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► To cite this version:

Vincent Niderkorn, Diego Morgavi, Estelle Pujos, Antoine Tissandier, Hamid Boudra. Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro model simulating corn silage. *Food Additives and Contaminants*, 2007, 24 (04), pp.406-415. 10.1080/02652030601101110 . hal-00577523

HAL Id: hal-00577523

<https://hal.science/hal-00577523>

Submitted on 17 Mar 2011

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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

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-167.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	27-Oct-2006
Complete List of Authors:	Niderkorn, Vincent; French Institute for Agricultural Research (INRA), Herbivore Research Unit; Lallemand SAS Morgavi, Diego; French Institute for Agricultural Research (INRA), Herbivore Research Unit Pujos, Estelle; French Institute for Agricultural Research (INRA), Research Unit for Protein-Energy Metabolism Tissandier, Antoine; French Institute for Agricultural Research (INRA), Herbivore Research Unit Boudra, Hamid; French Institute for Agricultural Research (INRA), Herbivore Research Unit
Methods/Techniques:	HPLC, LC/MS, Screening - microbial screening
Additives/Contaminants:	zearalenone, Fumonisins, Mycotoxins - fusarium, trichothecenes
Food Types:	Animal feedingstuffs

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3 1 **Screening of fermentative bacteria for their ability to bind and**
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6 2 **biotransform deoxynivalenol, zearalenone and fumonisins in an *in vitro***
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9 3 **model simulating corn silage**
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8 **Abstract**

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10 Fermentative bacteria can potentially be utilized to detoxify corn silage contaminated by
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12 *Fusarium* toxins. The objective of the present study was to test a large number of these
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14 bacteria for their ability to bind and/or biotransform deoxynivalenol (DON),
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16 zearalenone (ZEN), and fumonisins B₁ and B₂ (FB₁, FB₂) in conditions simulating corn
17
18 silage. A total of 202 strains were screened in contaminated, pH 4, corn infusion
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20 inoculated with 5×10^8 CFU ml⁻¹. Eight *Lactobacilli* and three *Leuconostoc*
21
22 biotransformed ZEN into α zearalenol, but no biotransformation was detected for DON
23
24 and fumonisins. In contrast, most strains were capable of binding *Fusarium* toxins. The
25
26 most effective genera were *Streptococcus* and *Enterococcus* capable of binding up to
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28 33%, 49%, 24% and 62% of DON, ZEN, FB₁ and FB₂, respectively. The ability to bind
29
30 *Fusarium* toxins seems to be a common property of fermentative bacteria and could
31
32 help to decrease their toxicity in animals.

33
34 **Keywords:** *Fusarium*, *mycotoxins*, *fermentative bacteria*, *probiotic*, *binding*,
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36 *biotransformation*, *corn silage*

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48 **Running headline:** *Fusarium* toxins removal by fermentative bacteria
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37 **Introduction**

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

53

54 In spite of the progress made in preventive approaches such as breeding of resistant corn
55 varieties (Mesterhazy 1989) and improvement in agronomic practices (Trenholm et al.
56 1989) to decrease *Fusarium* contamination, hazardous concentrations of *Fusarium*
57 toxins may occur in corn justifying the use of detoxification methods. Physical and
58 chemical methods such as ammonia treatment or the addition of adsorbents (Scott 1998;
59 Huwig et al. 2001) are generally expensive, drastic, not specific, or simply not adapted
60 to the treatment of corn destined to livestock. Inorganic adsorbents, although they have
61 a high affinity to bind aflatoxins, are not efficient for *Fusarium* toxins. In addition, these

