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Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro simulated corn silage model. — Source link

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Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an *in vitro* model simulating corn silage

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Abstract

Fermentative bacteria can potentially be utilized to detoxify corn silage contaminated by *Fusarium* toxins. The objective of the present study was to test a large number of these bacteria for their ability to bind and/or biotransform deoxynivalenol (DON), zearalenone (ZEN), and fumonisins B_1 and B_2 (FB_1, FB_2) in conditions simulating corn silage. A total of 202 strains were screened in contaminated, pH 4, corn infusion inoculated with 5×10^8 CFU ml^{-1}. Eight *Lactobacilli* and three *Leuconostoc* biotransformed ZEN into α zearalenol, but no biotransformation was detected for DON and fumonisins. In contrast, most strains were capable of binding *Fusarium* toxins. The most effective genera were *Streptococcus* and *Enterococcus* capable of binding up to 33%, 49%, 24% and 62% of DON, ZEN, FB_1 and FB_2, respectively. The ability to bind *Fusarium* toxins seems to be a common property of fermentative bacteria and could help to decrease their toxicity in animals.

Keywords: *Fusarium*, *mycotoxins, fermentative bacteria*, *probiotic*, *binding*, *biotransformation*, *corn silage*

Running headline: *Fusarium* toxins removal by fermentative bacteria

Introduction

Whole-plant corn silage and high moisture, fermented corn grain are widely utilized as ruminants and swine feeds, respectively. Under unfavourable climatic conditions, *Fusarium* toxins including deoxynivalenol (DON), zearalenone (ZEN), and fumonisins (FB) can be produced on corn plants in the field (Lepom et al. 1990) and be present in silage (Scudamore and Livesey 1998; Orsi et al. 2000). Ingestion of mycotoxin-contaminated corn by animals affects performances and health. Among farm animals, swine appear to be most susceptible to the toxic effects of *Fusarium* toxins, especially to ZEN (Diekman and Green 1992). Although ruminants are considered more resistant, the risk should not be underestimated as data of toxicity after chronic exposure or due to interactions among mycotoxins are limited (Seeling and Danicke 2005). ZEN is associated with estrogenic syndrome and DON causes vomiting in pigs, as well as decreased feed intake and digestive disorders in both pigs and ruminants, leading to losses of weight gain (D'Mello et al. 1999; Eriksen and Pettersson 2004). Fumonisins cause pulmonary edema in swine (Harrison et al. 1990) and hepatotoxicity in ruminants (Diaz et al. 2000).

In spite of the progress made in preventive approaches such as breeding of resistant corn varieties (Mesterhazy 1989) and improvement in agronomic practices (Trenholm et al. 1989) to decrease *Fusarium* contamination, hazardous concentrations of *Fusarium* toxins may occur in corn justifying the use of detoxification methods. Physical and chemical methods such as ammonia treatment or the addition of adsorbents (Scott 1998; Huwig et al. 2001) are generally expensive, drastic, not specific, or simply not adapted to the treatment of corn destined to livestock. Inorganic adsorbents, although they have a high affinity to bind aflatoxins, are not efficient for *Fusarium* toxins. In addition, these

agents can also bind important micronutrients like minerals or vitamins (Guerre 2000). Practical methods for the detoxification of silage are currently not available. A promising approach is the use of selected microorganisms to remove *Fusarium* toxins (Bata and Lasztity 1999). Detoxification can be achieved by microbial binding and/or biotransformation of mycotoxins into less toxic compounds (Styriak and Conkova 2002). Due to their role in the ensiling process, fermentative bacteria are appropriate candidates for the biological detoxification of corn silage. The bioconversion of *Fusarium* toxins by rumen microbes and intestinal microflora was reported (Kiessling et al. 1984; Kollarczik et al. 1994; Caloni et al. 2000) but the bacteria responsible for their biotransformation were not identified. In contrast, binding of DON and ZEN to the cell wall of probiotic strains *in vitro* has been recently reported (El-Nezami et al. 2002a; 2002b). This property can decrease the bioavailability of these compounds by reducing absorption and limit their toxic effects (El-Nezami et al. 1999). However, to date, only three strains were thoroughly investigated. In addition, they were effective at about 10^{10} CFU ml^{-1}, a high bacterial concentration that is difficult to obtain in fermented corn feeds.

In this study, a large number of strains of fermentative bacteria were screened for their ability to remove DON, ZEN, FB_1 and FB_2 from a medium that simulated silage in term of substrate, pH, and bacterial concentration. The test conditions chosen allowed to observe simultaneously binding and/or biotransformation.

