JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical Moieties and Interactions Involved in the Binding of Zearalenone to the Surface of *Lactobacillus rhamnosus* Strains GG

HANI EL-NEZAMI,*,†,‡ NEKTARIA POLYCHRONAKI,† YUAN KUN LEE,§ CAROLYN HASKARD,|| RISTO JUVONEN,⊥ SEPPO SALMINEN,# AND HANNU MYKKÄNEN†

Department of Clinical Nutrition, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland, Food and Health Research Center (ETTK), Mediteknia, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland, Department of Microbiology, Faculty of Medicine, National University of Singapore, Singapore 117597, Australian Water Quality Centre, Private Mail Bag 3, Salisbury, South Australia 5108, Australia, Department of Toxicology and Pharmacology, University of Kuopio, P.O.B. 1627, FIN-70211 Kuopio, Finland, and Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

Viable, heat-and acid-killed *Lactobacillus rhamnosus* strain GG (LGG) has shown high binding properties with zearalenone (ZEN). To identify the type of chemical moieties and interactions involved in binding with the ZEN, LGG was subjected to different chemical and enzymatical treatments, prior to the binding experiments. Pretreating the viable, heat- and acid-killed bacteria with *m*-periodate significantly decreased ZEN binding, suggesting that ZEN binds predominantly to carbohydrate components. Pretreatment with Pronase E had no effect on the ability of viable cells to bind ZEN, however, a reduction in the binding of ZEN by heat- and acid-killed cells, suggesting that the new binding sites exposed by heat or acid are proteins in nature. Pretreatment with urea also decreased binding, suggesting that hydrophobic interactions play a role in ZEN binding. The binding of ZEN in concentrations ranging from 0.79 to 62.82 μM and its subsequent dissociation by repetitive aqueous washes was also studied. The binding sites of the bacteria were not saturated by the maximum ZEN concentration studied.

KEYWORDS: Estrogenic mycotoxin; zearalenone; *Lactobacillus*; lactic acid bacteria; binding.

INTRODUCTION

Zearalenone (ZEN) is an estrogenic mycotoxin (*1*) known as 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid μ-lactone. It is a metabolite primarily associated with several *Fusarium* species (i.e., *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*), with *F. graminearum* being the species most responsible for the estrogenic effects commonly observed in farm animals (*2*). ZEN binds to estrogen receptors influencing estrogen-dependent transcription in the nucleus (*3*). Receptor binding by ZEN has been shown to inhibit the binding of estrogenic hormones in rat mammary tissues (*4*). Negative effects of ZEN on swine reproductive function have been reviewed (*5*). Swelling of the vulva and mammary glands and occasional vaginal and rectal prolapses in sexually mature gilts consuming feed contaminated with ZEN are among the effects of ZEN on pigs.

Recent studies (*4*, *6*) have demonstrated the potential for ZEN to stimulate growth of human breast cancer cells containing estrogen response receptors. In addition to estrogenic effects, hepatocellular adenomas and pituitary tumors were observed in long-term studies of ZEN carcinogenicity (*7*). However, these tumors were observed only at doses ($\geq$8−9 mg/kg body weight) greatly in excess of the concentrations that have hormonal effects. In another study, ZEN was measured in the endometrial tissue of 49 women. There were 27 endometrial adenocarcinomas, 11 endometrial hyperplasias, and 11 normal proliferative endometria, with ZEN levels of 48, 167 ng/mL and below the detection limit in the groups, respectively (*8*). Increased incidence of early telearche has been reported in the southeast region of Hungary, where ZEN concentrations of 19−104 μg/mL was found in serum samples and surplus food collected from the subjects (*9*).

Considering the above health consequences associated with exposure of humans and animals to ZEN, the ability of selected

* To whom correspondence should be addressed. Tel.: +358 17 16 3583. Fax: +358 17 16 2792. E-mail hani.el-nezami@uku.fi.
† Department of Clinical Nutrition, University of Kuopio.
‡ Food and Health Research Center (ETTK), Mediteknia, University of Kuopio.
§ National University of Singapore.
|| Australian Water Quality Centre.
⊥ Department of Toxicology and Pharmacology, University of Kuopio.
University of Turku.

