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Reduction *in vitro* of the minor *Fusarium* mycotoxin beauvericin employing different strains of probiotic bacteria

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ABSTRACT

The interaction between the minor *Fusarium* mycotoxins BEA and 13 bacterial strains characteristic of the gastrointestinal tract as *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium adolescentes*, *Lactobacillus rhamnosus*, *Lactobacillus casei-casei*, *Lactobacillus plantarum*, *Eubacterium crispatus*, *Salmonella fecalis*, *Salmonella termofilus*, *Lactobacillus ruminis*, *Lactobacillus casei* and *Lactobacillus animalis* was studied.

The fermentations were carried out in the liquid medium of MRS during 4, 12, 16, 24 and 48 h at 37 °C, under anaerobic conditions.

Levels of BEA in the fermentation liquid, on the cell walls and on the internal part of the cells were determined using liquid chromatography coupled to the mass spectrometry detector (LC-MS/MS). Results showed that the bacteria reduced the concentration of the BEA present in the medium, part of the mycotoxin was adsorbed by cell wall and part internalized by the bacteria. All the bacteria analyzed in this study showed a significant BEA reduction during the fermentation process, in particular the mean diminution resulted variable from 66 to the 83%.

1. Introduction

Beauvericin (BEA) is a cyclic hexadepsipeptide mycotoxin which was originally isolated from *Beauveria bassiana* and later from *Paecilomyces fumosoroseus* and *Fusarium lateritium* (Meca et al., 2010). BEA has strong antibacterial, antifungal, and insecticidal activities (Castlebury, Sutherland, Tanner, Henderson, & Cerniglia, 1999), and has also shown significant cytotoxic activity toward various human cancer cell lines. The notable antimicrobial and cytotoxic activities of BEA have attracted research interest in its application as a potential antibiotic and anticancer agent for human health care (Jow, Chou, Chen, & Tsai, 2004; Zhang, Yan, Zhang, Huang, & Chen, 2007).

BEA is most widely produced by many entomopathogenic *Fusarium* fungal species (Fotso, Leslie, & Smith, 2002). In addition to BEA, enniatins, which belong to another class of cyclicpeptides having entomopathogenic activities, are also produced by many of these *Fusarium* species. Nevertheless, BEA is the most common and abundant mycotoxin, and plays a major role in the insecticidal activities of these *Fusarium* species (Moretti, Mule, Ritieni, & Logrieco, 2007).

As regard the antibacterial activity of the BEA, Castlebury et al. (1999) studied the action of this bioactive compound on bacteria typically isolated from mammalian intestinal tract to understand the interaction of the BEA with the normal intestinal microbiota that has an important role in nutrition, physiology, and colonization resistance to invasive pathogens. Also, Fotso and Smith (2003) evaluated the BEA mutagenicity, utilizing the Ames test on *Salmonella typhimurium* standard tester strain.

BEA has exhibited minimum inhibitory concentration (MIC) values of 0.8–1.6 μg/mL against *Mycobacterium tuberculosis*, and IC_{50} values of 1.3–2.4 μg/mL against *Plasmodium falciparium* (Nilanota, Isaka, Kittakoop, & Trakulnaleamsai, 2002).

The potential toxic role of BEA is exemplified by results from *in vitro* studies using cell lines. For instance, BEA induces significant cell deaths in insect, murine, and human tumor cell lines (Calo et al., 2003; Mazziotti & Perlmutter, 1998). Furthermore, BEA is a potent and specific cholesterol acyltransferase inhibitor in rat liver microsomes (Tomoda et al., 1992).

In mammalian cell lines, cell deaths caused by BEA have been suggested to involve a Ca^{2+} dependent pathway, in which BEA induces a significant increase in intracellular Ca^{2+} concentration

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that leads death cell as a result of a combination of both apoptosis and necrosis (Jow et al., 2004; Lin et al., 2005; Logrieco et al., 1998; Nilanota et al., 2002; Ojcius, Zychlinsky, Zheng, & Young, 1991).

BEA is considered a normal contaminant of cereals and also of products composed by cereals (Zinedine, Meca, Mañes, & Font, 2011). In particular, the presence of BEA in food commodities has been recently reported during the last decade in some European countries (Finland, Norway, Spain, Slovakia, Croatia, Switzerland and Italy), in USA, in South Africa (Jestoi, 2008; Meca, Zinedine, Blesa, Font, & Mañes, 2010; Munkvold, Stahr, Logrieco, Moretti, & Ritieni, 1998; Ritieni et al., 1997; Shephard, Sewram, Nieuwoudt, Marasas, & Ritieni, 1999; Zinedine et al., 2011) in higher concentrations (milligrams per kilograms) respect to the classical legislate *Fusarium* mycotoxins as the fumonisins or the trichothecenes.

