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Adsorption mechanism of tenuazonic acid using inactivated lactic acid bacteria

Na Ge [b], Jingjing Xu [b], Bangzhu Peng [a, *], Siyi Pan [a]

[a] Key Laboratory of Environment Correlative Dietology, Ministry of Education, Huazhong Agricultural University, 430070, Wuhan, Hubei, China
[b] College of Food Science & Technology, Huazhong Agricultural University, 430070, Wuhan, Hubei, China

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ABSTRACT

Tenuazonic acid (TeA) is a fungal secondary metabolite that is produced by a number of Alternaria species and is therefore a natural contaminant of food and feed samples. The aim of this study was to investigate the adsorption mechanism of TeA using various modified inactivated lactic acid bacteria (LAB). The bacterial cells were characterized by Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM-EDS) and Transmission Electron Microscopy (TEM). The results indicated that increasing the surface area of the cell wall improves the adsorption capacity of TeA, and an ion-exchange reaction may occur during the adsorption process. Fourier Transform Infrared (FTIR) Spectroscopy and X-ray Diffraction (XRD) analysis indicated that C–O, O–H and N–H groups, which are related to protein and carbohydrate components, were obviously involved in the adsorption of TeA. The zeta potential indicated that TeA adsorption was related to the surface charge of the bacteria cells. Above all, polysaccharides and protein were demonstrated to be important components of the LAB cell wall and are involved in TeA removal.

1. Introduction

Tenuazonic acid ((5S, 8S)-3-acetyl-5-sec-butyltetramic acid, TeA) is a toxic metabolite produced by *Alternaria* spp., *Phoma sorghina* and *Pyricularia oryzae* (Meronuck, Steele, Mirocha, & Christensen, 1972; Umetsu, Kaji, & Tamari, 1972). TeA is thought to be a hybrid of 1 isoleucine and 2 acetates (Yun, Motoyama, & Osada, 2015). It can inhibit protein biosynthesis by suppressing the release of new proteins from the ribosome (Shigeura & Gordon, 1963). TeA has been reported to exert antiviral, antitumor, antibacterial, cytotoxic and phytotoxic properties and to be acutely toxic in mammals (Lou, Fu, Peng, & Zhou, 2013; Rychlik, Lepper, Weidner, & Asam, 2016; Yekeler, Bçitmçis, Ö, Doymaz, & Alta, 2001). The oral medium lethal dose (LD_{50}) of TeA was shown to be 225 mg/kg body weight (bw) in mice and 100–150 mg/kg bw in *Macaca fascicularis*; thus, TeA is considered to be the most toxic *Alternaria* mycotoxins (Ostry, 2008). TeA-producing fungi are ubiquitous in many biological environments and are able to infest most plant species (Gross, Curtui, Ackermann, Latif, & Usleber, 2011). Consequently, TeA was found in foods derived from plants, especially in flour and bakery products (Janic et al., 2016; Siegel, Rasenko, Koch, & Nehls, 2009; Zhao, Shao, Yang, Li, & Zhu, 2015), tomatoes and their processing products (Da Motta & Valente Soares, 2000; Siciliano et al., 2015), beverages (Abramson, Delaquis, & Smith, 2007; Siegel, Merkel, Koch, & Nehls, 2010), and even infant food (Asam & Rychlik, 2013; Gross, Asam, & Rychlik, 2017; Rychlik et al., 2016).

Mycotoxin decontamination by physical and chemical methods has been reviewed extensively elsewhere (Jouany, 2007; Kabak, Dobson, & Var , 2006). Although there are many different approaches available for mycotoxin decontamination, most of them are not popular due to high costs or practical difficulties involved in the detoxification process. In recent years, using biotechnology to control and remove mycotoxin contamination has received much attention and has gradually become a hot topic in the field of mycotoxin decontamination because of its potential applications (Bata & Lásztity, 1999; Dalié, Deschamps, & Richard-Forget, 2010; Ji, Fan, & Zhao, 2016; Shetty & Jespersen, 2006). Bacteria and yeasts have been studied for their potential ability to reduce the levels of mycotoxins, including aflatoxin B_1, ochratoxin A, zearalenone, *Fusarium* toxins, fumonisin, and patulin (Corassin, Bovo, Rosim, & Oliveira, 2013; El-Nezami, Kankaanpaa, Salminen, Ahokas, & Ahokas, 1998; El-Nezamiy, Chrevatidis, Auriola, Salminen, & Mykkanen,

* Corresponding author.
E-mail address: bzpeng@mail.hzau.edu.cn (B. Peng).