10.1021/jf049924m CCC: $27.50
Published on Web 06/18/2004

strains of lactic acid bacteria to bind ZEN has been investigated. *Lactobacillus rhamnosus* strain GG (LGG) was capable of removing 55% ZEN added to incubation medium (*10*). Both heat- and acid-killed bacteria were significantly capable of removing more ZEN compared to vial cells, indicating that binding, rather than metabolism, is the mechanism by which ZEN is removed from the medium. An understanding of the binding mechanism is required and will allow optimization and safe application of new methods for the decontamination of foods and feeds containing ZEN.

This paper attempts to identify the type of chemical moieties and interactions involved in the binding of ZEN by LGG. In addition, the mechanism of binding and dissociation of ZEN was investigated.

MATERIALS AND METHODS

Reagent Preparation. Pronase E (Protease from *Streptomyces griseus*, cat. no., P0652; Sigma, St. Louis, MO) and lipase (cat. no., L8906; Sigma, St. Louis, MO) were prepared as 0.5 mg/mL solutions in 0.01 M phosphate buffer (pH 7.6). A 10 mg/mL solution of sodium *m*-periodate (Sigma, St. Louis, MO) was prepared in 0.01 M acetate buffer (pH 4.5). As a control, a 10 mg/mL solution of iodate (Sigma, St Louis, MO) was also prepared in acetate buffer. Containers containing iodate and periodate solutions were wrapped in foil to protect the solutions from light. An aqueous solution of urea (8 M) was prepared.

ZEN (Sigma, St. Louis, MO.) was dissolved in methanol (solubility of ZEN in water is only 0.002 g/100 mL; it is slightly soluble in *n*-hexane and progressively more soluble in benzene, acetonitrile, methylene chloride, methanol, ethanol, and acetone (*11*)), and the concentration was determined spectrophotometrically at 236 nm ($\epsilon_{236\ nm} = 29700\ M^{-1}\ cm^{-1}$). An aliquot of this solution was taken and methanol evaporated with nitrogen. Methanol (50 μL) was added prior to making up to volume with aqueous solution. The ionic strength of ZEN solution was adjusted by adding NaCl or $CaCl_2$ (0.3, 0.5, 0.75, or 1 M, respectively). The pH of ZEN solutions was adjusted (3.5, 4.5, 5.5, 6.5, 7.5, and 8.5, respectively) using HCl or NaOH while maintaining 0.85% NaCl. All other ZEN solutions were prepared in phosphate buffered saline (PBS; pH 7.3, 0.01 M).

Bacterial Preparations. The bacterium used was *L. rhamnosus* strain GG (ATCC 53013) which obtained from Valio Ltd. (Helsinki, Finland) after lyophilization in the late exponential-early stationary phase. This strain was selected based on its common use by the food industry and on evidence of their ability to remove ZEN from solution (*10*).

Bacterial counts were determined by flow cytometry using a Coulter Electronics EPICS Elite ESP cytometer (Coulter, SF), equipped with an air-cooled 488 nm argon-ion laser at 15 mV. Total bacterial counts were enumerated using the fluorescent emission from SYTO9 (LIVE/DEAD *Bac*Light bacterial viability kit, L-7012, Molecular Probes, OR) at 3.34 μM per 10^6-10^7 bacteria. A 525-nm band-pass filter was used to collect the emission for both strains, and Fluoresbrite Beads (2.0 μm, Polysciences Inc., PA) were used as an internal calibration.

Bacteria (0.1 g, 10^{10} CFU) were either viable (incubated at 37 °C in 4 mL PBS for 1 h), heat-killed (boiled in 4 mL PBS for 1 h) or acid-killed (incubated at 37 °C in 4 mL 2 M HCl for 1 h). Bacterial samples were centrifuged (3000*g*, 10 min, <10 °C) and the supernatant removed. Acid-killed bacteria were washed twice with 4 mL PBS prior to use to avoid the effect of remaining acid on ZEN.

Treatments. For pretreatment, solutions of Pronase E, lipase, sodium *m*-periodate, phosphate buffer, sodium iodate or urea were added (1.5 mL) to the bacterial pellet and incubated (37 °C, 2 h). After centrifugation (3000*g*, 10 min, <10 °C), pellets were washed twice with PBS (4 mL), and the toxin association assay was performed. To observe the effects of varying ionic strength or pH on binding, ZEN solutions (1.5 mL) were added directly to the bacterial pellets, and the toxin association assay was performed.