As regards the methodologies employed for the reduction of this contaminant in food, in the scientific literature is present only a US patent (Duvick & Rod, 1998) on the biological detoxification of the minor *Fusarium* mycotoxin BEA. In particular the authors, employing as detoxification agent a strain of *Nocardia Glubera*, reduced the contamination by BEA in wheat kernels of 50% considering an initial contamination of the mycotoxin of 1000 mg/L.

The biological detoxification strategies for the reduction of the other *Fusarium* mycotoxins were studied by many authors. In particular Young, Zhou, Yu, Zhu, and Gong (2007) evaluated the degradation of 12 trichothecenes by chicken intestinal microbes observing a complete conversion to the deepoxy metabolites of the non-acylated trichothecenes and a deacetylation of the monoacetyl trichothecenes.

Guan et al. (2009), evaluated the transformation of several trichothecenes mycotoxins by microorganism isolated by fish digesta, evidencing a completely transformation of the mycotoxin deoxynivalenol (DON) to deepoxy DON (dE-DON) at 15 °C in full medium after 96 h incubation. The authors evidenced also that most of the other trichothecenes were transformed to deacetyl and/or deepoxy products.

Islam, Zhou, Young, Goodwin, and Pauls (2011) studied the aerobic and anaerobic deepoxydation of mycotoxin DON by bacteria originating from agricultural soil. In particular the bacteria isolated by the authors and related to the family of *Serratia*, *Clostridium*, *Citrobacter*, *Enterococcus*, *Stenotrophomonas*, *Streptomyces* produced through an enzyme process the de-epoxydized DON after 60 h of incubation. Bacterial deepoxydation of DON occurred also in the pH range 6.0–7.5, and a wide array of temperatures (12–40 °C).

Considering the lack of data on the biological detoxification of the minor *Fusarium* mycotoxins the aim of this study was to evaluate the reduction *in vitro* of the minor *Fusarium* mycotoxin BEA employing different strains of probiotic bacteria as detoxification agents.

2. Material and methods

2.1. Chemicals

A stock standard solution of BEA (98% of purity) (Sigma–Aldrich, St. Luis, USA) was prepared by dissolving 1 mg of standard in 1 mL of pure methanol, obtaining a 1 mg BEA/mL (1000 μg/mL) solution. This stock solution was then diluted with methanol in order to obtain the appropriated work solutions with concentrations of 1, 10 and 100 mg/L. All BEA solutions were stored in darkness at 4 °C until the LC-MS/MS analysis. Acetonitrile, methanol, water, ethyl acetate (all of LC grade) and acetic acid were purchased from Merck (Whitehouse Station, NJ, USA).

2.2. Strains and methodology

The study was carried out using 13 strains probiotic bacteria, named *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium adolescentes*, *Lactobacillus rhamnosus*, *Lactobacillus casei-casei*, *Lactobacillus plantarum*, *Eubacterium crispatus*, *Salmonella fecalis*, *Salmonella termofilus*, *Lactobacillus ruminis*, *Lactobacillus casei* and *Lactobacillus animalis*.

The strains 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.

The microbes were cultured in 15 mL sterile plastic centrifuge tubes utilizing as growth medium 10 mL of DeMan-Rogosa-Sharpe (MRS broth, Oxoid Madrid, Spain) and incubated at 37 °C in an anaerobic atmosphere with 95% CO_2 and 5% H_2 during 48 h. After that each bacterial suspensions at concentrations of 10^8 CFU/mL was added to a fresh 10 mL of MRS contaminated with 5 mg/L of the mycotoxin BEA, incubated at 37 °C in an anaerobic atmosphere with 95% CO_2 and 5% H_2 during 4, 12, 16, 24 and 48 h.

The mediums were analyzed in order to determinate the residual concentration of BEA present in the growth medium, the toxin concentration adsorbed by bacteria on the cells wall, and also the BEA amount internalized in the cells.

2.3. BEA extraction from fermented mediums

The fermentation tubes were centrifuged at 4000 rpm (Centrifuge 5810R, Eppendorf, Germany) during 5 min at 4 °C in order to separate the fermented medium from the cells. BEA contained in fermented medium was extracted as follows (Jestoi, 2008). Five mL of fermented MRS were introduced in a 20 mL plastic tube, and extracted three times with 5 mL of ethyl acetate using a vortex VWR international (Barcelona, Spain) during 1 min. After that the mixtures were centrifuged (Centrifuge 5810R, Eppendorf, Germany) at 4000 rpm for 10 min at 4 °C. The organic phases were completely evaporated by a rotary evaporator (Buchi, Switzerland) operating at 30 °C and 30 mbar pressure, resuspended in 1 mL of methanol, filtered with 0.22 μM filters (Pheomenex, Madrid, Spain) and analyzed by LC-MS/MS (Meca, Blaiotta, & Ritieni, 2010; Meca, Zinedine, et al., 2010).