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3 62 agents can also bind important micronutrients like minerals or vitamins (Guerre 2000).
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6 63 Practical methods for the detoxification of silage are currently not available. A
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8 64 promising approach is the use of selected microorganisms to remove *Fusarium* toxins
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10 65 (Bata and Lasztity 1999). Detoxification can be achieved by microbial binding and/or
11
12 66 biotransformation of mycotoxins into less toxic compounds (Styriak and Conkova
13
14
15 67 2002). Due to their role in the ensiling process, fermentative bacteria are appropriate
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17 68 candidates for the biological detoxification of corn silage. The bioconversion of
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19 69 *Fusarium* toxins by rumen microbes and intestinal microflora was reported (Kiessling et
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21 70 al. 1984; Kollarczik et al. 1994; Caloni et al. 2000) but the bacteria responsible for their
22
23 71 biotransformation were not identified. In contrast, binding of DON and ZEN to the cell
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25 72 wall of probiotic strains *in vitro* has been recently reported (El-Nezami et al. 2002a;
26
27 73 2002b). This property can decrease the bioavailability of these compounds by reducing
28
29 74 absorption and limit their toxic effects (El-Nezami et al. 1999). However, to date, only
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31 75 three strains were thoroughly investigated. In addition, they were effective at about 10^{10}
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33 76 CFU ml⁻¹, a high bacterial concentration that is difficult to obtain in fermented corn
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35 77 feeds.
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43 79 In this study, a large number of strains of fermentative bacteria were screened for their
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45 80 ability to remove DON, ZEN, FB₁ and FB₂ from a medium that simulated silage in term
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47 81 of substrate, pH, and bacterial concentration. The test conditions chosen allowed to
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49 82 observe simultaneously binding and/or biotransformation.
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54 84 **Materials and methods**

55 85 *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,
8
9 89 France; Lallemand SAS, Blagnac, France; Laboratory of Dynamics, Evolution and
10
11 90 Expression of Genomes of Microorganisms, University Louis Pasteur/CNRS FRE 2326,
12
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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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-
34
35 100 stoke, UK) containing 5% v/v of a 10% w/v lactose solution was used for the culture of
36
37 101 *Lactococci*, *Streptococci* and *Enterococci*. Yeast Extract Lactate (YEL) medium,
38
39 102 prepared according to Malik and Vedamuthu (1968), was used for the culture of
40
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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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
8 113 Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The most common derivative of
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10 114 DON, deepoxy-deoxynivalenol (DOM) was purchased from Biopure (Tulin, Austria).
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12 115 Derivatives of fumonisins, aminopentols (HFB₁ and HFB₂), were obtained by
13
14 116 hydrolysis of FB₁ and FB₂ according to Pagliuca et al. (2005). DON and ZEN (and
15
16 117 derivatives) were dissolved in acetonitrile and methanol, respectively. Concentration
17
18 118 was determined by measuring the absorbance at 218 nm for DON ($\epsilon = 6406 \text{ mmol}^{-1}$
19
20 119 cm^2) and at 274 nm for ZEN ($\epsilon = 13\,909 \text{ mmol}^{-1} \text{ cm}^2$). Since spectrophotometry is not
21
22 120 possible with FB₁ and FB₂, these compounds were dissolved in an exact volume of
23
24 121 acetonitrile-water 1:1(v/v) to achieve the desired concentration. Mycotoxin solutions of
25
26 122 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
27
28 123 prepared by evaporating solvents with nitrogen gas, redissolving in water (DON, FB₁,
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30 124 FB₂) or ethanol (ZEN) and adding corn infusion to reach the desired concentration. The
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32 125 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
48
49 131 of growth (30 or 37°C) for 24 h. *Propionibacteria* strains were incubated at 30°C for 72
50
51 132 h. At the end of incubation, the bacterial concentration of cultures was estimated by
52
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
11
12 140 one of the three mycotoxin solutions. Positive controls containing no bacteria and a
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14
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 $\text{supernatant} / \text{Peak area of mycotoxin in the positive control})]$.

32 33 34 149 35 36 150 *HPLC mycotoxins analysis*

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38 151 Separation and quantification of mycotoxins were performed by the method described
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40 152 previously (Niderkorn et al. 2006) with slight modifications in order to remove
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42 153 interferences. The HPLC system consisted of a P1000XR pump (SpectraSYSTEM, San
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44 154 Jose, California, USA) and an automatic sampler (SpectraPhysics, San Jose, California,
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46 155 USA). Separation was performed on C₁₈ reversed-phase columns (Macherey-Nagel,
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48 156 France); Nucleodur (125 × 4.6 mm, 5 μm) was used for DON and ZEN and ProntoSil
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50 157 (120 × 3.0 mm, 3 μm) was used for FB₁ and FB₂. The mobile phase used to separate
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52 158 DON consisted of a water–acetonitrile solution (95:5 v/v) and the flow rate was 1 ml
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54 159 min⁻¹. Detection was set at 220 nm and retention time was 12.1 min. After 14 min of
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60 160 run, the column was washed with a water–acetonitrile solution (80:20 v/v) to remove