ZEN Binding Assay. The bacterial pellet was suspended in PBS (1.5 mL) containing 4 μg/mL of ZEN (12.56 μM). The mixture was incubated (37 °C, 30 min) and centrifuged (3000*g*, 10 min, <10 °C) prior to analysis of the supernatant for ZEN by HPLC. All assays were performed in triplicate, and both positive controls (PBS substituted for bacteria) and negative controls (PBS substituted for ZEN) were included.

Table 1. Effect of Pre-Treating Viable, Heat-, and Acid-killed L. Rhamnosus Strain GG and (1010 CFU) with Various Chemicals on Its Ability to Remove Zearalenone (ZEN, 12.56 μM) from Aqueous Solution

bacterial pretreatment	% of ZEN removed (± SD)[a]		
	L. rhamnosus strain GG		
	viable[b]	heat-killed[c]	acid-killed[d]
phosphate buffer[e]	56 ± 1	64 ± 2	59 ± 1
pronase E[f]	58 ± 2	42 ± 3	22 ± 4
lipase[f]	56 ± 1	64 ± 3	59 ± 2
urea 8M	45 ± 1	55 ± 3	51 ± 3
iodate[g]	55 ± 3	53 ± 6	49 ± 1
m-periodate[g]	40 ± 2	44 ± 13	43 ± 8

[a] Results are the average of triplicate measurements. [b] Incubated in 4 mL of PBS (37 °C, 1 h). [c] Boiled in 4 mL of PBS (1 h). [d] Incubated in 4 mL of 2 M HCl (37 °C, 1 h). [e] (0.01 M, pH 7.6). [f] In phosphate buffer (0.01 M, pH 7.6). [g] In acetate buffer (0.01 M, pH 4.5).

ZEN Dissociation Assay. The dissociation of ZEN from the ZEN-bacteria complex was followed by repeated washes. Bacterial pellets that had been exposed to ZEN were suspended in Milli-Q water (1.5 mL) and incubated (room temperature, 10 min). Suspensions were centrifuged prior to quantification of ZEN in the supernatants by HPLC. The washing procedure was repeated up to six times. After the last wash, the pellet was suspended in methanol (1.5 mL) to recover any ZEN remaining bound.

Determination of ZEN by HPLC. Reverse-phase HPLC (Shimadzu Model LC-10ADvp solvent delivery system, Shimadzu Model SIL-10Advp auto-injector) was used to quantify ZEN remaining in the supernatant after bacterial incubation. ZEN was separated on an Allsphere ODS-2 column (250 by 4.6 mm, 5 μm; Alltech, Deerfield, IL) fitted with a spherisorb ODS-2 guard column (Alltech, IL), with a mobile phase of water−methanol (35:65, vol/vol) at a flow rate of 1 mL/min. Fluorescence was detected (RF-10AXL, Shimadzu) by excitation at 280 nm and emission at 440 nm, and quantified by a Class VP 5.0 software (Shimadzu Koyoto, Japan). The assay temperature was 30 °C and an injection volume of 10 μL was used. The retention time was 13.7 min. The percentage of toxin removed was calculated using the equation: $100 \times (1 - (\text{peak area of ZEN in the supernatant})/(\text{peak area of ZEN standard solution}))$.

Statistical Analysis. The percentage ZEN bound was determined for at least triplicate samples. Statistical analysis was carried out using SPSS 11.0 for Windows. Analysis of variance was used to test the differences in toxin binding between the treatments. The Fisher post hoc test was used to determine significant differences (the level of significance $P < 0.05$).

RESULTS

As shown in **Table 1**, pretreatment with *m*-periodate and 8 M urea produced significant decreases in ZEN removal by LGG regardless of the viability ($P < 0.05$). Pretreatment of the bacteria with Pronase E did not affect the ability of the viable bacteria to remove ZEN from solution, but significantly reduced the ability of nonviable (heat- and acid-killed) bacteria to remove ZEN ($P < 0.05$) (**Table 1**). Pretreatment with lipase had no significant effect on ZEN removal by viable, heat- and acid-killed LGG.

