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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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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1 Screening of fermentative bacteria for their ability to bind and
2 biotransform deoxynivalenol, zearalenone and fumonisins in an *in vitro*
3 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 B₁ and B₂ (FB₁, FB₂) 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, FB₁ and FB₂, 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, FB₁ and FB₂ 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

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3 86 Bacterial strains were obtained from different collections (LGC Promochem, Molsheim,
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5 87 France; Research Unit for Food Process Engineering and Microbiology, INRA,
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7 88 Thivernal-Grignon, France; Research Laboratory for Animal Husbandry, INRA, Corte,
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9 89 France; Lallemand SAS, Blagnac, France; Laboratory of Dynamics, Evolution and
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11 90 Expression of Genomes of Microorganisms, University Louis Pasteur/CNRS FRE 2326,
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13 91 Strasbourg, France; Cheese Research Laboratory, INRA, Aurillac, France). A total of
14
15 92 202 strains were tested: 137 *Lactobacilli*, 17 *Lactococci*, 6 *Leuconostoc*, 2 *Pediococci*, 5
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17 93 *Propionibacteria*, 31 *Streptococci*, and 4 *Enterococci*. The majority of strains were
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19 94 isolated from dairy and plant material. For long-term conservation, all isolates were
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21 95 stored at -80°C in 30% glycerol.
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27 96
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29 97 *Media*
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31 98 De Man, Rogosa, Sharpe broth (MRS, Oxoid Ltd, Basing-stoke, UK) was used for the
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33 99 culture of *Lactobacilli*, *Pediococci* and *Leuconostoc*. M17 broth (Oxoid Ltd, Basing-
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35 100 stoke, UK) containing 5% v/v of a 10% w/v lactose solution was used for the culture of
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37 101 *Lactococci*, *Streptococci* and *Enterococci*. Yeast Extract Lactate (YEL) medium,
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39 102 prepared according to Malik and Vedomuthu (1968), was used for the culture of
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41 103 *Propionibacteria*. A corn infusion was prepared by steeping dry whole-plant corn in
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43 104 water (6% w/v) at 60°C for 2 h. The infusion was then filtered through filter paper
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45 105 (Durieux, no. 120, VWR, Fontenay-sous-Bois, France), the filtrate was centrifuged
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47 106 (8500 g, 5 min) and the decanted supernatant was filtered through a 0.45 µm membrane.
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49 107 The filtered infusion was stored at 4°C and adjusted to pH 4 with lactic acid before
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51 108 using.
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59 110 *Mycotoxins and derivatives*
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3 111 DON, FB₁, FB₂, ZEN and its main derivatives α - and β -zearalenols (α and β ZOL), α -
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5 112 and β -zearalanols (α - and β - ZAL), and zearalanone (ZAN) were purchased from
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7 113 Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The most common derivative of
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9 114 DON, deepoxy-deoxynivalenol (DOM) was purchased from Biopure (Tulin, Austria).
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11 115 Derivatives of fumonisins, aminopentols (HFB₁ and HFB₂), were obtained by
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13 116 hydrolysis of FB₁ and FB₂ according to Pagliuca et al. (2005). DON and ZEN (and
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15 117 derivatives) were dissolved in acetonitrile and methanol, respectively. Concentration
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17 118 was determined by measuring the absorbance at 218 nm for DON ($\epsilon = 6406 \text{ mmol}^{-1}$
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19 119 cm^2) and at 274 nm for ZEN ($\epsilon = 13\,909 \text{ mmol}^{-1} \text{ cm}^2$). Since spectrophotometry is not
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21 120 possible with FB₁ and FB₂, these compounds were dissolved in an exact volume of
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23 121 acetonitrile-water 1:1(v/v) to achieve the desired concentration. Mycotoxin solutions of
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25 122 DON ($5 \mu\text{g ml}^{-1}$), ZEN ($5 \mu\text{g ml}^{-1}$), FB₁ + FB₂ ($5 \mu\text{g ml}^{-1}$ each) in corn infusion were
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27 123 prepared by evaporating solvents with nitrogen gas, redissolving in water (DON, FB₁,
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29 124 FB₂) or ethanol (ZEN) and adding corn infusion to reach the desired concentration. The
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31 125 water or ethanol solvent represented 5% (v/v) of the final mycotoxin solution.
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41 *Mycotoxin removal test*