Materials and methods

Bacterial strains

Bacterial strains were obtained from different collections (LGC Promochem, Molsheim, France; Research Unit for Food Process Engineering and Microbiology, INRA, Thivernal-Grignon, France; Research Laboratory for Animal Husbandry, INRA, Corte, France; Lallemand SAS, Blagnac, France; Laboratory of Dynamics, Evolution and Expression of Genomes of Microorganisms, University Louis Pasteur/CNRS FRE 2326, Strasbourg, France; Cheese Research Laboratory, INRA, Aurillac, France). A total of 202 strains were tested: 137 *Lactobacilli*, 17 *Lactococci*, 6 *Leuconostoc*, 2 *Pediococci*, 5 *Propionibacteria*, 31 *Streptococci*, and 4 *Enterococci*. The majority of strains were isolated from dairy and plant material. For long-term conservation, all isolates were stored at -80°C in 30% glycerol.

Media

De Man, Rogosa, Sharpe broth (MRS, Oxoïd Ltd, Basing-stoke, UK) was used for the culture of *Lactobacilli*, *Pediococci* and *Leuconostoc*. M17 broth (Oxoïd Ltd, Basing-stoke, UK) containing 5% v/v of a 10% w/v lactose solution was used for the culture of *Lactococci*, *Streptococci* and *Enterococci*. Yeast Extract Lactate (YEL) medium, prepared according to Malik and Vedamuthu (1968), was used for the culture of *Propionibacteria*. A corn infusion was prepared by steeping dry whole-plant corn in water (6% w/v) at 60°C for 2 h. The infusion was then filtered through filter paper (Durieux, no. 120, VWR, Fontenay-sous-Bois, France), the filtrate was centrifuged (8500 g, 5 min) and the decanted supernatant was filtered through a 0.45 µm membrane. The filtered infusion was stored at 4°C and adjusted to pH 4 with lactic acid before using.

Mycotoxins and derivatives

DON, FB_1, FB_2, ZEN and its main derivatives α- and β-zearalenols (α and β ZOL), α- and β-zearalanols (α- and β- ZAL), and zearalanone (ZAN) were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany). The most common derivative of DON, deepoxy-deoxynivalenol (DOM) was purchased from Biopure (Tulin, Austria). Derivatives of fumonisins, aminopentols (HFB_1 and HFB_2), were obtained by hydrolysis of FB_1 and FB_2 according to Pagliuca et al. (2005). DON and ZEN (and derivatives) were dissolved in acetonitrile and methanol, respectively. Concentration was determined by measuring the absorbance at 218 nm for DON (ε = 6406 $mmol^{-1}$ cm^2) and at 274 nm for ZEN (ε = 13 909 $mmol^{-1}$ cm^2). Since spectrophotometry is not possible with FB_1 and FB_2, these compounds were dissolved in an exact volume of acetonitrile-water 1:1(v/v) to achieve the desired concentration. Mycotoxin solutions of DON (5 µg ml^{-1}), ZEN (5 µg ml^{-1}), FB_1 + FB_2 (5 µg ml^{-1} each) in corn infusion were prepared by evaporating solvents with nitrogen gas, redissolving in water (DON, FB_1, FB_2) or ethanol (ZEN) and adding corn infusion to reach the desired concentration. The water or ethanol solvent represented 5% (v/v) of the final mycotoxin solution.

Mycotoxin removal test

Bacteria screened for their ability to bind and/or biotransform *Fusarium* toxins were prepared as follow. For each strain, one tube containing 20 ml of medium was inoculated with 0.1 ml of an overnight culture and was incubated at optimal temperature of growth (30 or 37°C) for 24 h. *Propionibacteria* strains were incubated at 30°C for 72 h. At the end of incubation, the bacterial concentration of cultures was estimated by measuring the absorbance at 600 nm as described previously (Niderkorn et al. 2006). A volume of culture containing 35×10^8 CFU was centrifuged (3000 g, 10 min, 5°C) and the supernatant was removed. The bacterial pellet was washed twice with 5 ml of

phosphate-buffered saline (PBS 0.01 M, pH 7.4). After the second wash, the bacterial pellet was resuspended in 7 ml PBS to obtain a concentration of 5×10^8 CFU ml^{-1}, and transferred to six polypropylene tubes (1 ml per tube). Tubes were centrifuged (3000 g, 10 min, 5°C), supernatants were removed, and bacteria were re-suspended in 1 ml of one of the three mycotoxin solutions. Positive controls containing no bacteria and a negative control per bacterial genus containing no mycotoxin were included. All the tubes were incubated at 25°C in microaerophilic conditions for 1 h with shaking (480 rpm), then for 23 h without shaking. At the end of the incubation period, tubes were centrifuged (3000 g, 10 min, 5°C) and supernatants were analysed for mycotoxins by reversed-phase HPLC. Assays and positive controls were performed in duplicate.

The percentage of mycotoxin removed was calculated by using the following formula: Mycotoxin removed (%) = 100 × [1 – (Peak area of mycotoxin in the supernatant / Peak area of mycotoxin in the positive control)].