The effects of different concentrations of both mono- (NaCl) and di-valent ($CaCl_2$) cations on ZEN binding by LGG are shown in **Figure 1**. Increasing salt concentrations slightly increased the binding of ZEN; however, this increase was not statistically significant ($P > 0.05$). The pH of the buffer solution,

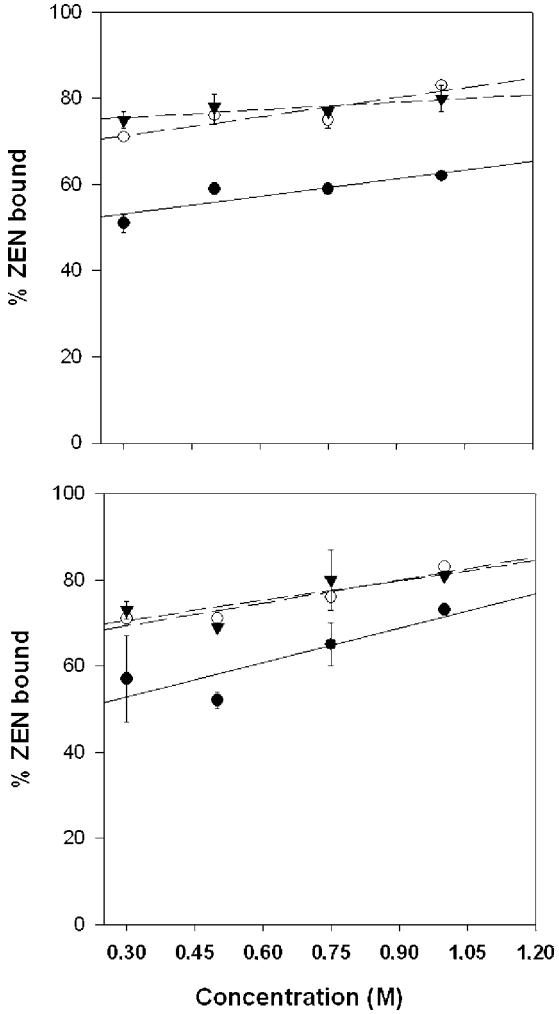

Figure 1. The effect of NaCl (upper panel) and $CaCl_2$ (lower panel) on zearalenone (ZEN) binding by *L. rhamnosus* strain GG. Viable (●), heat- (○), or acid-killed (▼) bacterial cells (10^{10} CFU) were incubated (37 °C, 30 min) with ZEN (12.56 μM, 1.5 mL). Data shown are the mean ± SD of triplicates.

in the range 3.5−8.5, representing that found in the human gastrointestinal tract, had no significant effect on ZEN binding by LGG (**Figure 2**).

The extent of ZEN binding over the range 0.79−62.82 μM (0.25−20 μg/mL) was studied for viable heat- and acid-killed LGG. The plots practically pass through the origin for all treatments (**Figure 3**). The concentration of ZEN bound was not directly proportional to the initial ZEN concentration. The increase in proportion of ZEN bound with increasing ZEN concentration resulted in an upward curve rather than a linear relationship, with the amount of curvature increasing in the order: acid-killed < heat-killed < viable. Heat- and acid-treatments significantly enhanced the binding ($P < 0.05$). Bacteria were not saturated at the highest concentration of ZEN studied.

Scatchard plots ((ZEN bound)/(ZEN free)) versus (ZEN bound) show a decrease at low ZEN concentrations (<10 μM) and then a curve upward at higher concentrations (**Figure 4**). The curved, rather than linear, relationship of these plots implies that there are multiple independent nonidentical binding sites on the bacterial surface.

To investigate the nature of dissociation of ZEN from the bacterial surface, the natural log value of bound ZEN was plotted against the number of washes with Milli-Q water (**Figure 5**).

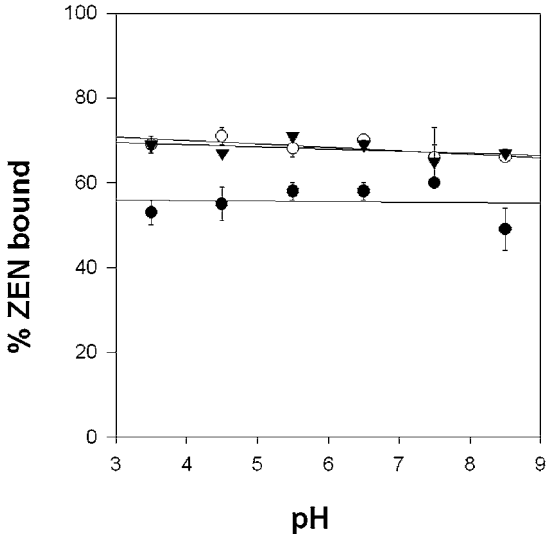

Figure 2. The effect of pH on zearalenone (ZEN) binding by *L. rhamnosus* strain GG. Viable (●), heat- (○), or acid-killed (▼) bacterial cells (10^{10} CFU) were incubated (37 °C, 30 min) with ZEN (12.56 μM, 1.5 mL). Data shown are the mean ± SD of triplicates.