2.4. BEA extraction from cells wall

The BEA adsorbed on the cell walls was extracted suspending the pellet, separated from the fermented medium, in 1 mL of saline solution (0.90% NaCl, w/v) in a 1.5 mL vial. The extraction was carried out by agitation with an orbital shaker (IKA Ks 260 basic, Stanfen, Germany) for 12 h, then the samples were centrifuged at 4000 rpm (Centrifuge 5810R, Eppendorf, Germany) for 10 min at 4 °C and the upper layer was filtered with 0.22 μM filters (Pheomenex, Madrid, Spain) and analyzed by LC-MS/MS (Meca, Blaiotta, et al., 2010; Meca, Zinedine, et al., 2010).

2.5. BEA extraction from cells

The BEA contained in the cells was determined as follows: cells were sonicated in a saline solution (0.90% NaCl, w/v) for 30 min. The pellet was suspended in 5 mL of saline solution, and 5 mL of ethyl acetate was added. After mixing with a vortex for 1 min and centrifuging (4000 rpm for 10 min at 4 °C, Centrifuge 5810R, Eppendorf, Germany), the upper layer, 5 mL approximately, were

Table 1
Beauvericin (BEA) contents (mg/L) in the fermentation medium MRS inoculated with different strains of probiotic bacteria during fermentation.

	Fermentation time					
	BEA mg/L					
	4 h	12 h	16 h	24 h	48 h	Reduction (%)
Bb. longum	3.6 ± 0.2	3.0 ± 0.2	2.2 ± 0.3	1.8 ± 0.1	1.3 ± 0.1	74.0
Bb. bifidum	3.1 ± 0.3	2.8 ± 0.3	1.9 ± 0.2	1.6 ± 0.1	1.3 ± 0.2	74.5
Bb. breve	3.3 ± 0.3	2.7 ± 0.2	2.0 ± 0.1	1.7 ± 0.2	1.3 ± 0.1	74.5
Bb. adolescentis	3.3 ± 0.3	3.2 ± 0.3	1.8 ± 0.3	1.4 ± 0.1	1.2 ± 0.1	76.2
Lb. rhamnosus	3.4 ± 0.1	3.0 ± 0.3	1.5 ± 0.2	1.2 ± 0.2	0.8 ± 0.1	83.1
Lb. casei-casei	2.3 ± 0.4	1.9 ± 0.1	2.0 ± 0.2	1.7 ± 0.2	1.3 ± 0.1	73.7
Lb. plantarum	3.5 ± 0.2	3.0 ± 0.3	2.0 ± 0.1	1.9 ± 0.2	1.2 ± 0.1	76.5
E. crispatus	3.4 ± 0.4	2.8 ± 0.3	1.5 ± 0.1	1.4 ± 0.3	1.2 ± 0.3	75.2
S. fecalis	3.3 ± 0.4	3.1 ± 0.4	2.9 ± 0,3	1.5 ± 0.1	1.7 ± 0.4	66.5
S. termofilus	3.7 ± 0.5	3.2 ± 0.3	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	73.4
Lb. ruminis	3.3 ± 0.2	2.0 ± 0.2	1.8 ± 0.3	1.6 ± 0.3	1.5 ± 0.3	69.4
Lb. casei	3.2 ± 0.1	3.0 ± 0.3	1.7 ± 0.3	1.7 ± 0.2	1.1 ± 0.2	77.7
Lb. animalis	3.7 ± 0.3	3.1 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	78.0

evaporated by a rotary evaporator (30 °C and 30 mbar pressure) (Buchi, Switzerland), resuspended in 1 mL of methanol, filtered with 0.22 μM filters (Pheomenex, Madrid, Spain) and analyzed by LC-MS/MS (Meca, Blaiotta, et al., 2010; Meca, Zinedine, et al., 2010).

2.6. LC-MS/MS analysis of BEA

LC analysis of BEA was carried out with a TQ mass spectrometer Quattro LC from Micromass (Manchester, UK), equipped with an LC Alliance 2690 system (Waters, Milford, MA) consisted of an autosampler and a quaternary pump, a pneumatically assisted electrospray probe, a Z-spray interface. Mass Lynx NT software 4.1 was used for data acquisition and processing. The autoinjector was programmed to inject 20 μL into the Luna C18 column (150 × 4.6 mm, 5 μm) Phenomenex maintained at 30 °C. The analytical separation for LC-MS/MS was performed using gradient elution with water as mobile phase A, and acetonitrile as mobile phase B, both containing 15 mM of ammonium formate. After an isocratic step of 65% B for 3 min, it was linearly increased to 75% B in 4 min and held constantly for 3 min. Flow rate was maintained at 0.3 mL min. Analysis was performed in positive ion modes. The ESI source values were as follows: capillary voltage, 3.20 kV; source temperature, 125 °C; desolvation temperature, 350 °C; desolvation gas (nitrogen, 99.99% purity) flow, 700 L/h. Identification and quantification, in which protonated molecule $[M + H]^+$ of the analyte (BEA 784,34) was fragmented in the collision cell to the product-ions (BEA 784,34-244,23).