HPLC mycotoxins analysis

Separation and quantification of mycotoxins were performed by the method described previously (Niderkorn et al. 2006) with slight modifications in order to remove interferences. The HPLC system consisted of a P1000XR pump (SpectraSYSTEM, San Jose, California, USA) and an automatic sampler (SpectraPhysics, San Jose, California, USA). Separation was performed on C_{18} reversed-phase columns (Macherey-Nagel, France); Nucleodur (125 × 4.6 mm, 5 mm) was used for DON and ZEN and Prontosil (120 × 3.0 mm, 3 µm) was used for FB_1 and FB_2. The mobile phase used to separate DON consisted of a water–acetonitrile solution (95:5 v/v) and the flow rate was 1 ml min^{-1}. Detection was set at 220 nm and retention time was 12.1 min. After 14 min of run, the column was washed with a water–acetonitrile solution (80:20 v/v) to remove

interferences and was equilibrated with the mobile phase for 6 min before the following injection. The mobile phase used to separate ZEN consisted of a water–methanol solution (40:60 v/v) and the flow rate was 1.2 ml min^{-1}. ZEN was detected by photo-diode-array (scan 200 – 380 nm) and fluorescence (λ_{exc} = 274 nm, λ_{em} = 440 nm) placed in series. The retention time of ZEN was 10.7 min. Fumonisins were derivatized before injection using *ortho*-phtalaldehyde (OPA) plus mercaptoethanol. Ten µl of sample were mixed with 90 µl of borate buffer pH 10, then 100 µl of derivatizing reagent were added. The preparation was mixed and allowed to react for 2 min before injection. Separation of FB_1 and FB_2 was performed with a gradient elution using acetonitrile (A) and a mixture of 0.05 mol l^{-1} dihydrogen phosphate–methanol (1:1 v/v) acidified at pH 3.35 (B). The gradient program was as follows: 10% to 50% A in 6 min, 50% A for 7 min, and 50% to 10% A in 1 min. The flow rate was 1 ml min^{-1} and detection was set at 336 nm excitation and 440 nm emission. The retention times were 10.0 and 12.7 min for FB_1 and FB_2, respectively.

HPLC-ESI-MS/MS analysis

A HPLC-MS/MS (TSQ Quantum Ultra AM, Thermo Electron, San Jose, California, USA) equipped with an electrospray ionization source (ESI) interface operating in the negative mode was used to identify the ZEN derivative produced by some strains. Chromatographic separation and MS analysis was performed on a 125 × 4.6 mm Prontosil C_{18} column with particle size of 3 µm (Bischoff, Leonberg, Germany), using a methanol – 1% acetic acid (65:35 v/v) mobile phase at a flow rate of 0.8 ml min^{-1}. Parameters of the MS were optimised using standard solutions of α ZOL in mobile phase. The test responses were recorded with electrospray voltage 3.0 kV, capillary temperature 400°C, sheath flow and auxiliary flow 50 and 10 arbitrary units,

respectively. Multiple reaction monitoring (MRM) was used to identify the metabolite. The most intense transitions from the fragmentation of ZEN, α ZOL and ZAN were determined. Six product ions were selected at different collision energies (CE) so as to characterize each molecule of interest (Table I). For analysis, 4 ml of corn infusion sample were extracted with 4 ml dichloromethane. The mixture was mixed for 15 min then centrifuged at 3000 g for 10 min. One ml of the organic layer was transferred into a new tube and evaporated to dryness under a stream of N_2. The dried residue was redissolved in 200 µl of mobile phase, and 5 µl of this solution were injected into the HPLC-MS/MS system. MS-analysis of extract was compared with ZEN, α ZOL and ZAN standards. [Insert Table I about here]

Statistical analysis

Data was subjected to the analysis of variance (ANOVA). A significant difference ($p < 0.05$) between means was determined by Duncan's multiple range test using the Statistical Analysis System software package (SAS Institute Inc., Cary, NC).

Results

The experimental model used in this work allowed us to observe at the same time biotransformation and binding of *Fusarium* toxins by fermentative bacteria. The HPLC method used, separated each mycotoxin as well as their main derivatives from the corn infusion medium matrix without an extraction step (Figure 1) and thus facilitated the screening. [Insert Figure 1 about here]

Biotransformation of mycotoxins

In incubations containing DON and fumonisins no biotransformation products were observed. There were not peaks corresponding to DOM and aminopentol—derivatives of DON and fumonisins, respectively—or any other unknown peak in the HPLC chromatograms. In contrast, in incubations containing ZEN for 11 out of the 202 strains tested (about 5.5%), an additional peak was observed in the chromatograms. This peak had the same retention time for all 11 strains suggesting that it was the same metabolite (Figure 2). Eight out of the 11 strains were *Lactobacilli* and the other three were *Leuconostoc*. These biotransforming *Lactobacilli* removed approximately twice more ZEN from the incubation media than strains from the same genus that only bound the mycotoxin (Table II and III). [Insert Figure 2 and Table II about here]