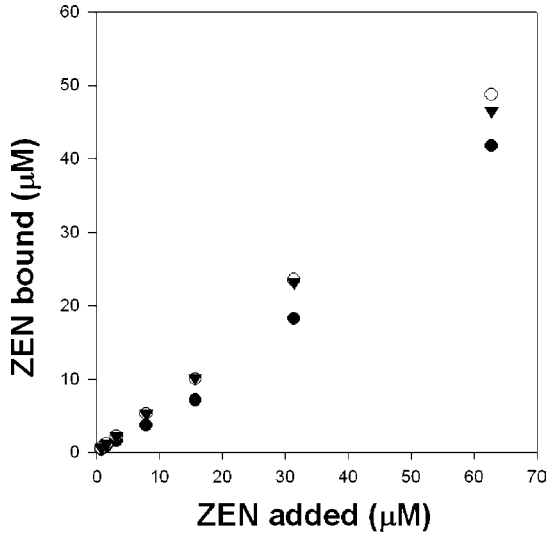

Figure 3. The amount of zearalenone (ZEN) bound by viable (●), heat- (○), or acid-killed (▼) *L. rhamnosus* strain GG (10^{10} CFU) after incubation (37 °C, 30 min) with increasing initial ZEN concentration.

In general, dissociation of ZEN from the viable cells of both lactobacilli was linear after each wash up to five washes. However, the dissociation of ZEN from heat- and acid-killed cells increased rapidly after the second wash, especially for heat-killed cells.

DISCUSSION

Chemical Moieties Types. The ability of lactic acid bacteria to bind different chemical carcinogens and mutagens was reported more than a decade ago, and several studies have postulated various theories on the binding of these compounds to bacterial cell walls. For example, it was reported that such binding may take place as a result of cation exchange mechanisms (*6*) or involvement of carbohydrate (*12*) or protein (*13*) structures. These theories were only speculations of some studies and have not provided any evidence.

Treatment with *m*-periodate, which oxidizes cis OH groups to aldehydes and carbon acid groups (*14*), caused the largest

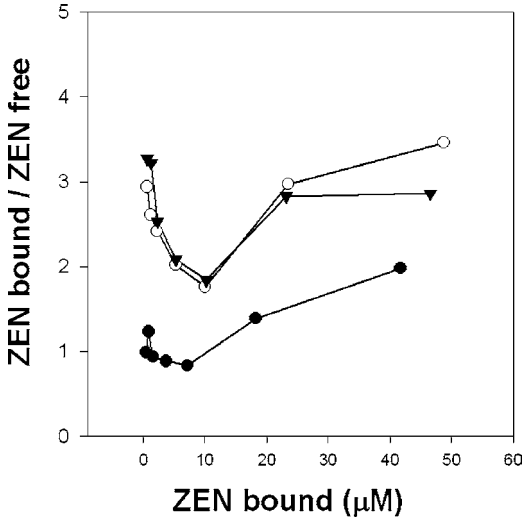

Figure 4. Scatchard plots of the data shown in **Figure 3** for viable (●), heat- (○), or acid-killed (▼) *L. rhamnosus* strain GG (10^{10} CFU) after incubation (37 °C, 30 min) with increasing initial ZEN concentration.