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43 128 Bacteria screened for their ability to bind and/or biotransform *Fusarium* toxins were
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45 129 prepared as follow. For each strain, one tube containing 20 ml of medium was
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47 130 inoculated with 0.1 ml of an overnight culture and was incubated at optimal temperature
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49 131 of growth (30 or 37°C) for 24 h. *Propionibacteria* strains were incubated at 30°C for 72
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51 132 h. At the end of incubation, the bacterial concentration of cultures was estimated by
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53 133 measuring the absorbance at 600 nm as described previously (Niderkorn et al. 2006). A
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55 134 volume of culture containing 35×10^8 CFU was centrifuged (3000 g, 10 min, 5°C) and
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57 135 the supernatant was removed. The bacterial pellet was washed twice with 5 ml of
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3 136 phosphate-buffered saline (PBS 0.01 M, pH 7.4). After the second wash, the bacterial
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5 137 pellet was resuspended in 7 ml PBS to obtain a concentration of 5×10^8 CFU ml⁻¹, and
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8 138 transferred to six polypropylene tubes (1 ml per tube). Tubes were centrifuged (3000 g,
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10 139 10 min, 5°C), supernatants were removed, and bacteria were re-suspended in 1 ml of
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12 140 one of the three mycotoxin solutions. Positive controls containing no bacteria and a
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15 141 negative control per bacterial genus containing no mycotoxin were included. All the
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17 142 tubes were incubated at 25°C in microaerophilic conditions for 1 h with shaking (480
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19 143 rpm), then for 23 h without shaking. At the end of the incubation period, tubes were
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21 144 centrifuged (3000 g, 10 min, 5°C) and supernatants were analysed for mycotoxins by
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23 145 reversed-phase HPLC. Assays and positive controls were performed in duplicate.

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27 146 The percentage of mycotoxin removed was calculated by using the following
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29 147 formula: Mycotoxin removed (%) = $100 \times [1 - (\text{Peak area of mycotoxin in the}$
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31 148 $\text{supernatant} / \text{Peak area of mycotoxin in the positive control})]$.

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36 150 *HPLC mycotoxins analysis*

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39 151 Separation and quantification of mycotoxins were performed by the method described
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41 152 previously (Niderkorn et al. 2006) with slight modifications in order to remove
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43 153 interferences. The HPLC system consisted of a P1000XR pump (SpectraSYSTEM, San
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45 154 Jose, California, USA) and an automatic sampler (SpectraPhysics, San Jose, California,
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47 155 USA). Separation was performed on C₁₈ reversed-phase columns (Macherey-Nagel,
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49 156 France); Nucleodur (125 × 4.6 mm, 5 mm) was used for DON and ZEN and Prontosil
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51 157 (120 × 3.0 mm, 3 μm) was used for FB₁ and FB₂. The mobile phase used to separate
52
53 158 DON consisted of a water–acetonitrile solution (95:5 v/v) and the flow rate was 1 ml
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55 159 min⁻¹. Detection was set at 220 nm and retention time was 12.1 min. After 14 min of
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59 160 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 photodiode-array (scan 200 – 380 nm) and fluorescence ($\lambda_{\text{exc}} = 274$ nm, $\lambda_{\text{em}} = 440$ nm) placed in series. The retention time of ZEN was 10.7 min. Fumonisin was derivatized before injection using *ortho*-phthalaldehyde (OPA) plus mercaptoethanol. Ten μ l of sample were mixed with 90 μ l of borate buffer pH 10, then 100 μ l of derivatizing reagent were added. The preparation was mixed and allowed to react for 2 min before injection. Separation of FB₁ and FB₂ 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 FB₁ and FB₂, 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 $\times$ 4.6 mm Prontosil C₁₈ column with particle size of 3 μ m (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,

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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 N₂. The dried residue was redissolved in 200 μ l of mobile phase, and 5 μ l 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