The ZEN metabolite showed a UV spectrum similar to ZEN with absorption maxima at 236, 274, and 316 nm (Figure 2, inset). Retention time (Rt) of this peak was compared to those of main ZEN derivatives using modified chromatographic conditions to improve separation. The changes in the chromatography were in the mobile phase, which consisted in methanol – 1% acetic acid mixture (65:35 v/v), and the flow rate, which was set at 0.8 ml min^{-1}. Under these conditions, the Rt of the unknown metabolite was 9.56 min, similar to α ZOL (9.50 min), while Rts of β ZOL, α and β ZAL, and ZAN standards were 6.10, 7.74, 4.35, and 9.84 min, respectively. This result indicated that α ZOL was most likely the metabolite produced. The Rt of ZAN was also near to that of the unknown metabolite. However, ZAN, at a concentration of 5 µg ml^{-1} had a peak area considerably lower than the peak area of the metabolite produced by active bacterial strains incubated with an equal concentration of ZEN and hence ZAN was unlikely the searched compound. Additional HPLC-MS/MS analyses were performed on ZEN-containing corn infusion extracts after incubation with biotransforming strains.

The LC-MS showed that the new peak had the same mass transitions characteristic (m/z = 319) as α ZOL (Figure 3) and thus unequivocally identified the metabolite. [Insert Figure 3 about here]

Binding of zearalenone, deoxynivalenol and fumonisins from acidified corn infusion

Table III shows the mycotoxin-binding capacity of the tested fermentative bacteria grouped by genera. Across all genera, FB_2 was the mycotoxin most efficiently removed followed by ZEN, DON, and FB_1. The average fraction of DON bound by the different genera ranged from 15 to 22% but these differences were not significant ($p > 0.05$). In contrast, large differences among genera were observed for the binding of ZEN and fumonisins ($p < 0.05$). It is interesting to note that, independently of the molecular structure of these mycotoxins, the genera *Streptococcus* and *Enterococcus* ranked always as the most efficient binders under the conditions of the assay. In particular, strains of *Streptococcus thermophilus*, which bound up to 49%, 33%, 24%, and 62% of ZEN, DON, FB_1 and FB_2, respectively. Out of the 202 strains tested about two third were *Lactobacilli*. This apparent bias has a biotechnological explanation because species of this genus are very active during the ensiling process and hence widely used as inoculants. *Lactobacilli* are dominant members of the bacterial population found in grass and corn silages (Langston and Bouma 1960; Dellaglio and Torriani 1986). *L. plantarum*, of which we tested 46 isolates, is a particularly common epiphytic specie, which is also found in many commercial silage additives (Weinberg and Muck 1996). All the other selected strains used in this work are representative of genera of fermentative bacteria regularly associated with silage (Moon et al. 1981; Driehuis and Elferink 2000). The binding activities of *Lactobacillus* spp. are shown in Table IV. Significant differences were observed among strains; however, compared to

Streptococcus and *Enterococcus* strains the binding capacity of the *Lactobacilli* was less important. [Insert Table III and Table IV about here]

Discussion

The ability of fermentative bacteria to biotransform or bind *Fusarium* toxins in whole-plant corn silage and fermented corn grain is a promising way to reduce their toxic effects on livestock. In this work, the conditions used to screen fermentative bacteria permitted to observe simultaneously binding and biotransformation in a liquid medium representative of corn silage.

The reduction of ZEN into α-ZOL can be carried out in the liver and also by the flora harboured in the digestive tract of animals (Olsen and Kiessling 1983; Kiessling et al. 1984; Kollarczik et al. 1994). However, it had never been observed with pure strains of lactic acid bacteria (LAB). In this study, this biotransformation was observed in 11 strains but it cannot be considered as a detoxification because α-ZOL is three to four times more estrogenic than ZEN (Mirocha et al. 1979). All the positive bacterial strains detected in this work were capable to produce large quantities of α-ZOL from ZEN. Even if strains possessing this property represent a relatively low percentage of the total fermentative bacteria, they could form part of the epiphytic population present in fermented feeds and convert significant amounts of ZEN into α-ZOL. This means that the absence of ZEN or its presence in non toxic concentrations does not guarantee feed innocuity, and therefore, it could be advisable to check for the presence of α-ZOL in fermented feeds. It could also be recommended to check if this property is not present in strains used as fermentation starters. The biotransformation of ZEN to α-ZOL might explain the results of a recent study where a significant decrease in the concentration of

ZEN was observed during the fermentation of corn meal by LAB but without a reduction in toxicity (Mokoena et al. 2005).

Binding of mutagens and mycotoxins by LAB was reported in numerous studies (Morotomi and Mutai 1986; El-Nezami et al. 1998). Binding of DON and ZEN by three probiotic strains was also recently reported, but they were not effective at the low densities found in fermented corn feeds (El-Nezami et al. 2002a; 2002b). The results of the present work showed that significant quantities of *Fusarium* toxins including fumonisins are bound by most species of fermentative bacteria at a density of 5×10^8 CFU ml^{-1}. We also confirmed previous observations (Niderkorn et al. 2006) showing that FB_2 was removed more efficiently than FB_1 in spite of their similarity in chemical structure.

