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A new insight into the adsorption mechanism of patulin by the heat-inactive lactic acid bacteria cells

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ABSTRACT

The primary objective of this study was to identify the characteristics of the heat-inactived lactic acid bacteria (LAB) cells involved in the adsorption of patulin. The bacterial cells were characterized by Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDS), Transmission Electron Microscopy (TEM) and Brunauer–Emmett–Teller (BET) technique. The patulin-exposed bacterial cells and patulin-unexposed bacterial cells were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Zeta Potential and Contact Angle Method. It was found that *Lactobacillus brevis* 20023 (LB-20023), which has the highest specific surface area and cell wall volume, showed the highest capacity to adsorb patulin from the aqueous solution. Five major elements (C, N, O, P, and S) were detected by SEM-EDS, and LB-20023 displayed the highest nitrogen-to-carbon (N/C) ratio (0.2938). LB-20023 exhibited the highest hydrophobicity, but the zeta potential was not prominent compared to other bacterial cells. The main functional groups involved in adsorbing patulin were C–O, OH and/or NH groups, suggesting that polysaccharides and/or protein were important functional components. Above all, the adsorption capacity of bacterial cells had close relationships with physical and chemical properties of cell surface, including specific surface area, cell wall volume, nitrogen-to-carbon (N/C) ratio, hydrophobicity and functional groups. Further study will be needed to find other additional functional factors.

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1. Introduction

Patulin is a water-soluble unsaturated lactone, produced by a number of fungal species belonging to the genera *Penicillum*, *Aspergillus* and *Byssochlamys* (Drusch, Kopka, & Kaeding, 2007; Ritieni, 2003). It is usually found in higher amount than other mycotoxins in fruits and vegetables, especially in apples and apple products (Drusch et al., 2007; Zoghi, Khosravi-Darani, & Sohrabvandi, 2014). It is believed that patulin exerted its cytotoxic effects mainly by reacting toward essential cellular thiol groups in proteins and also amino groups (Fliege & Metzler, 1999). Human exposure to mycotoxins by ingestion of contaminated products can lead to serious health problems, including immunosuppression and carcinogenesis (Drusch et al., 2007). Due to its toxicity, Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) have established a provisional maximum tolerable daily intake for patulin, i.e. 0.4 μg/kg body weight/day (World Health Organization, 1995). In this regard, European Union (EU) has recommended that the maximum levels of patulin were 50 μg/kg for fruit juices and their products, 25 μg/kg for solid apple products including apple compote and apple puree, and 10 μg/kg for products intended for infants (European Union, 2006).

Several physical and chemical approaches such as washing, clarification, filtration, chemical addition and ionizing radiation have been developed to reduce patulin contamination in apple juice and other apple products (Acar, Gökmen, & Taydas, 1998; Moake, Padilla-Zakour, & Worobo, 2005). However, the processing of fruits does not result in the complete removal of patulin; furthermore, most of these approaches are not widely available because of expensive equipment and monitoring system requirements, high reagent or energy requirements and trained operational personnel requirements involved in detoxification process (González-Osnaya, Soriano, Moltó, & Mañes, 2007; Sant'Ana, Rosenthal, & de Massaguer, 2008; Shetty & Jespersen, 2006). Other efficient, safe and reliable methods utilizing various micro-organisms, including lactic acid bacteria (LAB) (Fuchs et al., 2008; Hatab, Yue, & Mohamad, 2012; Peltonen, El-Nezami, Haskard, Ahokas, & Salminen, 2001; Topcu, Bulat, Wishah, & Boyacı,

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2010), *Gluconobacter oxydans* (Ricelli, Baruzzi, Solfrizzo, Morea, & Fanizzi, 2007) and *Saccharomyces cerevisiae* (Guo, Yuan, Yue, Hatab, & Wang, 2012; Yue, Dong, Guo, & Worobo, 2011), have been published for the adsorption and detoxification of patulin (Zoghi et al., 2014).

The effectiveness of microorganisms in the biodegradation of patulin was evaluated by several research groups. They observed that patulin can be degraded into potentially less toxic compounds (Castoria et al., 2011; Moss & Long, 2002; Ricelli et al., 2007). But the predominant degradation compound (E-Ascladiol) still retains a quarter of the toxicity of patulin, and can be considered a mycotoxin in itself (Castoria et al., 2011; Suzuki, Takeda, & Tanabe, 1971). In additionally, the active microbial cells, which can be used for biodegradation of toxins, are limited to fermented foods. Furthermore, the active microbial cells are themselves sensitive to patulin (Sumbu, Thonart, & Bechet, 1983). By contrast, inactive microbial cells can be applied more broadly to food.

