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Original Research Article

Influence of pro- and prebiotics on gastric, duodenal and colonic bioaccessibility of the mycotoxin beauvericin

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ABSTRACT

Beauvericin (BEA) is a bioactive compound produced by the secondary metabolism of several *Fusarium* strains and known to have various biological activities. This study investigates the influence of several dietary fibers (galactomanan, glucomannan, citrus fiber, bamboo fiber, carrot fiber, pie fiber, β-glucan, xilan, and cellulose) and probiotic strains (*Lactobacillus animalis*, *Lb. casei*, *Lb. casei*, *Lb. plantarum*, *Lb. rhuminis*, *Lb. casei casei*, *Bifidobacterium breve*, *Bf. Adolescents*, *Bf. bifidum*, *Corynebacterium vitaeruminis*, *Streptococcus faecalis*, *Eubacterium crispatus*, and *Saccharomyces cerevisiae*) on the minor *Fusarium* mycotoxin BEA bioaccessibility employing a model solution. The bioaccessibility was determined using a simulated gastrointestinal digestion that mimics the physiological conditions of the digestive tract until the colonic compartment. The determination of BEA in the intestinal fluids was carried out by liquid chromatography–mass spectrometry detection (LC–MS). The reduction of BEA bioaccessibility in the experiments carried out using the prebiotic compounds ranged from 60 to 80%, whereas in the trials carried out using the probiotic strains the bioaccessibility observed ranged from 30 to 85%. A BEA degradation product produced by colonic fermentation was identified using the technique of LC–MS-LIT.

1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide consisting of alternating D-α-hydroxy-isovaleryl and aromatic N-methyl-phenylalanine. This toxin is produced by various *Fusarium* species such as *Fusarium avenaceum*, *F. poae*, *F. oxysporum* and *F. proliferatum*, and naturally occurs on maize, wheat, barley, rice and oat (Logrieco et al., 1998; Uhlig et al., 2006; Jestoi, 2008; Sorensen et al., 2008; Kokkonen et al., 2010; Waskiewicz et al., 2010). BEA has been detected in grains throughout the world under different climates (South Africa, Poland, Norway, Spain, Croatia), with concentrations ranging from trace level up to 520 mg/kg in maize in Italy (Ritieni et al., 1997). Meca et al. (2010) have shown that BEA was present in cereals (barley, corn and rice) purchased in Spanish markets, with levels ranging from 0.51 to 11.78 mg/kg.

An in vivo study has shown that mice orally exposed to BEA presented an increase of mortality with a Lethal Dose 50 (LD_{50}) superior to 100 mg/kg bw (Jestoi, 2008). The cytotoxicity of BEA has been demonstrated in vitro in several cell line models, including human leukemia cells CCRF-CEM, human monocytic lymphoma cells U-937 and promyelocytic leukemia cells HL-60, monkey kidney epithelial cells Vero, Chinese hamster ovary cells CHO-K1 and murine macrophage J774 (Tomoda et al., 1992; Calo et al., 2004; Jow et al., 2004; Ruiz et al., 2011a,b).

In the analysis of the risk evaluation related to human health, food ingestion is considered one of the important routes of exposure of many contaminants (Carolien et al., 2005).

To achieve any effects in a specific tissue or organ, the mycotoxins must be available, which refers to the compound's tendency to be extracted from the food matrix, and they must then be absorbed from the gut via the intestinal cells (Fernández-García et al., 2009). The term bioaccessibility has been defined as the fraction of a bioactive compound present in a food matrix that is not modified structurally through the reactions related to the gastrointestinal digestion and thus become available for intestinal absorption (Fernández-García et al., 2009).

Probiotics are defined as 'live microorganisms which when administered in adequate amount confer health benefits to the host' (FAO/WHO, 2002). Alternatively, probiotics have been defined as live microbial feed supplements that beneficially affect

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the host animal by improving its intestinal microbial balance. Probiotics were originally used to improve the health of both animals and humans through the modulation of the intestinal microbiota. At present, several well-characterized strains of Lactobacilli and Bifidobacteria are available for human use to reduce the risk of gastrointestinal (GI) infections or treat such infections (Salminen et al., 2005). Some of the beneficial effects of probiotic consumption include improvement of intestinal health by the regulation of microbiota, and stimulation and development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing symptoms of lactose intolerance, and reducing the risk of certain other diseases (Kumar et al., 2010, 2011; Nagpal et al., 2007, 2010; Yadav et al., 2008).

The concept and understanding of prebiotics have been evolving over time as new information emerges. 'Prebiotic' was first defined as a non-digestible food ingredient that beneficially affected the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon. Recent literature, however, does not restrict the colon as the only action site and defines a prebiotic as a selectively fermented ingredient that allows specific changes in the composition and/or activity of the gastrointestinal microbiota that confer benefits upon health and wellbeing of the host (Figueroa-Gonzalez et al., 2011). Thus not only are prebiotics being examined for antipathogenic effects (such as inhibiting adhesion of pathogenic organisms to the gut mucosa), but they are also being developed to decrease fecal transit time, lower cholesterol and the glycemic response, improve bone health, lower daily energy (fat) intake, relieve symptoms of inflammatory bowel disease, and attempt to lower colon cancer rates (Pineiro et al., 2008).

In the scientific literature, only a few articles are available on the influence of prebiotics on the bioaccessibility of the minor *Fusarium* mycotoxins (Meca et al., 2012a,b), whereas the influence of the probiotics on the stability of this bioactive compound during gastrointestinal digestion has never been studied. For these reasons the aims of this study were (a) to evaluate the influence of several soluble and insoluble prebiotics on BEA bioaccessibility, (b) to evaluate how different probiotic strains can influence BEA's bioaccessibility and (c) to determine the possible adduct with BEA and fibers or the degradation products produced by bacteria by LC–MS-LIT.