Since most strains of fermentative bacteria tested seem to have the ability to bind DON, ZEN and fumonisins, it is probable that these *Fusarium* toxins are also partially bound by the epiphytic microflora of silage or by inoculants already utilized to improve silage fermentation or aerobic stability. Although *Lactobacilli* are widely utilized as silage additives, none of the strains tested was particularly efficient at binding *Fusarium* toxins. From the isolates tested in this work, the use of fermentative bacteria to bind *Fusarium* toxins in silage appear to be more advantageous for *Streptococci* and *Enterococci*, which bound more ZEN and fumonisins than other genera. Consequently, an advantageous use of fermentative bacteria to bind *Fusarium* toxins in silage would be limited to these two genera. The higher capacity to bind *Fusarium* toxins by *Streptococci* and *Enterococci* cannot be explained by their size as they are smaller than other genera with lower binding capacity such as *Lactobacillus*. The composition of the

cell wall may confer the *Streptococci* and *Enterococci* their superior binding property. The mechanism of binding of mycotoxins by bacteria is still not clear. In the case of ZEN, it was recently suggested that this mycotoxin predominantly binds to carbohydrate moieties of the cell wall of LAB by means of hydrophobic interactions (El-Nezami et al. 2004). As hydrophobic interactions are relatively weak, this bacterium-mycotoxin complex could be unstable. Nevertheless, these authors also suggested that ZEN binding is not limited to hydrophobic links and other type of interactions could be important.

Conclusions

The use of selected strains of fermentative bacteria could be a method to limit the toxic effects of *Fusarium* toxins present in corn silage. Our results have shown that the biodegradation of mycotoxins is possible. However, the resulting compound is not always less toxic than the parent mycotoxin. Binding seems to be the most promising mode of action, but for the practical application of this technology, it is essential that the bacterium-mycotoxin complex remain stable during the digestion process. Further studies are planned to evaluate the stability of the complex in the different parts of the gastrointestinal tract of farm animals.

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Table captions

Table I. Product ions of zearalenone (ZEN), α zearalenol (α ZOL), and zearalanone (ZAN) for HPLC-ESI-MS/MS analysis.

Table II. Lactic acid bacteria biotransforming zearalenone (ZEN) to α zearalenol (α ZOL).

Table III. *In vitro* binding of zearalenone (ZEN), deoxynivalenol (DON), and fumonisins B_1 and B_2 (FB_1, FB_2) by different genera of fermentative bacteria.

Table IV. *In vitro* binding of zearalenone, deoxynivalenol, and fumonisins B_1 and B_2 by *Lactobacillus* species.

Figure captions

Figure 1. HPLC chromatograms of corn infusion supernatants incubated with bacteria (5×10^8 CFU ml^{-1}) at 25°C for 24 h without and with zearalenone (ZEN, 5 µg ml^{-1}) (a), without and with fumonisins B_1 and B_2 (FB_1 and FB_2, 5 µg ml^{-1} each) (b), without and with deoxynivalenol (DON, 5 µg ml^{-1}) (c).

Figure 2. HPLC chromatograms of corn infusion supernatants containing zearalenone (ZEN, 5 µg ml^{-1}) incubated at 25°C for 24 h without bacteria (a) and with *Lactobacillus brevis* R0002 (5×10^8 CFU ml^{-1}) (b). ZEN (retention time = 10.4 min) and metabolite (retention time = 9.2 min) showed a similar UV spectrum (inset).

Figure 3. HPLC-MS spectra of zearalenone, ZEN (a) and α zearalenol, α ZOL (b) standards alone, and the extract from corn infusion supernatant containing ZEN and the produced metabolite (c). Spectra of peaks 1 and 2 showed parent mass of m/z 319.08 and 317.06, respectively.

Table I. Product ions of zearalenone (ZEN), α zearalenol (α ZOL), and zearalanone (ZAN) for HPLC-ESI-MS/MS analysis

Compound	Retention time (min)	Transition	CE (V)
ZEN	11.01	317 → 130	46
		317 → 131	35
		317 → 149	35
		317 → 175	34
		317 → 187	30
		317 → 273	27
α-ZOL	9.59	319 → 130	54
		319 → 131	55
		319 → 160	37
		319 → 174	13
		319 → 275	31
		319 → 301	27
ZAN	9.90	319 → 137	52
		319 → 161	35
		319 → 163	38
		319 → 205	31
		319 → 259	37
		319 → 275	28

Table II. Lactic acid bacteria biotransforming zearalenone (ZEN) to α zearalenol (α ZOL)*

Taxon	Source	Used in	ZEN biotransformed (%)
Lactobacillus			
L. brevis	LGMPA - C11	Cheese making	41 (1)
L. brevis	Lallemand - R0002	Animal nutrition	47 (2)
L. brevis	Lallemand - L62	Baking	24 (1)
L. casei casei	LGMPA - B1	Cheese making	49 (4)
L. fermenti	LGMPA - 34	Cheese making	19 (0)
L. plantarum	LGMPA - J1	Cheese making	41 (2)
L. plantarum	Lallemand - R1113	Baking	50 (3)
L. spp.	LGMPA - F7	Cheese making	23 (1)
Leuconostoc			
Ln. mesenteroides or *Ln. pseudomesenteroides*	URF - 171	Cheese making	16 (2)
Ln. mesenteroides or *Ln. pseudomesenteroides*	URF - 365	Cheese making	8 (0)
Ln. mesenteroides	URF - 1118	Cheese making	9 (0)

*Bacteria (5×10^8 CFU ml^{-1}) were incubated for 24 h at 25°C in corn infusion, pH 4, containing ZEN (5 µg ml^{-1}). Data shown are the means (SD) of duplicate.