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3 161 interferences and was equilibrated with the mobile phase for 6 min before the following
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5 162 injection. The mobile phase used to separate ZEN consisted of a water–methanol
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7 163 solution (40:60 v/v) and the flow rate was 1.2 ml min⁻¹. ZEN was detected by photo-
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9 164 diode-array (scan 200 – 380 nm) and fluorescence ($\lambda_{\text{exc}} = 274$ nm, $\lambda_{\text{em}} = 440$ nm) placed
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11 165 in series. The retention time of ZEN was 10.7 min. Fumonisin were derivatized before
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13 166 injection using *ortho*-phtalaldehyde (OPA) plus mercaptoethanol. Ten μ l of sample
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15 167 were mixed with 90 μ l of borate buffer pH 10, then 100 μ l of derivatizing reagent were
16
17 168 added. The preparation was mixed and allowed to react for 2 min before injection.
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19
20 169 Separation of FB₁ and FB₂ was performed with a gradient elution using acetonitrile (A)
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22 170 and a mixture of 0.05 mol l⁻¹ dihydrogen phosphate–methanol (1:1 v/v) acidified at pH
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24 171 3.35 (B). The gradient program was as follows: 10% to 50% A in 6 min, 50% A for 7
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26 172 min, and 50% to 10% A in 1 min. The flow rate was 1 ml min⁻¹ and detection was set at
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28 173 336 nm excitation and 440 nm emission. The retention times were 10.0 and 12.7 min for
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30 174 FB₁ and FB₂, respectively.
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176 *HPLC-ESI-MS/MS analysis*

41 177 A HPLC-MS/MS (TSQ Quantum Ultra AM, Thermo Electron, San Jose, California,
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43 178 USA) equipped with an electrospray ionization source (ESI) interface operating in the
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45 179 negative mode was used to identify the ZEN derivative produced by some strains.
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47 180 Chromatographic separation and MS analysis was performed on a 125 × 4.6 mm
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49 181 Prontosil C₁₈ column with particle size of 3 μ m (Bischoff, Leonberg, Germany), using a
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51 182 methanol – 1% acetic acid (65:35 v/v) mobile phase at a flow rate of 0.8 ml min⁻¹.
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53 183 Parameters of the MS were optimised using standard solutions of α ZOL in mobile
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55 184 phase. The test responses were recorded with electrospray voltage 3.0 kV, capillary
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57 185 temperature 400°C, sheath flow and auxiliary flow 50 and 10 arbitrary units,
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3 186 respectively. Multiple reaction monitoring (MRM) was used to identify the metabolite.
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5 187 The most intense transitions from the fragmentation of ZEN, α ZOL and ZAN were
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7
8 188 determined. Six product ions were selected at different collision energies (CE) so as to
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10 189 characterize each molecule of interest (Table I). For analysis, 4 ml of corn infusion
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12 190 sample were extracted with 4 ml dichloromethane. The mixture was mixed for 15 min
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14
15 191 then centrifuged at 3000 g for 10 min. One ml of the organic layer was transferred into a
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17 192 new tube and evaporated to dryness under a stream of N₂. The dried residue was
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19 193 redissolved in 200 μ l of mobile phase, and 5 μ l of this solution were injected into the
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21 194 HPLC-MS/MS system. MS-analysis of extract was compared with ZEN, α ZOL and
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23 195 ZAN standards. [Insert Table I about here]
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28 29 197 *Statistical analysis*

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31 198 Data was subjected to the analysis of variance (ANOVA). A significant difference ($p <$
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33 199 0.05) between means was determined by Duncan's multiple range test using the
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35 200 Statistical Analysis System software package (SAS Institute Inc., Cary, NC).
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40 41 202 **Results**

42
43 203 The experimental model used in this work allowed us to observe at the same time
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45 204 biotransformation and binding of *Fusarium* toxins by fermentative bacteria. The HPLC
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47 205 method used, separated each mycotoxin as well as their main derivatives from the corn
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49 206 infusion medium matrix without an extraction step (Figure 1) and thus facilitated the
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51 207 screening. [Insert Figure 1 about here]
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56 57 209 *Biotransformation of mycotoxins*