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2002; Fuchs et al., 2008; Guo, Yuan, Yue, Hatab, & Wang, 2012, 2013; Hernandez-Mendoza, Garcia, & Steele, 2009; Joannis-Cassan, Tozlovanu, Hadjeba-Medjdoub, Ballet, & Pfohl-Leszkowicz, 2011; Sangsila, Faucet-Marquis, Pfohl-Leszkowicz, & Itsaranuwat, 2016; Shetty, Hald, & Jespersen, 2007). Lactic acid bacteria (LAB), due in large part to their Generally Recognized as Safe (GRAS) status and use as probiotics, are of particular interest for reducing mycotoxins (Elsanhoty, Salam, Ramadan, & Badr, 2014; Haskard, El-Nezami, A P E KSalminen, & Ahokas, 2001; Hatab, Yue, & Mohamad, 2012). Recently, various physical, chemical and enzymatic treatments have been used to ascertain the potential mycotoxin adsorption sites on bacterial cells. Although the LAB mechanism for binding mycotoxins is not fully understood, some authors have supposed that the primary cellular components involved in this phenomenon are parietal poly-saccharides (Luo et al., 2015; Ringot et al., 2007; Vijayaraghavan & Yun, 2008; Wang et al., 2015). The LAB cell wall consists of a peptidoglycan matrix that forms a major structural component of the cell wall, which houses other components, such as teichoic and lipoteichoic acid, a proteinaceous S layer and neutral polysaccharides (Delcour, Ferain, Deghorain, Palumbo, & Hols, 1999). These components have various functions, including adhesion and macromolecular binding, especially the fibrillar network of teichoic acids and neutral polysaccharides. Adsorption on the cell wall surface is an interaction between the toxins and functional groups of the cell surface, based on physical adsorption, ion exchange and complexation. The cell walls harbouring polysaccharides (glucan, mannan), proteins and lipids exhibit numerous different and easily accessible adsorption centers as well as different binding mechanisms including hydrogen bonds, ionic or hydrophobic interactions (Faucet-Marquis, Joannis-Cassan, Hadjeba-Medjdoub, Ballet, & Pfohl-Leszkowicz, 2014). Yiannikouris et al. (2006) found that hydroxyl, ketone, and lactone groups are involved in the formation of both hydrogen bonds and van der Waals interactions between aflatoxins B1, deoxynivalenol, ZEA and patulin, and β-D-glucans.

The aim of this work was to further characterize the possible mechanisms involved in the removal of TeA by inactived LAB used as a biological adsorbent in an aqueous system. Fourier transform infrared (FTIR) spectroscopy analysis was used to search for the potential binding sites and possible functional groups of the tested strains. Then, Energy Dispersive X-ray Spectroscopy (SEM-EDS), transmission electron microscopy (TEM), zeta potential, and X-ray Diffraction (XRD) were used to assess the inactivated LAB before and after TeA adsorption.

2. Materials and methods

2.1. Chemicals and media

Lactobcillus brevis CICC 20023 (LAB-20023) was obtained from the China Center of Industrial Culture Collection (CICC) (Beijing, China). Stock cultures were maintained at −80 °C in 25% (v/v) glycerol.

Tenuazonic acid, copper (II) salt (purity 98%), was purchased from Toronto Research Chemicals (TRC, Canada). (CAS Registry No. 610-88-8; M.W. 197; Molecular formula $C_{10}H_{15}O_3N$). HPLC gradient grade acetonitrile (ACN) was purchased from Fisher Chemical (Fisher Scientific China); all other chemicals utilized in this study were of analytical-reagent grade, purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing China).