Table III. *In vitro* binding of zearalenone (ZEN), deoxynivalenol (DON), and fumonisins B_1 and B_2 (FB_1, FB_2) by different genera of fermentative bacteria*

Genus	Number of strains	Fraction bound (%)†			
		ZEN‡	DON	FB1	FB2
Enterococcus	4	35 (2) ^{c}	22 (8)	14 (7) ^{b}	43 (8) ^{b}
Lactobacillus	137	20 (4) ^{b}	15 (9)	10 (6) ^{ab}	24 (11) ^{a}
Lactococcus	17	17 (4) ^{ab}	18 (8)	11 (4) ^{ab}	19 (8) ^{a}
Leuconostoc	6	28 (7) ^{bc}	18 (2)	4 (3) ^{a}	18 (5) ^{a}
Pediococcus	2	17 (2) ^{ab}	18 (3)	4 (3) ^{a}	23 (10) ^{a}
Propionibacterium	5	10 (3) ^{a}	18 (6)	10 (7) ^{ab}	15 (7) ^{a}
Streptococcus	31	40 (4) ^{c}	18 (7)	15 (5) ^{b}	48 (9) ^{b}

*Bacteria (5×10^8 CFU ml^{-1}) were incubated for 24 h at 25°C in corn infusion, pH 4, containing ZEN, 5 µg ml^{-1}, DON, 5 µg ml^{-1}, and a mixture of FB_1 and FB_2, 5 µg ml^{-1} each. Each strain was tested for each mycotoxin in duplicate.

†Values are the means (SD) of mycotoxin bound for all strains belonging to the same genus. Data within a column with unlike letter are not significantly different ($p < 0.05$).

‡To show only binding, strains biotransforming ZEN were not included.

Table IV. *In vitro* binding of zearalenone, deoxynivalenol, and fumonisins B_1 and B_2 by *Lactobacillus* species*

Lactobacillus species	Number of strains	Fraction bound (%)†			
		ZEN‡	DON	FB1	FB2
L. acidophilus	7	19 (4)	22 (4) ^{c}	13 (6) ^{b}	22 (10) ^{abc}
L. brevis	5	20 (1)	19 (5) ^{c}	9 (4) ^{ab}	27 (9) ^{bcd}
L. buchneri	3	20 (2)	4 (4) ^{ab}	11 (1) ^{ab}	28 (12) ^{cd}
L. casei . subsp. *alactosus*	1	18 (0)	10 (0) ^{abc}	3 (0) ^{a}	9 (0) ^{a}
L. casei . subsp. *casei*	21	18 (4)	17 (9) ^{c}	11 (5) ^{ab}	25 (11) ^{bcd}
L. casei . subsp. *rhamnosus*	4	18 (6)	19 (6) ^{c}	12 (7) ^{ab}	13 (3) ^{ab}
L. delbruekii . subsp. *bulgaricus*	5	28 (4)	11 (9) ^{abc}	12 (5) ^{ab}	26 (7) ^{bcd}
L. delbruekii . subsp. *lactis*	3	22 (5)	19 (9) ^{c}	9 (4) ^{ab}	23 (9) ^{abc}
L. fermentum	10	21 (7)	13 (8) ^{abc}	13 (12) ^{b}	25 (11) ^{bcd}
L. helveticus	8	19 (2)	17 (7) ^{c}	11 (6) ^{ab}	31 (9) ^{cd}
L. jugurti	7	19 (4)	20 (9) ^{c}	12 (7) ^{ab}	34 (9) ^{cd}
L. paraplantarum	7	22 (2)	13 (8) ^{abc}	5 (4) ^{ab}	10 (6) ^{a}
L. pentosus	1	23 (0)	2 (0) ^{a}	4 (0) ^{ab}	32 (0) ^{cd}
L. plantarum	46	19 (3)	13 (9) ^{abc}	9 (5) ^{ab}	22 (10) ^{abc}
L. reuteri	1	17 (0)	15 (0) ^{bc}	21 (0) ^{c}	37 (0) ^{d}
L. unidentified	8	20 (2)	19 (9) ^{c}	8 (5) ^{ab}	26 (6) ^{bcd}

*Test conditions were similar to those shown in table I.
†Values are the means (SD) of mycotoxin bound for strains belonging to the same species. Data within a column with unlike letter are not significantly different ($p < 0.05$).
‡To show only binding, strains biotransforming ZEN were not included.