Since many LAB strains are food grade microorganisms, using their cells in foods has an obvious advantage over non-food grade bacteria. Previous work showed that some heat-inactived LAB cells were capable of adsorbing patulin, but the mechanism of the adsorption interaction between the bacterial cells and patulin remains to be clarified (Hatab et al., 2012; Topcu et al., 2010). Recently, various physical, chemical and enzymatic treatments have been used to ascertain the potential adsorption sites for mycotoxin on bacterial cells. The results showed that the adsorption process was due to noncovalent interaction between toxins and carbohydrates and protein moieties of the cell walls (Dalié, Deschamps, & Richard-Forget, 2010; Guo et al., 2012; Haskard, Binnion, & Ahokas, 2000; Lahtinen, Haskard, Ouwehand, Salminen, & Ahokas, 2004; Topcu et al., 2010). Moreover, cell wall structural integrity of the bacterial cells was required for adsorbing mycotoxins (Guo et al., 2012; Hernandez-Mendoza, Guzman-de-Peña, & Garcia, 2009; Pizzolitto, Salvano, & Dalcero, 2012). Therefore, the direct information of surface characteristics of intact bacterial cells is very important and needed to be clarified.

This research tried to interpretate the mechanism of the adsorption of patulin by intact heat-inactived LAB cells based on investigating the relationship of the adsorption with the zeta potential, surface hydrophobicity and functional groups of bacterial cells surfaces. Also, the second goal was to give more possible associations between adsorption of patulin and cell size, specific surface area and volume content of cell wall of heat-inactived LAB cells to future research.

2. Material and methods

2.1. Chemicals and media

The LAB strains were cultured in de Mann Rogosa Sharpe (MRS) broth and agar (Oxoid, Basingstoke, UK). Standard patulin was purchased from Sigma–Aldrich (St. Louis, MO, USA). Other chemicals were obtained from Chemical Reagents Company (Yangling, China).

2.2. Bacterial cells preparation

Six LAB strains (*Lactobacillus curvatus* 21019, *Lactobacillus rhamnosus* 6133, *L. rhamnosus* 6224, *Lactobacillus brevis* 20023, *Enterococcus faecium* 20420 and *E. faecium* 21605) were used in this study. These strains were supplied by China Center of Industrial Culture Collection (CICC) (Beijing, China), as dry active strains and stored at −80 °C and coded as LC-21019, LR-6133, LR-6224, LB-20023, EF-20420 and EF-21605, respectively.

The LAB strains were cultured on MRS agar and incubated at 37 °C for 24 h. Two loopfuls of activate bacterial strains were incubated into MRS broth (24 h, 37 °C, 150 r/min). Thereafter, 2 mL of culture from first round shaking culture was transferred into a new MRS broth (150 mL) for a second shaking culture under the same conditions as the first shaking culture. After second incubation, bacterial cells were centrifuged, killed (121 °C for 20 min), washed at least three times with distilled water and freeze-dried at −54 °C for 26 h by a vacuum freeze-dryer (MCFD5505; SIM International Group Co. Ltd) (Hatab et al., 2012). Next, bacterial cells were powdered using mortar and pestle. These heat-inactived and powdered bacterial cells were used for the adsorption experiments.

2.3. Patulin binding assay

A standard stock solution of patulin (100 mg/L) was prepared in ethyl acetate and stored at −40 °C. A working solution of patulin (4 mg/L) was prepared by completely evaporating the ethyl acetate at 40 °C in a water bath, then the residue was immediately dissolved in acetic acid solution (pH 4.0).

Adsorption studies were conducted in batches with 0.01 g bacterial cells in a 50-mL plastic tube containing 2 mL patulin working solution (4 mg/L). The reaction mixtures were shaken on an orbital shaker with 150 rpm, 37 °C for 48 h. Subsequently, the incubation was terminated by centrifugation, and the supernatants were collected for analysis of residual toxin concentration by High Performance Liquid Chromatography (HPLC). All assays were performed in triplicate, negative controls (bacterial cells suspended in acetic acid solution) and positive controls (patulin working solution) were also performed. Part of the patulin-exposed bacterial cells and patulin-unexposed bacterial cells (bacterial cells obtained from negative controls) were dried and characterized using Fourier Transform Infrared Spectroscopy (FTIR) (Spectrum GX FT-IR, Perkin–Elmer).

2.4. HPLC analysis

The HPLC system (Shimadzu LC-20AD pump, CTO-20A column oven, and SPD-M20A UV/Vis detector; Shimadzu Scientific Instruments, Columbia, MD) and an Alltima reversed-phase column C18 (250 × 4.6 mm i.d., 5 μm particles) were used to determine residual patulin. All samples were filtered through a 0.22-μm pore membrane before analysis. A 20 μL sample was injected, and HPLC grade acetonitrile/water (10:90, v/v) was used as isocratic mobile phase with a flow rate of 1 mL/min at 30 °C. The detection wavelength was set at 276 nm. The percentage of patulin bound to the bacteria was calculated using the following equation:

$$Y = (1 - A/A_0) \times 100 \quad (1)$$

Where Y is the removal rate of patulin, A is the peak area of patulin in supernatant and A_0 is the peak area of patulin in positive control.

2.5. Characteristics of bacterial cells

Morphology and elementary composition of bacterial cells were determined by Scanning Electron Microscopy coupled with Energy Dispersive X-Ray Spectroscopy (SEM-EDS) and Transmission Electron Microscopy (TEM), and the volume content of cell wall in per gram bacterial cells was estimated according to the volume calculating formulas.

2.5.1. Characteristics of individual cell

For SEM-EDS, bacterial cells were gold-sputtered (using Scan-Coat six equipment-Oxford), observed and photographed with a

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