The samples corresponding to the BEA present in the fermented medium were also injected in the modality full scan with a *m*/*z* of 150–1500, to verify also the presence in the processed mediums of some possible adducts produced by the interaction of the mycotoxin BEA with the cell wall of the probiotic bacteria (Meca et al., 2010).

3. Results and discussions

The potential reduction of the bioactive compound was monitored during 4, 12, 16, 24 and 48 h. In the Table 1, are represented the data related to the BEA present in the fermentation mediums during the incubation time employed. In the first 4 h incubation, BEA decrease from 5 mg/L until a mean value of 3.31 mg/L. The highest reduction value was evidenced by the strain of *Lb. casei-casei* with a residual concentration of the bioactive compound in the fermentation medium of 2.3 ± 0.4 mg/L. At 12 h incubation the mean BEA quantity decrease from 5 mg/L until 2.8 mg/L. In the fermentation mediums fermented by *Lb. casei-casei* and *Lb. ruminis* the residual BEA concentration was of 1.9 ± 0.1 and 2.0 ± 0.2 mg/L respectively, evidencing at only 12 h incubation more than 50% of degradation of the mycotoxin studied. From 16 to 24 h BEA decreased meanly in the fermentation mediums from 1.8 to 1.5 mg/L. In these incubation hours was possible to evidence a good degradation activity by the strains of *Lb. rhamnosus*, *Eubacterium crispatus* and *Lb. animalis* that evidenced at 24 h incubation, residual BEA concentrations of 1.2 ± 0.2, 1.4 ± 0.3 and 1.3 ± 0.2 mg/L respectively. At 48 h incubation the probiotic bacteria employed produced a mean BEA percent reduction of 74.8%, considering the 5 mg/L introduced as initial concentration. The bacterium that reduced mainly the compound studied was the strain of *Lb. rhamnosus*, with 83.1% of reduction. Good reduction values were also evidenced by the strains of *Lb. plantarum*, *Lb. casei* and *Lb. animalis* with 76.5, 77.7 and 78.0% respectively. As possible to evidence in the Table 2, was also determined the percent of the BEA adsorbed on the cell wall by the probiotic bacteria employed in this study. In particular, the concentrations adsorbed are in the order of micrograms per liters, very low concentrations considering the 5 mg/L introduced of the initial concentration. In particular as remarked in the Table 2, at 48 h incubation, the mean adsorption value was of 4.2% considering all the strains employed. The highest absorption properties were evidenced by the strains of *Lb. rhamnosus* and *Lb. animalis* with 8.8 and 7.7% respectively. Summarizing, very few quantities of the bioactive compound studied were adsorbed by the bacteria employed.

Table 2
BEA adsorbed on the cell wall during the fermentation process.

	Fermentation time					
	BEA μg/L					
	4 h	12 h	16 h	24 h	48 h	Absorption (%)
Bb. longum	46.5 ± 2.4	57.3 ± 2.2	76.2 ± 2.3	97.3 ± 2.2	127.2 ± 2.2	2.5
Bb. bifidum	120.0 ± 3.2	125.3 ± 3.2	162.3 ± 3.5	161.2 ± 2.3	198.3 ± 2.6	4.0
Bb. breve	64.2 ± 2.6	101.2 ± 3.3	182.2 ± 3.3	205.4 ± 3.3	229.5 ± 3.1	4.6
Bb. adolescentis	76.6 ± 2.6	111.0 ± 3.2	143.5 ± 3.4	163.5 ± 3.6	176.7 ± 1.8	3.5
Lb. rhamnosus	42.6 ± 1.6	57.1 ± 3.2	214.3 ± 3.6	396.4 ± 4.2	434.5 ± 3.5	8.7
Lb. casei-casei	65.7 ± 3.2	102.0 ± 2.4	126.3 ± 4.2	139.2 ± 3.6	180.4 ± 1.6	3.6
Lb. plantarum	51.0 ± 2.5	55.1 ± 2.6	127.1 ± 2.4	170.2 ± 1.6	198.4 ± 2.3	4.0
E. crispatus	56.8 ± 2.2	96.2 ± 2.7	141.2 ± 2.6	172.4 ± 2.4	201.4 ± 2.2	4.0
S. fecalis	25.7 ± 2.3	48.0 ± 1.5	52.8 ± 1.6	92.6 ± 1.2	123.6 ± 1.6	2.5
S. termofilus	27.5 ± 1.5	48.4 ± 1.6	109.0 ± 2.2	136.5 ± 1.5	146.8 ± 1.5	2.9
Lb. ruminis	71.0 ± 2.3	78.4 ± 2.5	103.2 ± 2.3	167.2 ± 1.4	187.7 ± 1.5	3.7
Lb. casei	96.6 ± 3.2	99.9 ± 2.4	105.4 ± 2.6	144.2 ± 1.2	169.4 ± 1.8	3.4
Lb. animalis	43.0 ± 2.5	45.4 ± 2.1	53.9 ± 1.6	361.4 ± 2.8	387.2 ± 3.2	7.7