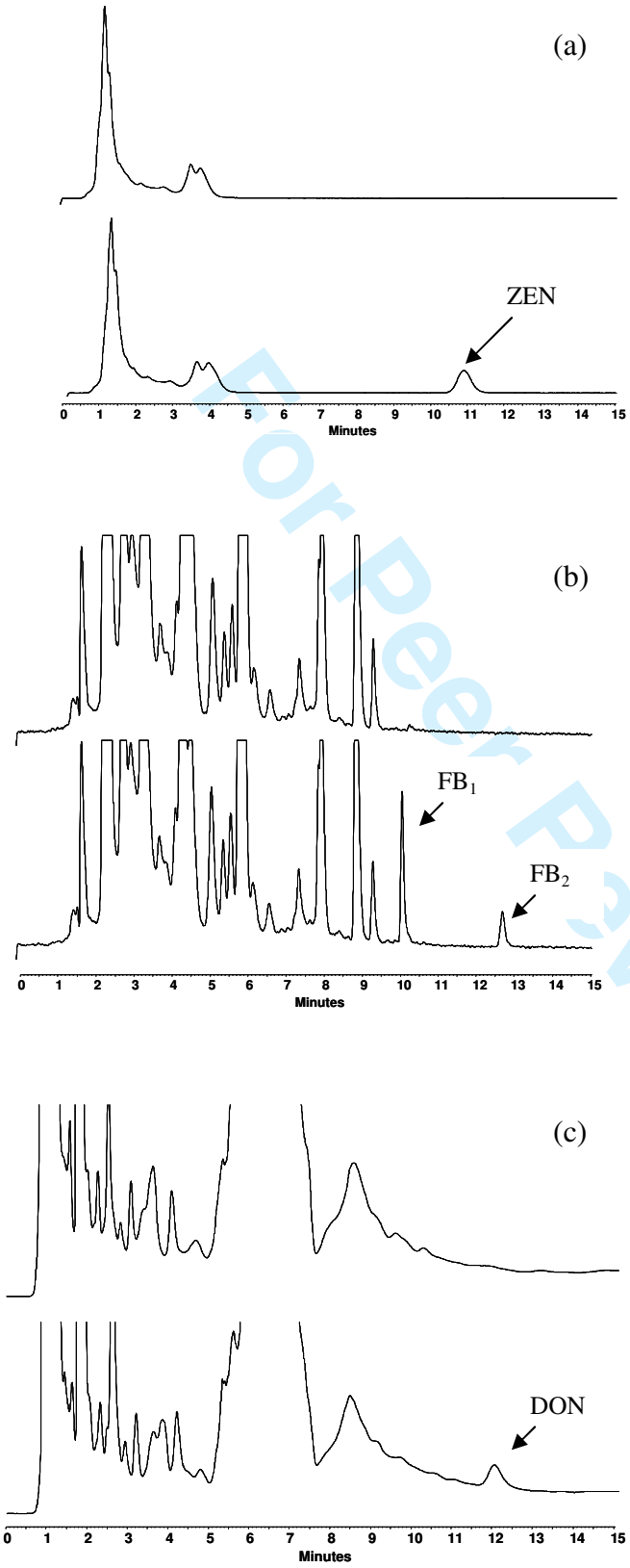
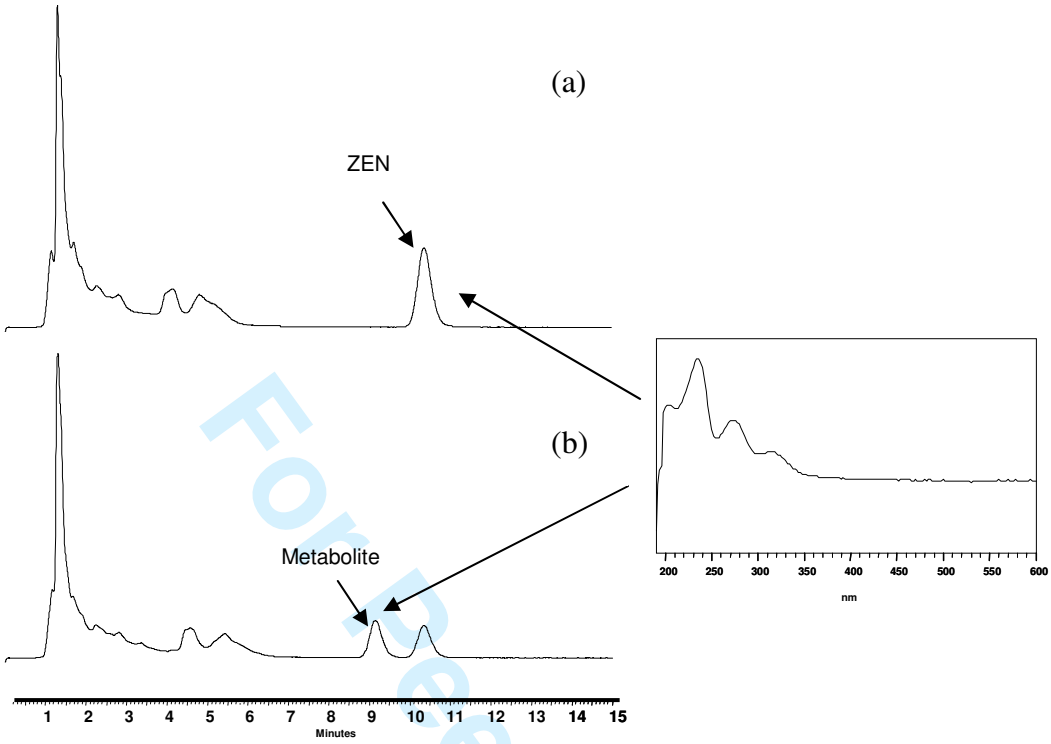