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

221 The ZEN metabolite showed a UV spectrum similar to ZEN with absorption maxima at
222 236, 274, and 316 nm (Figure 2, inset). Retention time (R_t) of this peak was compared
223 to those of main ZEN derivatives using modified chromatographic conditions to
224 improve separation. The changes in the chromatography were in the mobile phase,
225 which consisted in methanol – 1% acetic acid mixture (65:35 v/v), and the flow rate,
226 which was set at 0.8 ml min^{-1} . Under these conditions, the R_t of the unknown metabolite
227 was 9.56 min, similar to α ZOL (9.50 min), while R_t s of β ZOL, α and β ZAL, and ZAN
228 standards were 6.10, 7.74, 4.35, and 9.84 min, respectively. This result indicated that α
229 ZOL was most likely the metabolite produced. The R_t of ZAN was also near to that of
230 the unknown metabolite. However, ZAN, at a concentration of $5 \mu\text{g ml}^{-1}$ had a peak
231 area considerably lower than the peak area of the metabolite produced by active
232 bacterial strains incubated with an equal concentration of ZEN and hence ZAN was
233 unlikely the searched compound. Additional HPLC-MS/MS analyses were performed
234 on ZEN-containing corn infusion extracts after incubation with biotransforming strains.

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

238

239 *Binding of zearalenone, deoxynivalenol and fumonisins from acidified corn infusion*

240 Table III shows the mycotoxin-binding capacity of the tested fermentative bacteria
241 grouped by genera. Across all genera, FB₂ was the mycotoxin most efficiently removed
242 followed by ZEN, DON, and FB₁. The average fraction of DON bound by the different
243 genera ranged from 15 to 22% but these differences were not significant ($p > 0.05$). In
244 contrast, large differences among genera were observed for the binding of ZEN and
245 fumonisins ($p < 0.05$). It is interesting to note that, independently of the molecular
246 structure of these mycotoxins, the genera *Streptococcus* and *Enterococcus* ranked
247 always as the most efficient binders under the conditions of the assay. In particular,
248 strains of *Streptococcus thermophilus*, which bound up to 49%, 33%, 24%, and 62% of
249 ZEN, DON, FB₁ and FB₂, respectively. Out of the 202 strains tested about two third
250 were *Lactobacilli*. This apparent bias has a biotechnological explanation because
251 species of this genus are very active during the ensiling process and hence widely used
252 as inoculants. *Lactobacilli* are dominant members of the bacterial population found in
253 grass and corn silages (Langston and Bouma 1960; Dellaglio and Torriani 1986). *L.*
254 *plantarum*, of which we tested 46 isolates, is a particularly common epiphytic specie,
255 which is also found in many commercial silage additives (Weinberg and Muck 1996).
256 All the other selected strains used in this work are representative of genera of
257 fermentative bacteria regularly associated with silage (Moon et al. 1981; Driehuis and
258 Elferink 2000). The binding activities of *Lactobacillus* spp. are shown in Table IV.
259 Significant differences were observed among strains; however, compared to

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

262

263 Discussion

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

269

270 The reduction of ZEN into α -ZOL can be carried out in the liver and also by the flora
271 harboured in the digestive tract of animals (Olsen and Kiessling 1983; Kiessling et al.
272 1984; Kollarczik et al. 1994). However, it had never been observed with pure strains of
273 lactic acid bacteria (LAB). In this study, this biotransformation was observed in 11
274 strains but it cannot be considered as a detoxification because α -ZOL is three to four
275 times more estrogenic than ZEN (Mirocha et al. 1979). All the positive bacterial strains
276 detected in this work were capable to produce large quantities of α -ZOL from ZEN.
277 Even if strains possessing this property represent a relatively low percentage of the total
278 fermentative bacteria, they could form part of the epiphytic population present in
279 fermented feeds and convert significant amounts of ZEN into α -ZOL. This means that
280 the absence of ZEN or its presence in non toxic concentrations does not guarantee feed
281 innocuity, and therefore, it could be advisable to check for the presence of α -ZOL in
282 fermented feeds. It could also be recommended to check if this property is not present in
283 strains used as fermentation starters. The biotransformation of ZEN to α -ZOL might
284 explain the results of a recent study where a significant decrease in the concentration of