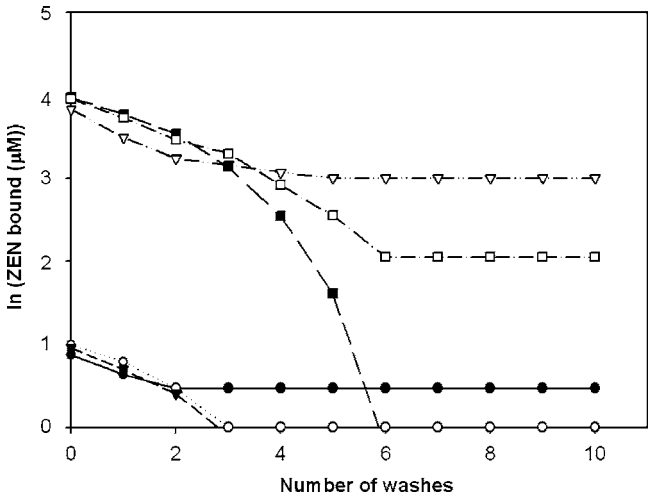

Figure 5. Zearalenone (ZEN) remaining bound when either 3.14 μM or 62.82 μM of ZEN is incubated with viable (●, ▽), heat- (○, ■), or acid-killed (▼, □) *L. rhamnosus* strain GG (10^{10} CFU), after repeated washes with Milli-Q water (1.5 mL) expressed in natural log values.

decrease in ZEN binding (**Table 1**), suggesting that a polysaccharide component is essential for binding. For viable LGG, the decrease in ZEN binding resulted in 15% reduction in ZEN binding ($P < 0.05$) when compared to the iodate control. For heat- and acid killed LGG, a decrease of 6−9% in ZEN binding is observed. Cell wall peptidoglycans and polysaccharides have been suggested to be important elements responsible for the binding of amino acid pyrolysates (*15*) and aflatoxin B_1 (*14*) by lactic acid bacteria. Cell wall polysaccharides contain the amino acid pyrolysates binding sites of *L. gasseri* (*16*). Their intact glucose molecules have a significant role in the binding (*16*). In a number of studies, cell walls have bound amino acid pyrolysates more effectively than pure peptidoglycan, and this has been contributed to the removal of surface polysaccharides in the extraction of peptidoglycan (*15*, *17*).

The absence of a significant effect from viable bacterial pretreatment with Pronase E implies that proteins have negligible involvement in ZEN binding by viable bacteria. However, Pronase E treatment of the heat- and acid-killed LGG significantly decreased ZEN binding. Since Pronase E fragments proteins (*14*), this result suggests that the newly exposed ZEN binding sites on cell surface after heat- and acid-treatments are proteins. This effect of pretreatment with Pronase E was not observed for viable LGG, suggesting that these ZEN binding sites in intact viable bacterial cells were shielded from Pronase E, while heat- and acid-treatment exposed the proteins. The absence of a significant effect from bacterial pretreatment with lipase implies that involvement of lipids, such as lipoteichoic acid, in the binding of ZEN is unlikely.

Although the treatments used decreased binding, in all cases, ZEN binding still occurred in substantial amounts. This may have been due to the involvement of multiple components in binding. The effects of heat and acid on the bacteria, and possible effects on ZEN binding, have been studied (*18*). Heat and acid treatments may change the original binding site of the viable bacteria and expose new binding sites.

Chemical Interactions. Pretreatment of viable, heat- or acid-killed LGG with urea, an anti-hydrophobic agent, resulted between 6 and 11% reductions in ZEN binding. Since urea is an antihydrophobic agent, this implies that hydrophobic interactions are involved in the binding mechanism. Heat and acid treatments may cause protein denaturation, leading to exposure of more hydrophobic surfaces. However, as discussed above, the binding interaction appears to occur predominantly with polysaccharides. Polysaccharides occur in three main forms in the cell wall of these bacteria; cell wall polysaccharide, peptidoglycan, and teichoic or lipoteichoic acids (*19*). All but the lipoteichoic acids are generally considered to be hydrophilic. Urea also denaturates proteins, however, this effect does not appear to be significant, as the binding by viable LGG is least affected. The formation of hydrophobic bonds has been suggested in the binding of Trp-P-1 by a *Lactococcus* strain (*13*). Urea is known to break hydrogen bonds, in addition to being an anti-hydrophobic agent, however, as will be discussed later, it appears that the effect of urea on binding that observed in this study results from hydrophobic interactions.