Table 3
BEA internalized in the cells during the fermentation process.

	Fermentation time					
	BEA mg/Kg					
	4 h	12 h	24 h	16 h	48 h	Internalized (%)
Bb. longum	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.2	1.7 ± 0.2	3.8 ± 0.3	76.0
Bb. bifidum	0.9 ± 0.09	1.8 ± 0.3	2.1 ± 0.2	2.2 ± 0.3	4.0 ± 0.5	79.8
Bb. breve	1.6 ± 0.07	1.7 ± 0.1	2.4 ± 0.3	2.7 ± 0.1	3.7 ± 0.6	74.3
Bb. adolescentis	1.0 ± 0.08	1.1 ± 0.1	2.0 ± 0.3	2.3 ± 0.2	3.8 ± 0.4	75.2
Lb. rhamnosus	0.6 ± 0.07	1.2 ± 0.2	1.9 ± 0.2	3.0 ± 0.3	3.9 ± 0.3	77.8
Lb. casei-casei	0.9 ± 0.08	1.5 ± 0.2	1.9 ± 0.4	2.7 ± 0.4	3.8 ± 0.5	75.6
Lb. plantarum	1.0 ± 0.1	2.1 ± 0.3	2.2 ± 0.1	2.6 ± 0.3	3.9 ± 0.5	77.6
E. crispatus	0.6 ± 0.07	0.6 ± 0.08	1.8 ± 0.3	2.1 ± 0.1	2.2 ± 0.2	44.0
S. fecalis	0.5 ± 0.05	0.6 ± 0.07	1.7 ± 0.3	2.0 ± 0.3	2.1 ± 0.3	42.0
S. termofilus	0.7 ± 0.07	1.3 ± 0.3	1.6 ± 0.2	1.8 ± 0.2	1.0 ± 0.1	68.3
Lb. ruminis	1.0 ± 0.1	1.1 ± 0.07	1.4 ± 0.1	1.9 ± 0.3	3.2 ± 0.3	64.4
Lb. casei	0.9 ± 0.1	1.1 ± 0.1	1.3 ± 0.3	1.6 ± 0.1	3.6 ± 0.5	72.5
Lb. animalis	1.2 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.9 ± 0.3	2.5 ± 0.3	50.8

To understand if part of the BEA degraded by the probiotic bacteria employed can be absorbed by the strains tested, also the BEA internalized by the cells was analyzed (Table 3). BEA concentrations internalized by the cells increase during the fermentation times and in particular from the mean value of 0.9 mg/kg evidenced in the first 4 h, until to a mean value of 3.3 mg/kg at 48 h incubation. Evaluating the absorption data at 48 h incubation, the mean BEA percentage internalized by the cells employed was of 67.5%. As is possible to show in the Table 3, the majority part of the bioactive compound employed in this study was absorbed by the cells, reducing its concentration from the fermentation medium.

To identify some possible adduct between the BEA and some cells metabolites, the extracts corresponding to the BEA present in the fermented mediums were injected in the LC-MS in the full scan mode with a *m*/*z* a range variable from 150 to 1500 Da.

As is possible to observe in the Fig. 1, near BEA peak is possible to visualize another peak, not present in the control test, (medium BEA free and fermented at the same incubation times), with a mass spectra is evidenced in the Fig. 2.