285 ZEN was observed during the fermentation of corn meal by LAB but without a
286 reduction in toxicity (Mokoena et al. 2005).
287
288 Binding of mutagens and mycotoxins by LAB was reported in numerous studies
289 (Morotomi and Mutai 1986; El-Nezami et al. 1998). Binding of DON and ZEN by three
290 probiotic strains was also recently reported, but they were not effective at the low
291 densities found in fermented corn feeds (El-Nezami et al. 2002a; 2002b). The results of
292 the present work showed that significant quantities of *Fusarium* toxins including
293 fumonisins are bound by most species of fermentative bacteria at a density of 5×10^8
294 CFU ml⁻¹. We also confirmed previous observations (Niderkorn et al. 2006) showing
295 that FB₂ was removed more efficiently than FB₁ in spite of their similarity in chemical
296 structure.
297
298 Since most strains of fermentative bacteria tested seem to have the ability to bind DON,
299 ZEN and fumonisins, it is probable that these *Fusarium* toxins are also partially bound
300 by the epiphytic microflora of silage or by inoculants already utilized to improve silage
301 fermentation or aerobic stability. Although *Lactobacilli* are widely utilized as silage
302 additives, none of the strains tested was particularly efficient at binding *Fusarium*
303 toxins. From the isolates tested in this work, the use of fermentative bacteria to bind
304 *Fusarium* toxins in silage appear to be more advantageous for *Streptococci* and
305 *Enterococci*, which bound more ZEN and fumonisins than other genera. Consequently,
306 an advantageous use of fermentative bacteria to bind *Fusarium* toxins in silage would
307 be limited to these two genera. The higher capacity to bind *Fusarium* toxins by
308 *Streptococci* and *Enterococci* cannot be explained by their size as they are smaller than
309 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.

318

319 **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.

328

329

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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 B₁ and B₂ (FB₁, FB₂) by different genera of fermentative bacteria.

Table IV. *In vitro* binding of zearalenone, deoxynivalenol, and fumonisins B₁ and B₂ 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 μ g ml⁻¹) (a), without and with fumonisins B₁ and B₂ (FB₁ and FB₂, 5 μ g ml⁻¹ each) (b), without and with deoxynivalenol (DON, 5 μ g ml⁻¹) (c).

Figure 2. HPLC chromatograms of corn infusion supernatants containing zearalenone (ZEN, 5 μ g 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.

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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 $\rightarrow$ 130	46
		317 $\rightarrow$ 131	35
		317 $\rightarrow$ 149	35
		317 $\rightarrow$ 175	34
		317 $\rightarrow$ 187	30
		317 $\rightarrow$ 273	27
α -ZOL	9.59	319 $\rightarrow$ 130	54
		319 $\rightarrow$ 131	55
		319 $\rightarrow$ 160	37
		319 $\rightarrow$ 174	13
		319 $\rightarrow$ 275	31
		319 $\rightarrow$ 301	27
ZAN	9.90	319 $\rightarrow$ 137	52
		319 $\rightarrow$ 161	35
		319 $\rightarrow$ 163	38
		319 $\rightarrow$ 205	31
		319 $\rightarrow$ 259	37
		319 $\rightarrow$ 275	28

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

Taxon	Source	Used in	ZEN biotransformed (%)
<i>Lactobacillus</i>			
<i>L. brevis</i>	LGMPA - C11	Cheese making	41 (1)
<i>L. brevis</i>	Lallemand - R0002	Animal nutrition	47 (2)
<i>L. brevis</i>	Lallemand - L62	Baking	24 (1)
<i>L. casei casei</i>	LGMPA - B1	Cheese making	49 (4)
<i>L. fermenti</i>	LGMPA - 34	Cheese making	19 (0)
<i>L. plantarum</i>	LGMPA - J1	Cheese making	41 (2)
<i>L. plantarum</i>	Lallemand - R1113	Baking	50 (3)
<i>L. spp.</i>	LGMPA - F7	Cheese making	23 (1)
<i>Leuconostoc</i>			
<i>Ln. mesenteroides</i> or <i>Ln. pseudomesenteroides</i>	URF - 171	Cheese making	16 (2)
<i>Ln. mesenteroides</i> or <i>Ln. pseudomesenteroides</i>	URF - 365	Cheese making	8 (0)
<i>Ln. mesenteroides</i>	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 μ g ml⁻¹). Data shown are the means (SD) of duplicate.

