability to bind mycotoxins (Niderkorn et al., 2006; Pizzolitto et al., 2012).

Several food or animal-original LAB strains have been tested for their ability to bind aflatoxins and other mycotoxins to their surface (El-Nezami et al., 2002; Fuchs et al., 2008). Twenty-nine strains, including LAB and propionic acid bacteria (PAB) could remove deoxynivalenol (DON) and zearalenone, and the LAB strains removed zearalenone up to 88% while the PAB strains are less efficient (Niderkorn et al., 2006). Meanwhile, it has been a burning environmental issue on removing fumonisins from the surroundings, although more physical or chemical methods are chosen to reduce the damage of various mycotoxins to human and animals.

Therefore, the objectives of this study were: (1) to determine the removal of FB1 and FB2 by *Lactobacillus*; (2) to investigate the nature of the mechanism involved in FBs-Microorganism; (3) to evaluate the removal ability *Lactobacillus* using Caco-2 cells system. *Lactobacillus* strains used in this paper were isolated from fermented corn products.

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2. Materials and methods

2.1. Bacterial strains

Lactobacillus strains used in this study were listed in Table 1. The strains were cultured in MRS (De Man, Rogosa, and Sharpe) medium for 18 h at 37 °C under anaerobic condition. Viability was confirmed by standard count methods using MRS agar at 37 °C for 48 h.

2.2. Cell culture and treatment scheme

Caco-2 cells (Third Hospital of Beijing, China) were cultured in DMEM (Hyclone, US) supplemented with 10% fetal calf serum and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin) at 37 °C, 5% CO_2. Caco-2 cells were plated at the density of 1.5×10^6 to perform the binding assay. To study the effects of bacterial cells on removing fumonisins, Caco-2 cells were washed twice with PBS(Hyclone, US) and treated with 2 mL bacterial suspension (approx. 5.0×10^9 CFU/mL) mixed Fumonisins (5 μg/mL) for 3 h. After incubation, the cells were washed six times with PBS to remove non-bound bacteria. The cells were harvested with trypsin (Sigma) and treated with 0.5 mL TritonX-100 (0.1%) to determine the concentration of bound bacteria using plate count methods. DMSO (10 mL/L) was used as the solvent control.

The percentage of cells adhesion was calculated by using the following equation:

Adhesion rate (%) = bound bacteria/cells number in plate × 100%.

The bacterial cells (approx. 5.0×10^9 CFU/mL) were obtained by centrifugation (4000*g*, 10 min, 4 °C) and wash twice with PBS. Then the pellets was suspended in DMEM supplemented with 10% fetal calf serum and fumonisins (5 μg/mL). After incubated with 1 h, the suspension was centrifugated (4000*g*, 10 min, 4 °C), and the supernatant was added into Caco-2 cells to incubated for 4 h at 27 °C. Finally, the cells were harvested with trypsin and suspended in 3 mL DMSO. After propidium iodide staining, the cells were determined with flow cytometry(FCM).

2.3. Fumonisin removal assay

Fumonisins (B1 + B2) (Pribolab) was dissolved in sterile double-distilled H_2O to obtain a concentration of 5 μg/mL fumonisin solution.

Fumonisin B1 and B2 (Pribolab) were dissolved in 1 mL acetonitrile (Mreda)/water (1:1, v/v) respectively to obtain a concentration of 1 mg/mL stock solutions. Working solution FB1 was obtained by dissolving stock solution in sterile double-distilled H_2O. Working solution FB2 was obtained as the same way.

Cells of *Lactobacillus* strains were collected by centrifugation at 4000*g* for 10 min at 4 °C. The pellets were washed twice with sterile double-distilled H_2O. The cell concentration was determined using plate count methods. Then culture (approx. 5.0×10^9 CFU/mL) was suspended in 1.0 mL of fumonisin solution and incubated for 1 h at 37 °C. The cells were removed by centrifugation (4000*g*, 10 min, 4 °C) and supernatant were collected and analyzed by fumonisin ELISA kit (Aokin, Beijing Thai LeQi biotechnology co., LTD, China).

The strains selected by fumonisin ELISA kit were re-suspended in 1.0 mL working solution which contained 5 μg/mL FB1 or FB2, and incubated for 1 h at 37 °C. The bacterial cells were centrifugated (4000*g*, 10 min, 5 °C), and the supernatants were obtained to analyze by reversed-phase high performance liquid chromatography (HPLC, LC-20A, Shimadzu Corporation, Japan).

Positive (sterile H_2O + Fumonisin) and negative (sterile H_2O + cells) controls were included for all experiments. The percentage of strains removing fumonisins was calculated by using the following equation:

Fumonisins removed (%) = 100 × (1-fumonisins peak area of sample/fumonisins peak area of positive control).

2.4. The effect of temperature, pH, time and cell viability on removing fumonisins

To determine the effect of temperature on binding ability, cells and fumonisins were incubated for 4 h at 4 °C, 20 °C, 37 °C and 50 °C, respectively.

To determine the effect of pH on removing ability, cells were incubated in 0.1 M sodium citrate buffer contained fumonisins. The pH of the buffer was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with 0.1 M Tris—HCl, respectively. After incubation for 1 h at 37 °C, samples were analyzed by HPLC. analyzed by HPLC.

In order to determine the effect of incubation time on removing Fumonisins, bacterial cells and fumonisins were co-incubated at 37°C for 10 min, 30 min, 1 h and 1.5 h, and analyzed by HPLC.

The effect of cell viability on removing ability was conducted by heat-treatment (121 °C, 15 min) of the cells before incubation with fumonisins. After treated, the cells were washed three times with sterile double-distilled H_2O. Nonviable cells were monitored by plating in MRS agar. Then the samples with fumonisins above were analyzed by HPLC.

2.5. The effect of HCl, TCA and lysozyme on removing fumonisins

The effect of HCl was conducted in HCl (1.0 mol/L) and kept in boiling water for 15 min. To determine the effect of TCA on removing fumonisins, 10% TCA was added into pellets and kept in boiling water for 15 min. Lysozyme (45,000 U, Sigma) was shaking-incubated (90 r/min, 37 °C, 18 h). After treated, cells of *Lactobacillus* were collected by centrifugation at 4000*g* for 10 min at 4 °C. Then washed twice with sterile double-distilled H_2O and adjusted pH to 4.0. Finally, the samples with fumonisins were analyzed by HPLC.

2.6. The binding ability of different cellular structures

In this part, some cell components which may involve in binding process were obtained to determine the binding ability. The details were as follows:

Table 1
The binding ability of different strains by Fumonisin ELISA kit.

Strains	Source[a]	Removal effect[b]
L. pentosus X8	CICC	+
L. pentosus CH8	CICC	− +
L. pentosus ZJ28	CICC	− +
L. plantarum ZJ46	CICC	− +
L. plantarum B7	CICC	+
L. acidophilus Ind-1	CICC	− +
L. lactis subsp. *lactis* BY21	CICC	+
E. faecium RS8	CICC	−
E. faecium RS30	CICC	−
E. hirae RS18	CICC	−
E. hirae RS21	CICC	−
L. acidophlus NCFM	DuPont	−
Bacillus subtilis 168	HEBAU	+

[a] CICC, China center of industrial culture collection; DuPont, E. I. du Pont de Nemours and Company, NYSE: DD; HEBAU, Agricultural University of Hebei Province.

[b] "−", strains have no removal ability for Fumonisin; "− +", strains have weak removal ability for Fumonisin; "+", strains have strong removal ability for Fumonisin.