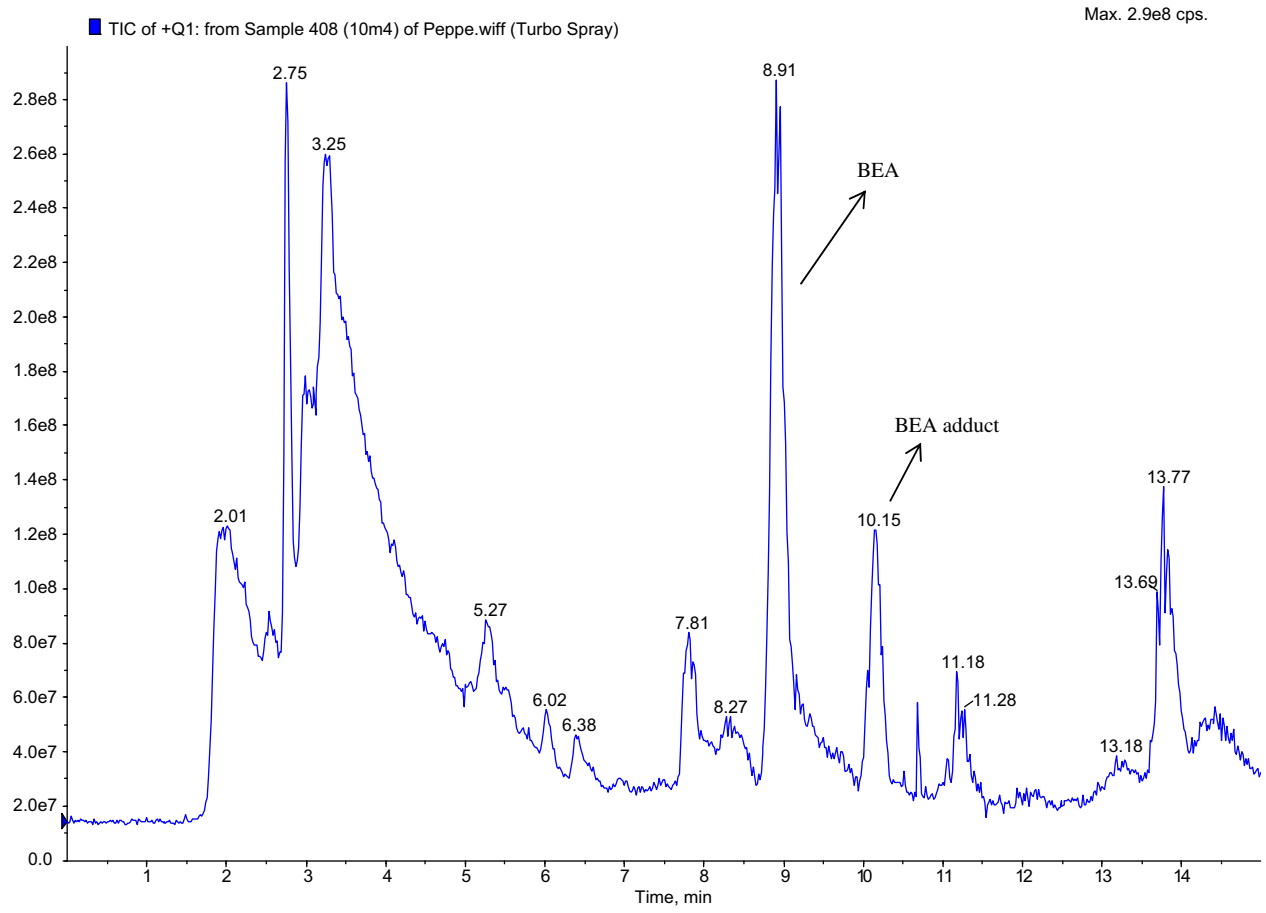

Fig. 1. LC-MS scan chromatogram of the BEA present in the fermented mediums.