2. Materials and methods

2.1. Materials

Potassium chloride (KCl), potassium thiocyanate (KSCN), monosodium phosphate (NaH_2PO_4), sodium sulfate (Na_2SO_4), sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), urea, a-amylase, hydrochloric acid (HCl), sodium hydroxide (NaOH), formic acid, pepsin, pancreatin, bile salts, phosphate buffer saline (PBS, pH 7.5), galactomanan, β-glucan, xylan, cellulose high molecular weight (HMW), and cellulose medium molecular weight (MMW) were obtained from Sigma–Aldrich (Madrid, Spain). Glucomannan high molecular weight (HMW), glucomannan fine powder, citrus fiber, bamboo fiber, carrot fiber, and pie fiber were generously provided by Prof. Alberto Ritieni of the University of Naples "Federico II".

Acetonitrile, methanol and ethyl acetate were purchased from Fisher Scientific (Madrid, Spain). Deionized water (<18 MΩ cm resistivity) was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Chromatographic solvents and water were degassed for 20 min using a Branson 5200 (Branson Ultrasonic Corp., CT, USA) ultrasonic bath. The BEA used in this study were produced and purified according to the method of Meca et al. (2010).

2.2. Bacterial strains and growth conditions

Thirteen commercial probiotic strains were obtained for the in vitro system that simulates the physiologic condition of the colonic intestinal compartment. In particular *Lactobacillus paracasei* CECT 277, *Lb. casei* CECT 4180, *Lb. rhamnosus* CECT 278T, *Lb. plantarum* CECT 220, *Lb. ruminis* CECT 4061T, *Lb. casei casei* CECT 277, *Bifidobacterium breve* CECT 4839T, *Bf. adolescentes* CECT 5781T and *Bf. bifidum* CECT 870T, *Bf. Longum* CECT 4551, *Corynebacterium vitaeruminis* CECT 537, *Eubacterium crispatus* CECT 4840, *Saccharomyces cerevisiae* CECT 1324 were obtained at the Spanish Type Culture Collection (CECT Valencia, Spain), in sterile 18% glycerol. For longer survival and higher quantitative retrieval of the cultures, they were stored at −80 °C. When needed, recovery of strains was undertaken by two consecutive subcultures in appropriate media prior to use (Meca et al., 2012a).

2.3. Model solution preparation

The stock model solution used in this study to reproduce the food model was composed of water, glucose (1%), starch (5%), albumin (3%), sodium chloride (NaCl) (0.1%), and triolein (2%). The model solutions (with fibers) were prepared in 100 mL Erlenmeyer flasks and spiked with 1 and 5 g of each dietary fiber. In the study of the probiotic strains influence on the BEA bioaccessibility, the model solution was inoculated with 2×10^6 of each bacterial strain tested. Solutions were mixed using ultrasound bath (Lab Police, Barcelona, Spain) operating at a temperature of 30 °C, and then 10 mL of each solution were contaminated with 10 mg BEA/L. Contamination of the solutions was carried out using a BEA solution stock (1 g/L) in methanol.

2.4. In vitro digestion model

The procedure was adapted from the method outlined by Gil-Izquierdo et al. (2002), with slight modifications. The method consists of three sequential steps; an initial saliva/pepsin/HCl digestion for 2 h at 37 °C, to simulate the mouth and the gastric conditions, followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate duodenal digestion (Fig. 1). The colonic conditions were simulated by adding to the duodenal simulated fluid some bacteria representative of the gastrointestinal tract. For the saliva/pepsin/HCl digestion, 10 mL of the model solution or 10 g of the crispy bread contaminated with 5 and 25 mg/kg of BEA, were mixed with 6 mL of artificial saliva composed by: 10 mL of KCl 89.6 g/L, 10 mL of KSCN 20 g/L, 10 mL of NaH_2PO_4 88.8 g/L, 10 mL of Na_2SO_4 57 g/L, 1.7 mL of NaCl 175.3 g/L, 20 mL $NaHCO_3$ 84.7 g/L, 8 mL of urea 25 g/L, and 290 mg of a-amylase. The pH of this solution was corrected to 6.8 with NaOH 0.1 N. These mixtures composed of model solutions and the artificial saliva were placed in plastic bags, containing 40 mL of water and homogenized using a Stomacher IUL Instruments (Barcelona, Spain) during 30 s.

To this mixture, 0.5 g of pepsin (14,800 U) dissolved in 25 mL of HCl 0.1 N was added. The pH of the mixture was corrected to a value of 2 with 6 N HCl, and then incubated in a 37 °C orbital shaker (250 rpm) (Infors AG CH-4103, Bottmingen, Switzerland) for 2 h.

After gastric digestion, pancreatic digestion was simulated. The pH was increased to 6.5 with NaHCO3 (0.5 N) and then 5 mL of (1:1; v/v) pancreatin (8 mg/mL):bile salts (50 mg/mL), dissolved in 20 mL of water, was added and incubated in a 37 °C orbital shaker (250 rpm) for 2 h. An aliquot of 5 mL of the duodenal fluid was sampled for the extraction of the BEA and the determination of the duodenal bioaccessibility.

To mimic the colonic compartment bacterial strains (previously described) were grown in a sterile plastic centrifuge tube overnight at 37 °C in MRS broth (Oxoid, Madrid, Spain) under anaerobic