The influence of electrostatic and electrochemical interactions on the binding of ZEN were studied. These interactions appear to inhibit ZEN binding somewhat, as evidenced by the increase in removal seen in the presence of increasing salt concentration (**Figure 2**). The effect was more pronounced with $CaCl_2$ (I = 0.9−3.0 M) than with NaCl (I = 0.3−1.0 M), probably as a result of the divalent cation providing a greater ionic strength effect. Metal ions have previously been reported to inhibit the binding of amino acid pyrolysates, and monovalent ions had the least effect. (*6*, *13*). Salt concentration is expected to influence bacterial surface charge. The ability of bacterial cell walls to bind cations, especially divalent cations, arises from the presence of acidic centers in the cell wall structure; such as the teichoic and lipoteichoic acids that protrude from the cell surface. The results obtained show that electrostatic interactions do have minor effects on binding. The previously proposed cation exchange mechanism of binding amino acid pyrolysates to the cell wall peptidoglycan of lactic acid bacteria (*6*) does not appear to be operating in the binding of ZEN to viable, heat- or acid-killed LGG. This is not surprising, considering the structural difference between ZEN and these compounds.

The pH tested in this study represents the pH in different parts along the gastrointestinal tract; this is especially important if these strains are applied to reduce the absorption of ZEN from the small intestine. The binding of ZEN to viable, heat- or acid-killed LGG is independent of solution pH in the range 3.5−8.5 (**Figure 1**). This suggests that hydrogen bonding is not important and that bacterial binding of ZEN should occur at any point along the gastrointestinal tract.

Mechanism of Binding and Dissociation of ZEN. The plots of bound ZEN concentration against the initial ZEN concentration in solution show curves bending upward at high ZEN concentrations, suggesting the absorption of ZEN on bacterial cell surface is not a simple interaction between ZEN and a single type of adsorption sites, which would have shown a linear relationship. The Scatchard plots of the data show nonlinear curves with negative slopes (instead of linear relationship) at bound ZEN concentrations of $<10\ \mu$M and then positive slopes at higher concentrations, indicating that there is more than one type of binding interaction. The negative slope of the Scatchard plot is numerically equal to the association constant (affinity) of the binding interaction. A curve suggests that the affinity of ZEN for receptors changes with the amount of ZEN bound. The positive slope at bound ZEN concentrations of $>10\ \mu$M suggests that a positive cooperative effect may take place (i.e., adsorption of ZEN molecules on the cell surface receptors leads to enhanced affinity for further adsorption of ZEN).

The total number of ZEN molecules that can be bound to single bacteria of LGG was calculated from **Figure 3**. The number has been estimated to exceed 10^{10} for viable cells and 10^{11} for heat-or acid-killed.

In the dissociation study, the release of ZEN from viable LGG was relatively linear with respect to the number of washes. The dissociation constant, which could be estimated from the slope, was 0.2/wash at low (3.14 μM) and 0.15/wash at high (62.82 μM) ZEN concentrations. For heat- and acid-killed cells, the initial rate of dissociation of ZEN was comparable with that of the viable cells (first 2 washes); however, the dissociation constant increased with number of washes, especially for heat-killed cells reaching a high value of 0.64/wash. A higher dissociation constant implies lower affinity binding sites for ZEN. Release of loosely bound ZEN from cell surface after the strongly bound ZEN is unexpected. This would lead to the release of ZEN bound onto the lower affinity receptors only after the ZEN bound onto higher affinity receptors was released. This could also explain the apparent positive cooperative effect at high ZEN ($>10\ \mu$M) concentrations, at which the binding of one ZEN molecule led to the slower release (higher affinity) of another ZEN molecule. Alternatively, the ZEN molecules may have affinity for one another as well as the bacteria. Another explanation could be that the lower affinity receptors are topographically beneath the higher affinity receptors.

In conclusion, although heat and acid treatment appear to enhance the capability of LGG to remove ZEN, the binding of ZEN, especially by acid-killed cells, becomes sensitive to the activity of the proteases, which could lead to more bound ZEN being released from the cell surface in the intestinal tract. Consequently, for the future application of this approach to food and feeds matrixes, it is perhaps preferable to use viable LGG for removal of ZEN. The study also concludes that multiple binding sites are involved in the removal of ZEN by LGG. Further studies using mutants deficient in the target site (e.g., mutants deficient in different carbohydrate moieties) are to be conducted, to fully understand the mechanism of binding prior to the practical application.

ACKNOWLEDGMENT

We would like to thank Valio Ltd, Helsinki, Finland for providing the LGG strain used in this study.

LITERATURE CITED

(1) Diekman, M. A.; Green, M. L. Mycotoxins and reproduction in domestic livestock. *J. Anim. Sci.* **1992**, *70*, 1615−1627.