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3 210 In incubations containing DON and fumonisins no biotransformation products were
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5 211 observed. There were not peaks corresponding to DOM and aminopentol—derivatives
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8 212 of DON and fumonisins, respectively—or any other unknown peak in the HPLC
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10 213 chromatograms. In contrast, in incubations containing ZEN for 11 out of the 202 strains
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12 214 tested (about 5.5%), an additional peak was observed in the chromatograms. This peak
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14 215 had the same retention time for all 11 strains suggesting that it was the same metabolite
15
16 216 (Figure 2). Eight out of the 11 strains were *Lactobacilli* and the other three were
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18 217 *Leuconostoc*. These biotransforming *Lactobacilli* removed approximately twice more
19
20 218 ZEN from the incubation media than strains from the same genus that only bound the
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22 219 mycotoxin (Table II and III). [Insert Figure 2 and Table II about here]
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29 221 The ZEN metabolite showed a UV spectrum similar to ZEN with absorption maxima at
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31 222 236, 274, and 316 nm (Figure 2, inset). Retention time (Rt) of this peak was compared
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33 223 to those of main ZEN derivatives using modified chromatographic conditions to
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35 224 improve separation. The changes in the chromatography were in the mobile phase,
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37 225 which consisted in methanol – 1% acetic acid mixture (65:35 v/v), and the flow rate,
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39 226 which was set at 0.8 ml min⁻¹. Under these conditions, the Rt of the unknown metabolite
40
41 227 was 9.56 min, similar to α ZOL (9.50 min), while Rts of β ZOL, α and β ZAL, and ZAN
42
43 228 standards were 6.10, 7.74, 4.35, and 9.84 min, respectively. This result indicated that α
44
45 229 ZOL was most likely the metabolite produced. The Rt of ZAN was also near to that of
46
47 230 the unknown metabolite. However, ZAN, at a concentration of 5 $\mu\text{g ml}^{-1}$ had a peak
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49 231 area considerably lower than the peak area of the metabolite produced by active
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51 232 bacterial strains incubated with an equal concentration of ZEN and hence ZAN was
52
53 233 unlikely the searched compound. Additional HPLC-MS/MS analyses were performed
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55 234 on ZEN-containing corn infusion extracts after incubation with biotransforming strains.
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3 235 The LC-MS showed that the new peak had the same mass transitions characteristic (m/z
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5 236 = 319) as α ZOL (Figure 3) and thus unequivocally identified the metabolite. [Insert
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7
8 237 Figure 3 about here]

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11
12 239 *Binding of zearalenone, deoxynivalenol and fumonisins from acidified corn infusion*

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14
15 240 Table III shows the mycotoxin-binding capacity of the tested fermentative bacteria
16
17 241 grouped by genera. Across all genera, FB₂ was the mycotoxin most efficiently removed
18
19 242 followed by ZEN, DON, and FB₁. The average fraction of DON bound by the different
20
21 243 genera ranged from 15 to 22% but these differences were not significant ($p > 0.05$). In
22
23 244 contrast, large differences among genera were observed for the binding of ZEN and
24
25 245 fumonisins ($p < 0.05$). It is interesting to note that, independently of the molecular
26
27 246 structure of these mycotoxins, the genera *Streptococcus* and *Enterococcus* ranked
28
29 247 always as the most efficient binders under the conditions of the assay. In particular,
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31 248 strains of *Streptococcus thermophilus*, which bound up to 49%, 33%, 24%, and 62% of
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33 249 ZEN, DON, FB₁ and FB₂, respectively. Out of the 202 strains tested about two third
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35 250 were *Lactobacilli*. This apparent bias has a biotechnological explanation because
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37 251 species of this genus are very active during the ensiling process and hence widely used
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39 252 as inoculants. *Lactobacilli* are dominant members of the bacterial population found in
40
41 253 grass and corn silages (Langston and Bouma 1960; Dellaglio and Torriani 1986). *L.*
42
43 254 *plantarum*, of which we tested 46 isolates, is a particularly common epiphytic specie,
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45 255 which is also found in many commercial silage additives (Weinberg and Muck 1996).
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47 256 All the other selected strains used in this work are representative of genera of
48
49 257 fermentative bacteria regularly associated with silage (Moon et al. 1981; Driehuis and
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51 258 Elferink 2000). The binding activities of *Lactobacillus* spp. are shown in Table IV.
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53 259 Significant differences were observed among strains; however, compared to