Figure 1. HPLC chromatograms of corn infusion supernatants incubated with bacteria (5×10^8 CFU ml^{-1}) at 25°C for 24 h without and with zearalenone (ZEN, 5 µg ml^{-1}) (a), without and with fumonisins B_1 and B_2 (FB_1 and FB_2, 5 µg ml^{-1} each) (b), without and with deoxynivalenol (DON, 5 µg ml^{-1}) (c).



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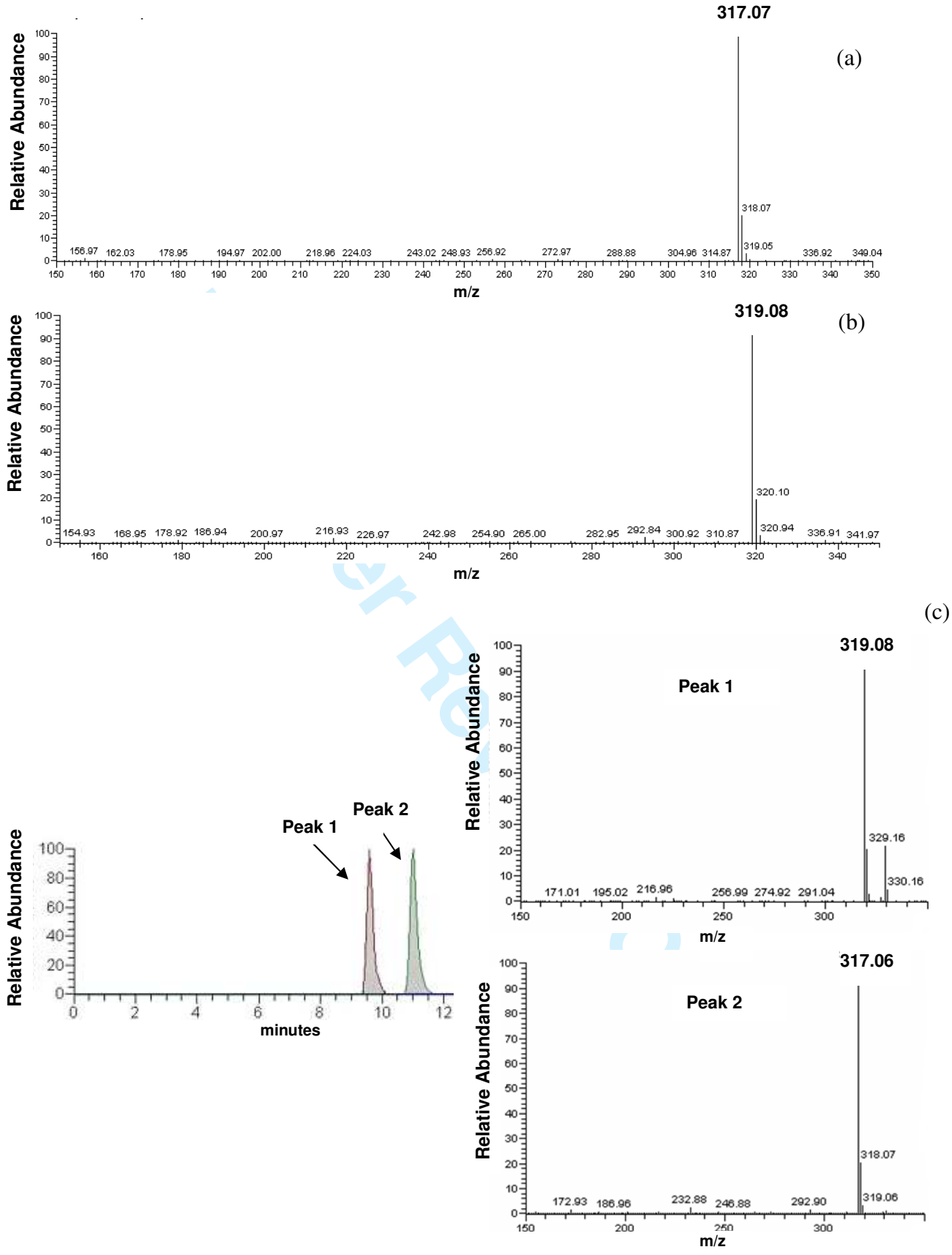

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