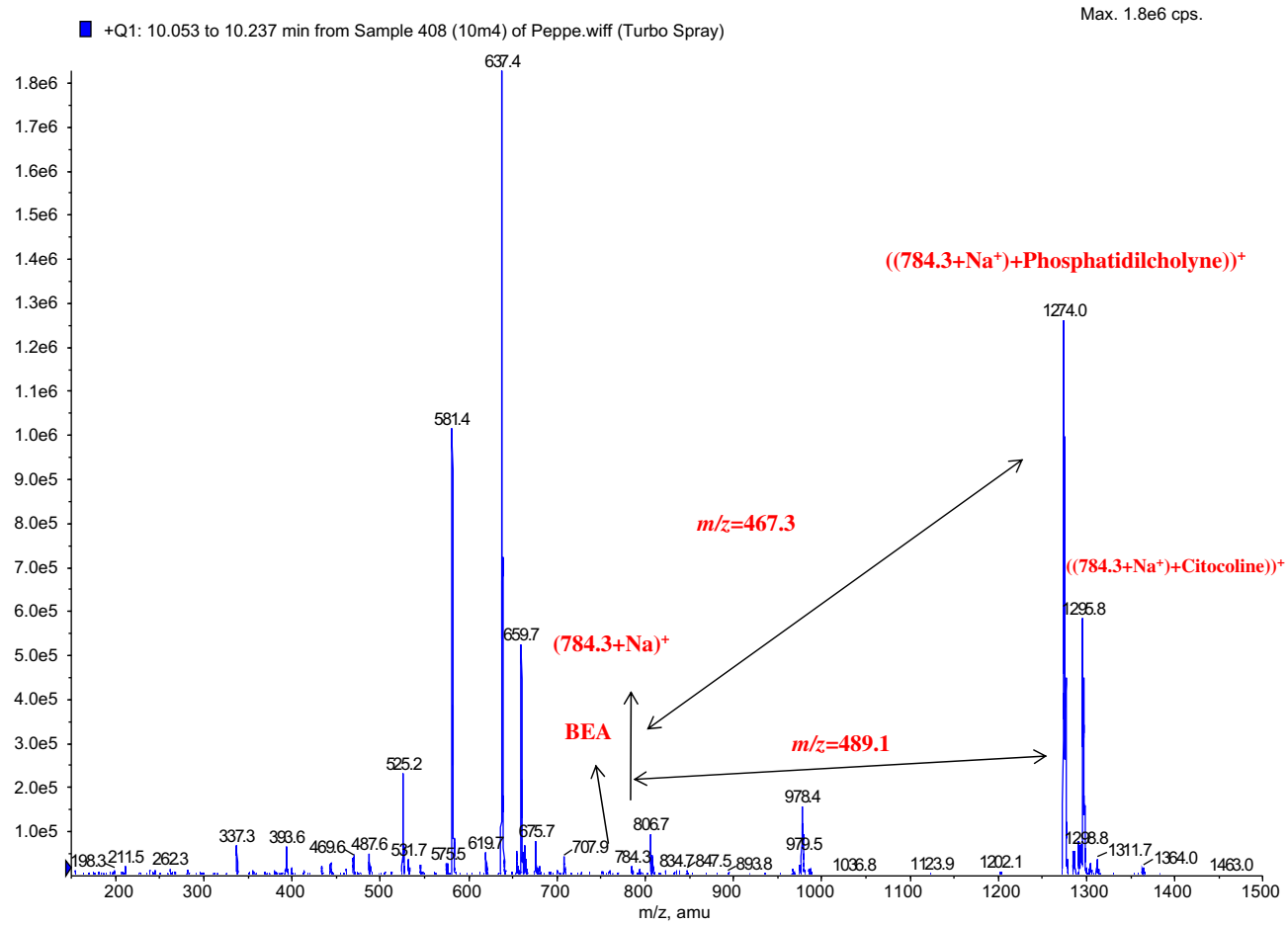

Fig. 2. Mass spectra fragments (m/z 200–1500) of the BEA adduct. It's possible to evidence in the mass spectra the presence of some fragments with a molecular weight highest than the BEA, and probably related with an adducts with the minor *Fusarium* mycotoxin BEA and some phospholipids of the bacterial cell walls.

In the mass spectra is possible to evidence a fragment corresponding to the molecular weight of the BEA (m/z 784.3) and the classical sodium adduct of BEA corresponding to the signal with a m/z of 806.7 $(783.3 + Na)^+$. These two fragments evidenced the presence in this adduct of a BEA part. In the right part of the mass spectra is possible to visualize two important diagnostic signals with a m/z of 1274.0 and 1295.8 respectively. The pseudomolecular ion with m/z of 1274.0 represents an adduct of the $(783.3 + Na)^+$ with the phosphatidilcoline, whereas the fragment with m/z 1295.8 represents an adduct $(783.3 + Na)^+$ with the citocoline, an intermediate of the synthesis of the phosphatidilcoline. The identification of these two adducts was verified calculating the molecular weight of the two compounds and relating these data with the BEA. The presence of these two fragments in the mass spectra demonstrated that the BEA can interact with some component of the bacterial cell wall, as the phospholipids, probably losing its toxicity.

The neoformed compound evidenced in the chromatogram has been quantified during the fermentation process, and the data are evidenced in the Table 4.

This study can be considered the first work, where the degradation of the minor *Fusarium* mycotoxins BEA by probiotic bacteria was evaluated, whereas the microbial degradation of other *Fusarium* mycotoxins was studied by several authors.

The degradation of 12 trichothecenes mycotoxins by chicken intestinal microbes was monitored by Young et al. (2007) applying the liquid chromatography–ultraviolet-mass spectrometry detection under positive ion mode. The fermentations were operated by the authors in the medium growth of L10, during 96 h at 37 °C under aerobic conditions. The two principally degradation pathways evidenced were: deacylation and deepoxydation. The authors evidenced also that the deepoxydation was the prevalent reaction in HT-2 toxin and T-2 triol, whereas T2 toxin showed only deacetylation. The percent of reduction considering as start concentration for each toxin employed the concentration of 50 mg/L, resulted variable from 40 to 95 %.