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3 260 *Streptococcus* and *Enterococcus* strains the binding capacity of the *Lactobacilli* was less
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5 261 important. [Insert Table III and Table IV about here]
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10 263 **Discussion**

11
12 264 The ability of fermentative bacteria to biotransform or bind *Fusarium* toxins in whole-
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14 265 plant corn silage and fermented corn grain is a promising way to reduce their toxic
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16 266 effects on livestock. In this work, the conditions used to screen fermentative bacteria
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18 267 permitted to observe simultaneously binding and biotransformation in a liquid medium
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20 268 representative of corn silage.
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25
26 270 The reduction of ZEN into α -ZOL can be carried out in the liver and also by the flora
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28 271 harboured in the digestive tract of animals (Olsen and Kiessling 1983; Kiessling et al.
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30 272 1984; Kollarczik et al. 1994). However, it had never been observed with pure strains of
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32 273 lactic acid bacteria (LAB). In this study, this biotransformation was observed in 11
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34 274 strains but it cannot be considered as a detoxification because α -ZOL is three to four
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36 275 times more estrogenic than ZEN (Mirocha et al. 1979). All the positive bacterial strains
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38 276 detected in this work were capable to produce large quantities of α -ZOL from ZEN.
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40 277 Even if strains possessing this property represent a relatively low percentage of the total
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42 278 fermentative bacteria, they could form part of the epiphytic population present in
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44 279 fermented feeds and convert significant amounts of ZEN into α -ZOL. This means that
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46 280 the absence of ZEN or its presence in non toxic concentrations does not guarantee feed
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48 281 innocuity, and therefore, it could be advisable to check for the presence of α -ZOL in
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50 282 fermented feeds. It could also be recommended to check if this property is not present in
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52 283 strains used as fermentation starters. The biotransformation of ZEN to α -ZOL might
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54 284 explain the results of a recent study where a significant decrease in the concentration of
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3 285 ZEN was observed during the fermentation of corn meal by LAB but without a
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5 286 reduction in toxicity (Mokoena et al. 2005).

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10 288 Binding of mutagens and mycotoxins by LAB was reported in numerous studies
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12 289 (Morotomi and Mutai 1986; El-Nezami et al. 1998). Binding of DON and ZEN by three
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14 290 probiotic strains was also recently reported, but they were not effective at the low
15
16 291 densities found in fermented corn feeds (El-Nezami et al. 2002a; 2002b). The results of
17
18 292 the present work showed that significant quantities of *Fusarium* toxins including
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20 293 fumonisins are bound by most species of fermentative bacteria at a density of 5×10^8
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22 294 CFU ml⁻¹. We also confirmed previous observations (Niderkorn et al. 2006) showing
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24 295 that FB₂ was removed more efficiently than FB₁ in spite of their similarity in chemical
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26 296 structure.

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33 298 Since most strains of fermentative bacteria tested seem to have the ability to bind DON,
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35 299 ZEN and fumonisins, it is probable that these *Fusarium* toxins are also partially bound
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37 300 by the epiphytic microflora of silage or by inoculants already utilized to improve silage
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39 301 fermentation or aerobic stability. Although *Lactobacilli* are widely utilized as silage
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41 302 additives, none of the strains tested was particularly efficient at binding *Fusarium*
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43 303 toxins. From the isolates tested in this work, the use of fermentative bacteria to bind
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45 304 *Fusarium* toxins in silage appear to be more advantageous for *Streptococci* and
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47 305 *Enterococci*, which bound more ZEN and fumonisins than other genera. Consequently,
48
49 306 an advantageous use of fermentative bacteria to bind *Fusarium* toxins in silage would
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51 307 be limited to these two genera. The higher capacity to bind *Fusarium* toxins by
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53 308 *Streptococci* and *Enterococci* cannot be explained by their size as they are smaller than
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55 309 other genera with lower binding capacity such as *Lactobacillus*. The composition of the
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3 310 cell wall may confer the *Streptococci* and *Enterococci* their superior binding property.
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5 311 The mechanism of binding of mycotoxins by bacteria is still not clear. In the case of
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7 312 ZEN, it was recently suggested that this mycotoxin predominantly binds to
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9 313 carbohydrate moieties of the cell wall of LAB by means of hydrophobic interactions
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11 314 (El-Nezami et al. 2004). As hydrophobic interactions are relatively weak, this
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13 315 bacterium-mycotoxin complex could be unstable. Nevertheless, these authors also
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15 316 suggested that ZEN binding is not limited to hydrophobic links and other type of
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17 317 interactions could be important.
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319 **Conclusions**