Table III. *In vitro* binding of zearalenone (ZEN), deoxynivalenol (DON), and fumonisins B₁ and B₂ (FB₁, FB₂) by different genera of fermentative bacteria*

Genus	Number of strains	Fraction bound (%) [†]			
		ZEN [‡]	DON	FB1	FB2
<i>Enterococcus</i>	4	35 (2) ^c	22 (8)	14 (7) ^b	43 (8) ^b
<i>Lactobacillus</i>	137	20 (4) ^b	15 (9)	10 (6) ^{ab}	24 (11) ^a
<i>Lactococcus</i>	17	17 (4) ^{ab}	18 (8)	11 (4) ^{ab}	19 (8) ^a
<i>Leuconostoc</i>	6	28 (7) ^{bc}	18 (2)	4 (3) ^a	18 (5) ^a
<i>Pediococcus</i>	2	17 (2) ^{ab}	18 (3)	4 (3) ^a	23 (10) ^a
<i>Propionibacterium</i>	5	10 (3) ^a	18 (6)	10 (7) ^{ab}	15 (7) ^a
<i>Streptococcus</i>	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 FB₁ and FB₂, 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 B₁ and B₂ by *Lactobacillus* species*

<i>Lactobacillus</i> species	Number of strains	Fraction bound (%) [†]			
		ZEN [‡]	DON	FB1	FB2
<i>L. acidophilus</i>	7	19 (4)	22 (4) ^c	13 (6) ^b	22 (10) ^{abc}
<i>L. brevis</i>	5	20 (1)	19 (5) ^c	9 (4) ^{ab}	27 (9) ^{bcd}
<i>L. buchneri</i>	3	20 (2)	4 (4) ^{ab}	11 (1) ^{ab}	28 (12) ^{cd}
<i>L. casei</i> . subsp. <i>alactosus</i>	1	18 (0)	10 (0) ^{abc}	3 (0) ^a	9 (0) ^a
<i>L. casei</i> . subsp. <i>casei</i>	21	18 (4)	17 (9) ^c	11 (5) ^{ab}	25 (11) ^{bcd}
<i>L. casei</i> . subsp. <i>rhamnosus</i>	4	18 (6)	19 (6) ^c	12 (7) ^{ab}	13 (3) ^{ab}
<i>L. delbruekii</i> . subsp. <i>bulgaricus</i>	5	28 (4)	11 (9) ^{abc}	12 (5) ^{ab}	26 (7) ^{bcd}
<i>L. delbruekii</i> . subsp. <i>lactis</i>	3	22 (5)	19 (9) ^c	9 (4) ^{ab}	23 (9) ^{abc}
<i>L. fermentum</i>	10	21 (7)	13 (8) ^{abc}	13 (12) ^b	25 (11) ^{bcd}
<i>L. helveticus</i>	8	19 (2)	17 (7) ^c	11 (6) ^{ab}	31 (9) ^{cd}
<i>L. jugurti</i>	7	19 (4)	20 (9) ^c	12 (7) ^{ab}	34 (9) ^{cd}
<i>L. paraplantarum</i>	7	22 (2)	13 (8) ^{abc}	5 (4) ^{ab}	10 (6) ^a
<i>L. pentosus</i>	1	23 (0)	2 (0) ^a	4 (0) ^{ab}	32 (0) ^{cd}
<i>L. plantarum</i>	46	19 (3)	13 (9) ^{abc}	9 (5) ^{ab}	22 (10) ^{abc}
<i>L. reuteri</i>	1	17 (0)	15 (0) ^{bc}	21 (0) ^c	37 (0) ^d
<i>L. unidentified</i>	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 B₁ and B₂ (FB₁ and FB₂, 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, $5 \mu\text{g ml}^{-1}$) incubated at 25°C for 24 h without bacteria (a) and with *Lactobacillus brevis* R0002 ($5 \times 10^8 \text{ CFU ml}^{-1}$) (b). ZEN (retention time = 10.4 min) and metabolite (retention time = 9.2 min) showed a similar UV spectrum (inset).

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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.