(2) Marasas, W. Toxigenic Fusaria. In *Mycotoxins and Animal Foods*; R. Anderson, Ed.; CRC Press: Boca Raton, FL, 1991; pp 119−139.

(3) Kolb, E. Recent knowledge on the mechanism of action and metabolism of mycotoxins. *Z. Gesamte Inn Med.* **1984**, *39*, 353−358.

(4) Boyd, P. A.; Wittliff, J. L. Mechanism of Fusarium mycotoxin action in mammary gland. *J. Toxicol. Environ. Health* **1978**, *4*, 1−8.

(5) Hussein, H. S.; Brasel, J. M. Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology* **2001**, *167*, 101−134.

(6) Morotomi, M.; Mutai, M. In vitro binding of potent mutagenic pyrolysates by intestinal bacteria. *J. Natl. Cancer Inst.* **1986**, *77*, 195−201.

(7) *NTP Carcinogenicity bioassay of zearalenone in F344/N rats and F6C3F1 mice*. National Toxicology Program Technical Report Series 235, 1982.

(8) Tomaszewski, J.; Miturski, R.; Semczuk, A.; Kotarski, J.; Jakowicki, J. Tissue zearalenone concentration in normal, hyperplastic, and neoplastic human endometrium. *Ginekol Pol* **1998**, *69*, 363−366.

(9) Szuets, P.; Mesterhazy, A.; Falkay, G.; Bartok, T. Early telarche symptoms in children and their relations to zearalenone contamination in foodstuffs. *Cereal Res. Commun.***1997**, *25*, 429−436.

(10) El-Nezami, H.; Polychronaki, N.; Salminen, S.; Mykkanen, H. Binding rather than metabolism may explain the interaction of two food-Grade Lactobacillus strains with zearalenone and its derivative (′)alpha-earalenol. *Appl. Environ. Microbiol.* **2002**, *68*, 3545−3549.

(11) Kuiper-Goodman, T.; Scott, P. M.; Watanabe, H. Risk assessment of the mycotoxin zearalenone. *Regul. Toxicol. Pharmacol.* **1987**, *7*, 253−306.

(12) Hosono, A.; Tanabe, T.; Otani, H. Binding properties of lactic acid bacteria isolated from kefir milk with mutagenic amino acid pyrolysates. *Milchwissenschaft* **1990**, *45*, 647−651.

(13) Tanabe, T.; Suyama, K.; Hosono, A. Effect of sodium dodecylsulphate on the binding of *Lactococcus lactis* ssp. lactis with 3-amino-1,4-dimethyl-5*H*-pyrido[3,4-*b*]indole. *J. Dairy Res.* **1994**, *61*, 311−315.

(14) Haskard, C.; Binnion, C.; Ahokas, J. Factors affecting the sequestration of aflatoxin by Lactobacillus rhamnosus strain GG. *Chem. Biol. Interact.* **2000**, *128*, 39−49.

(15) Sreekumar, O.; Hosono, A. Antimutagenicity and the influence of physical factors in binding Lactobacillus gasseri and Bifidobacterium longum cells to amino acid pyrolysates. *J. Dairy Sci.* **1998**, *81*, 1508−1516.

(16) Sreekumar, O.; Hosono, A. The heterocyclic amino binding receptors of Lactobacillus gasseri cells. *Mutat. Res.* **1998**, *421*, 65−72.

(17) Sreekumar, O.; Hosono, A. Amino acid pyrolysates competitive and combination binding with Lactobacillus gasseri cells. *Milchwissenschaft* **1998**, *53*, 73−76.

(18) El-Nezami, H.; Polychronaki, N.; Salminen, S.; Mykkanen, H. Binding rather than metabolism may explain the interaction of two food-Grade Lactobacillus strains with zearalenone and its derivative α-zearalenol. *Appl. Environ. Microbiol.* **2002**, *68*, 3545−3549.

(19) Delcour, J.; Ferain, T.; Deghorain, M.; Palumbo, E.; Hols, P. The biosynthesis and functionality of the cell-wall of lactic acid bacteria. *Antonie van Leeuwenhoek* **1999**, *76*, 159−184.

Received for review January 15, 2004. Revised manuscript received May 3, 2004. Accepted May 13, 2004. This work was supported by a grant to H.M. from the Academy of Finland (Grant 1574).

JF049924M