26
27 320 The use of selected strains of fermentative bacteria could be a method to limit the toxic
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29 321 effects of *Fusarium* toxins present in corn silage. Our results have shown that the
30
31 322 biodegradation of mycotoxins is possible. However, the resulting compound is not
32
33 323 always less toxic than the parent mycotoxin. Binding seems to be the most promising
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35 324 mode of action, but for the practical application of this technology, it is essential that the
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37 325 bacterium-mycotoxin complex remain stable during the digestion process. Further
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39 326 studies are planned to evaluate the stability of the complex in the different parts of the
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41 327 gastrointestinal tract of farm animals.
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51 **Acknowledgements**

52
53 331 V. Niderkorn is the recipient of a CIFRE Lallemand SAS research fellowship. The
54
55 332 authors are thankful to the Research Unit for Food Process Engineering and
56
57 333 Microbiology, INRA Thivernal-Grignon, France, the Research Laboratory for Animal
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59 334 Husbandry, INRA Corte, France, the Laboratory of Dynamics, Evolution and
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3 335 Expression of Genomes of Microorganisms, University Louis Pasteur/CNRS FRE 2326,
4
5 336 Strasbourg, France, the Cheese Research Laboratory, INRA, Aurillac, France, and
6
7 337 Lallemand SAS, Blagnac, France, for providing the strains of fermentative bacteria.
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9
10 338 They also thank Henri Durand, Frederique Chaucheyras-Durand, and Corinne Pouyet
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12 339 for the scientific assistance in this project.
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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.

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.

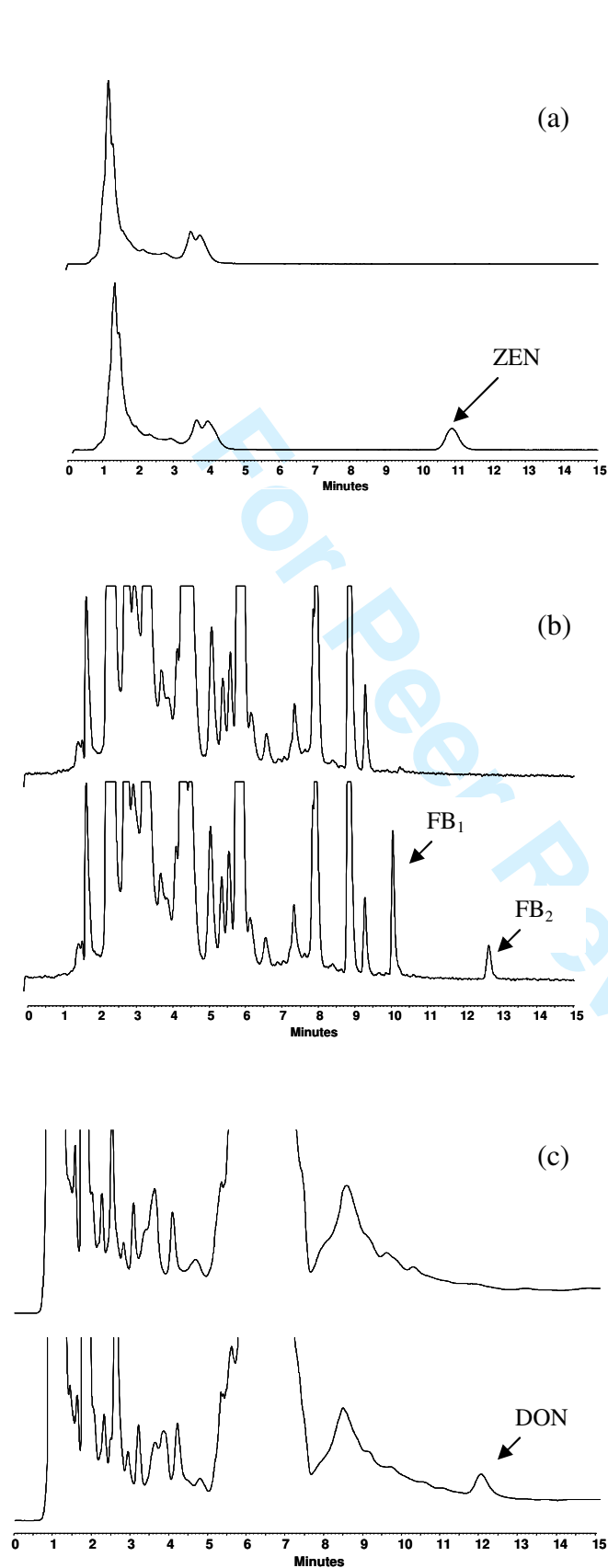
Table IV. *In vitro* binding of zearalenone, deoxynivalenol, and fumonisins B₁ and B₂ by *Lactobacillus* species*