Table 4
BEA adduct expressed in mg/L of BEA present in the fermented medium during the reduction process.

	Fermentation time				
	BEA mg/L				
	4 h	12 h	16 h	24 h	48 h
Bb. longum	0.5 ± 0.01	0.3 ± 0.02	0.3 ± 0.04	0.2 ± 0.04	nd
Bb. bifidum	0.6 ± 0.02	0.5 ± 0.01	0.4 ± 0.02	0.3 ± 0.02	nd
Bb. breve	0.7 ± 0.01	0.6 ± 0.02	0.5 ± 0.01	0.3 ± 0.01	nd
Bb. adolescentis	0.7 ± 0.02	0.6 ± 0.03	0.5 ± 0.03	0.3 ± 0.01	nd
Lb. rhamnosus	0.5 ± 0.03	0.5 ± 0.02	0.4 ± 0.03	0.3 ± 0.02	nd
Lb. casei-casei	0.7 ± 0.01	0.4 ± 0.03	0.3 ± 0.01	0.3 ± 0.02	nd
Lb. plantarum	0.6 ± 0.01	0.5 ± 0.03	0.4 ± 0.04	0.2 ± 0.02	nd
E. crispatus	0.6 ± 0.02	0.5 ± 0.02	0.3 ± 0.01	0.2 ± 0.04	nd
S. fecalis	0.8 ± 0.03	0.8 ± 0.03	0.5 ± 0.02	0.4 ± 0.02	nd
S. termofilus	0.6 ± 0.01	0.4 ± 0.02	0.3 ± 0.03	0.2 ± 0.01	nd
Lb. ruminis	0.6 ± 0.02	0.4 ± 0.02	0.3 ± 0.02	0.2 ± 0.03	nd
Lb. casei	0.6 ± 0.01	0.5 ± 0.02	0.3 ± 0.03	0.2 ± 0.01	nd
Lb. animalis	0.8 ± 0.03	0.7 ± 0.01	0.6 ± 0.04	0.4 ± 0.02	nd

Guan et al. (2009), evaluated the degradation of the *Fusarium* mycotoxin DON, employing microorganisms of fish gut capable to transform the trichothecenes in a less toxic compounds. The detection of the DON and of the degradation products was carried out by the authors employing the technique of the LC-MS. The fermentation were carried out in different mediums as full medium, nutrient broth and corn meal broth to study also how the nutrients present in the mediums can influence the degradation of the mycotoxins employed. The fermentations were operated in a range of temperature variable from 4 to 25 °C in aerobic conditions. The authors evidenced that the microbial community from one catfish *Ameiurus nebulosus*, namely microbial culture C133, completely transformed DON to deepoxy DON (dE-DON) at 15 °C in full medium after 96 h incubation.

Awad, Ghareeb, Bohm, and Zentek (2010), explained the detoxification properties of an *Eubacterium* strain (BBSH 797) isolated from bovine rumen fluid, to transformate completely the mycotoxin DON into 3a,7a,l5-trihydroxytrichothec-9, 12-dien-8-one (-de-epoxy DON), which has been named DOM-1 *in vitro* and *in vivo*.

Bacterial mixtures originating from the rumen of dairy cow showed both deacetylation and deepoxydation functions (Awad et al., 2010).

Microbial transformations were obtained after the tested mycotoxin was incubated in bacterial suspensions at 4.9×10^7 per mL anaerobically at 38 °C for 24 or 48 h.

Very effective deepoxydation activity was achieved when DON was mixed with the digesta of the large intestines of chickens; DON was completely transformed to DOM-1 (He, Young, & Forsberg, 1992). It was shown that the deepoxydation function of the intestinal microorganisms of chickens may vary greatly among the samples collected from different chicken varieties, individuals and intestinal regions. The variation was also noticed in DON deepoxydation activity by microorganisms from swine intestine, either no activity or with activity (Kollarczik, Gareis, & Hanelt, 1994).

Islam et al. (2011), employed different soil samples to realize a screening of the micrograms capable to transform DON to dE-DON. Microbial transformation was monitored employing of the liquid chromatography coupled to the ultraviolet–mass spectrometry detection. The authors evidenced that a microbial culture originated by a soil sample showed the 100% DON to dE-DON biotransformation in mineral salt broth (MSB), after 144 h incubation at the temperature of 25 °C. Gene sequence analysis of the bacteria in the enriched culture indicated the presence of at least six bacterial genera, namely *Serratia*, *Clostridium*, *Citrobacter*, *Enterococcus*, *Stenotrophomonas* and *Streptomyces*.

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