2.2. Preparation of TeA working solution

Commercial TeA copper (II) salt was dissolved in acetonitrile to obtain an approximate concentration of 100 μg/mL as standard stock solutions. Working solutions (500 μg/L) were prepared by further dilution with acidified water (pH4.0). All solutions were stored in the dark at −20 °C to ensure stability.

2.3. Bacterial cells preparation

Lactobacillus brevis 20023 (LAB-20023) was selected as the test strain because it has a higher specific surface area and cell wall volume (Wang et al., 2015) and showed higher capacity to adsorb TeA from the aqueous solution based on our report (Ge, Xu, Li, Peng, & Pan, 2017). LAB-20023 was cultured in a liquid medium and incubated at 30 °C and 120 rpm for 24 h. The composition of the culture medium for LAB-20023 was glucose, 10% (w/v); yeast extract, 0.75% (w/v); peptone, 0.75% (w/v); potassium hydrogen phosphate, 0.2% (w/v); and polysorbate 80, 0.05% (v/v). The pH of the solution was adjusted to 7 using 1 N NaOH. Then, a first round shaking culture was transferred into a new liquid medium as a second shaking culture under the same conditions as the first shaking culture. After the second incubation, bacterial cells were collected by centrifugation (5804R, Eppendorf Ltd., Germany) at 6000×*g* for 10 min, and the collected biomass was washed at least three times with distilled water.

2.4. Physical and chemical treatments of LAB cells

Heat treatment: LAB-20023 cells were killed at 121 °C for 20 min. Cell viability was detected with methylene blue staining. A drop of methylene blue (1%, w/v) was mixed with a drop of cells onto a microscope slide and viewed by light microscopy after 10 and 30 min (Guo et al., 2013).

Lysozyme treatment: LAB-20023 cells were chemically treated in vitro with glycine and lysozyme to induce their conversion to the cell wall defective (CWD). Briefly, mid log-phase cells were pre-treated with a 1% glycine solution in a liquid medium for 16 h at 30 °C. Glycine is an analog of D-alanine (an amino acid of the cell wall structure) that, when incorporated by bacteria, leads to the formation of leaky cell walls (pre-CWD cells). Pre-CWD forms were then harvested by centrifugation at 6000 × g for 10 min at room temperature and washed 2 times with phosphate buffered saline (PBS). Cells were re-suspended in a PBS solution supplemented with 20 mg/mL lysozyme. The lysozyme treatment was achieved for 2 h at 37 °C. Cells were then centrifuged at 6000×*g* for 6 min to remove intact bacillary forms and an autoclave (121 °C, 20 min) (Rosu, Bandino, & Cossu, 2013).

Formaldehyde treatment: 10.0 g of inactivated (121 °C, 20 min) LAB-20023 cells was suspended in a 250-mL Erlenmeyer flask containing 50 mL of formaldehyde and 100 mL of formic acid under magnetic stirring for 6 h at room temperature. The main reaction is as follows:

$$RCH_2NH_2 \xrightarrow{HCHO+HCOOH} RCH_2N(CH_3) + CO_2 + H_2O$$

The chemical reaction is mainly to shield the amino group of the cell wall to reveal the other functional groups.

Acetone treatment: 10.0 g of inactivated bacteria was suspended in 100 mL of acetone under magnetic stirring for 6 h. Acetone treatment was performed to dissolve organic matter inside cells to obtain cell walls.

Caustic treatment: Caustic treated cells were prepared by mixing 10.0 g of inactivated cells with 100 mL of 0.1 M NaOH under magnetic stirring for 6 h. NaOH treatment can hydrolyze the ester groups of the cell wall to form carboxyl and hydroxyl groups.

Methanol treatment: The 10.0 g of inactivated bacteria was mixed with 100 mL of anhydrous methanol and 2 mL of concentrated hydrochloric acid under magnetic stirring for 6 h. The main