<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.



56 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),
57 without and with fumonisins B₁ and B₂ (FB₁ and FB₂, 5 µg ml⁻¹ each) (b), without and
58 without and with deoxynivalenol (DON, 5 µg ml⁻¹) (c).
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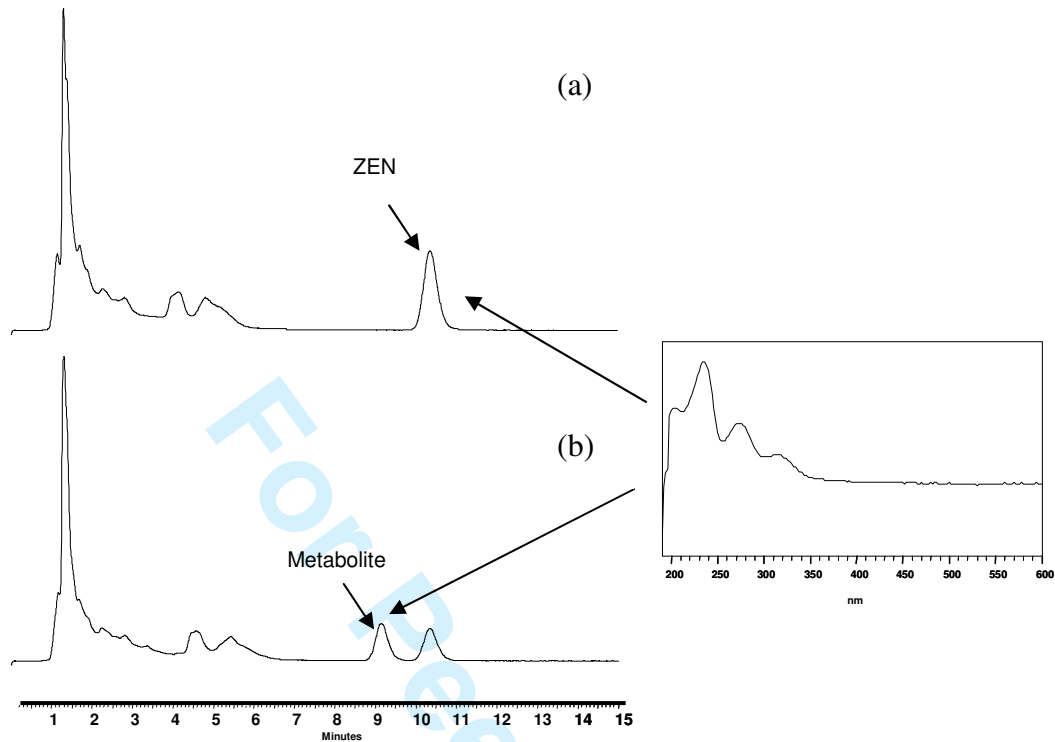


Figure 2. HPLC chromatograms of corn infusion supernatants containing zearalenone (ZEN, $5 \mu\text{g ml}^{-1}$) incubated at 25°C for 24 h without bacteria (a) and with *Lactobacillus brevis* R0002 ($5 \times 10^8 \text{ CFU ml}^{-1}$) (b). ZEN (retention time = 10.4 min) and metabolite (retention time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

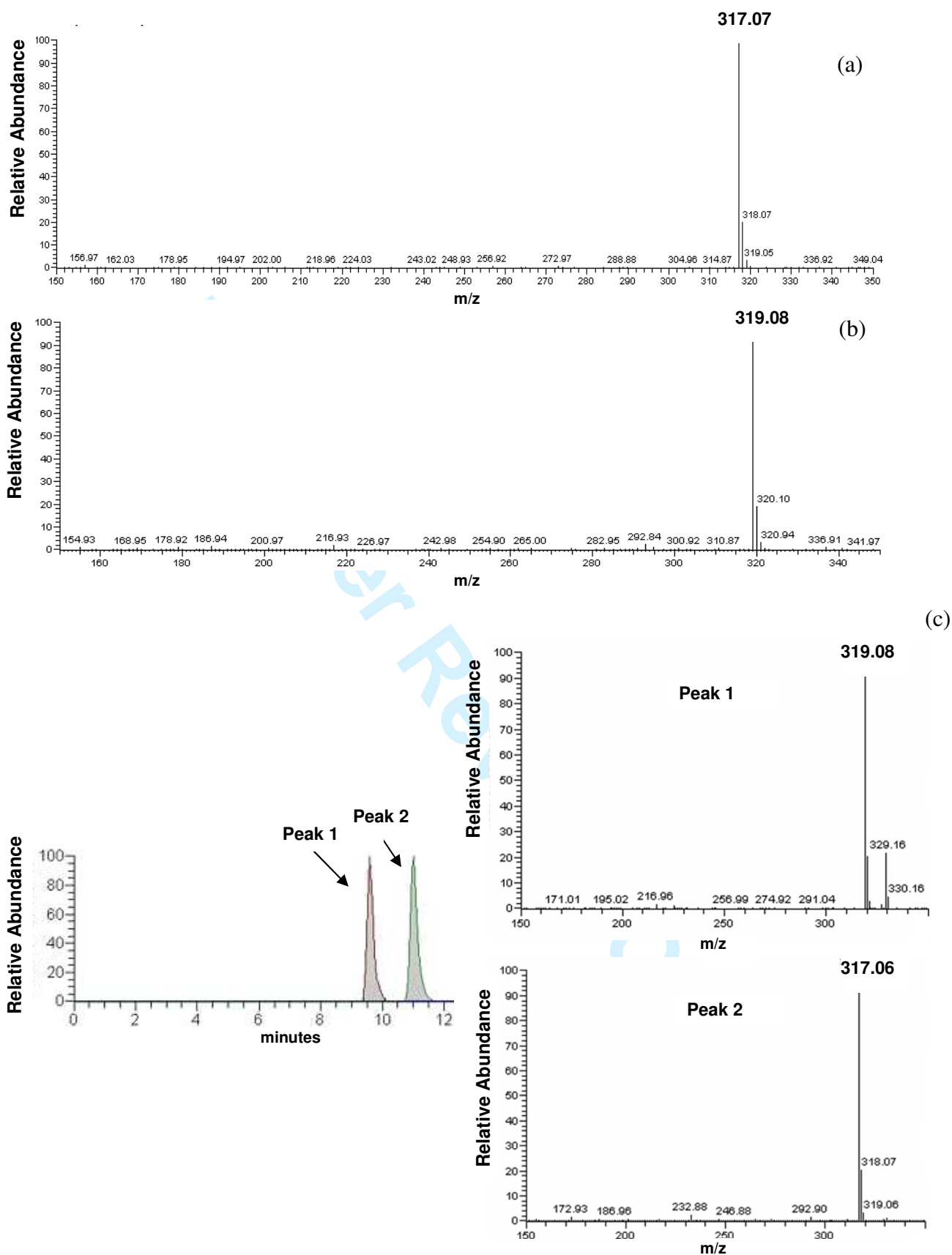


Figure 3. HPLC-MS spectra of zearalenone, ZEN (a) and α zearalenol, α ZOL (b) standards alone, and the extract from corn infusion supernatant containing ZEN and the produced metabolite (c). Spectra of peaks 1 and 2 showed parent mass of m/z 319.08 and 317.06, respectively.