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Microbial Transformation of Deoxynivalenol (Vomitoxin)

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Microbial inocula from rumen fluid, soil, and contents of the large intestines of chickens (CLIC) and of swine (SLIC) were tested for their ability to transform deoxynivalenol (vomitoxin) in vitro. Microorganisms in (CLIC) completely transformed pure vomitoxin, and this activity was retained through six serial subcultures. No alteration of the toxin by incubation with SLIC was detected, whereas 35% of the vomitoxin was metabolized in the original culture of rumen fluid and 50% was metabolized by the soil sample, though metabolism was decreased in subsequent subcultures of either sample. A single metabolite was isolated and identified as deepoxy vomitoxin. The increase in concentration of deepoxy vomitoxin in the culture medium corresponded with the decrease in vomitoxin concentration. The vomitoxin transformation rate was not affected by either the ratio of CLIC to vomitoxin (5 to 0.2 g of CLIC per mg of vomitoxin) or the initial concentration of vomitoxin (14 to 1,400 ppm) in the medium. Biotransformation of vomitoxin was completely inhibited when the pH in the medium was lowered to 5.20. Sodium azide at a 0.1% (wt/vol) concentration in the medium blocked the transformation of vomitoxin, suggesting that the deepoxidation of vomitoxin is an energy-dependent process. About 50% of the vomitoxin in moldy corn in culture medium was transformed by microorganisms from CLIC. The vomitoxin transformation rate in moldy corn was not affected when the concentration of CLIC changed from 0.2 to 0.8 g/ml of medium. Vomitoxin in the moldy corn was not transformed when CLIC were added to corn without culture medium. An acid pH due to accumulation of fermentation products, such as organic acids, may be a major factor which inhibits the complete transformation of vomitoxin in moldy corn treated with CLIC.

Deoxynivalenol (3α,7α,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) (DON, or vomitoxin), a mycotoxin produced by *Fusarium* spp., may cause emesis and reduced feed intake in swine (1, 3, 9, 25). It has been suggested that the epoxide group on the trichothecene nucleus of vomitoxin is important for its effect (11). A deepoxy compound, deepoxy vomitoxin (DOM-1), has been produced by rats (23) and ruminants (1) fed vomitoxin as well as rumen fluid incubated in vitro with vomitoxin (6). A decrease in vomitoxin, when it is incubated with contents of the ceca and colons of hens, has been observed (8).

This research was conducted to further evaluate sources of microbial cultures capable of transforming vomitoxin.

MATERIALS AND METHODS

Microbial cultures. Growing pigs and hens were obtained from the Arkell Research Station, University of Guelph. The animals were killed, and the large intestines (including ceca) of hens and segments of the large intestines of swine were tied, removed, placed in glass containers, and immediately taken to the laboratory. Rumen fluid was obtained from a cannulated cow, placed in a sealed glass container which had previously been flushed with nitrogen gas, and taken to the laboratory.

Soil samples were obtained in late May 1988 from a field where an experimental infection of corn with the mold *Fusarium graminearum* was conducted the previous year. Several areas adjacent to rotten corn cobs were sampled to a depth of 7 to 10 cm; the samples were placed in plastic bags, kept at ambient temperature, and used within 2 h.

Anaerobic culture conditions. All anaerobic incubations were conducted in an anaerobic chamber (model 1024; Forma Scientific, Marietta, Ohio). The atmosphere in the chamber was 10% (vol/vol) H_2 and 90% (vol/vol) CO_2; the temperature was set at 37°C. The anaerobic incubation medium (AIM) was a modification of the culture medium of Scott and Dehority (12) and contained the following components (per liter of distilled water): K_2HPO_4, 0.9 g; NaCl, 0.9 g; $(NH_4)_2SO_4$, 0.9 g; $CaCl_2$, 50 mg; $MgSO_4$, 50 mg; $MnSO_4 \cdot H_2O$, 20 mg; $FeSO_4 \cdot 7H_2O$, 20 mg; $ZnSO_4 \cdot 7H_2O$, 20 mg; $CoCl \cdot 6H_2O$, 2 mg; pyridoxine · HCl, 2 mg; riboflavin, 2 mg; thiamine · HCl, 2 mg; nicotinamide, 2 mg; calcium D-pantothenate, 2 mg; *para*-aminobenzoic acid, 0.1 mg; folic acid, 0.05 mg; biotin, 0.05 mg; cobalamine, 0.005 mg; isobutyric acid, 0.063 ml; valeric acid, 0.076 ml; resazurin, 1 mg; Na_2CO_3, 4 g; and cysteine · HCl, 1 g. Agar, when included, was at a final concentration of 1.5% (wt/vol). The final pH of the CO_2-equilibrated medium was 6.6 to 6.8.

Collected samples of intestinal material were transferred into the anaerobic chamber, where the contents of the large intestines chickens (CLIC) and of swine (SLIC) were removed and placed in glass containers. One milligram of vomitoxin (deoxynivalenol, purchased from Myco-lab, Chesterfield, Mo.) was mixed with 1 ml of AIM to give a vomitoxin concentration of 1,000 ppm. One milliliter of this vomitoxin solution was mixed with a 2-g sample of CLIC or SLIC in a 4-ml vial with a screw lid. Rumen fluid was filtered through four layers of cheesecloth, and 0.5 ml of the filtrate was mixed with 1 mg of vomitoxin and 0.5 ml of AIM in the vial. The final concentration of vomitoxin in the medium was 1,000 ppm.

Aerobic culture conditions. Aerobic incubation was conducted in an incubator with the temperature set at 30°C. The incubation medium was basal mineral medium (BMM) containing the following ingredients (per liter of distilled water):

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K_2HPO_4, 0.8 g; KH_2PO_4, 0.2 g; $CaSO_4 \cdot 2H_2O$, 0.05 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $(NH_4)_2SO_4$, 1.0 g; and yeast extract, 0.5 g (7). The pH was adjusted to 7.2. Agar, when included, was at a concentration of 1.5% (wt/vol). Two grams of the soil sample was mixed with 1 ml of BMM (containing vomitoxin at 1,000 ppm) and placed in a 4-ml vial with a loose lid.

The incubation time was 96 h for both anaerobic and aerobic samples. Vomitoxin recovery was determined at the end of the incubation. To ensure that microorganisms were responsible for the transformation of vomitoxin and to eliminate possible interference by enzymes from either the host animal or the soil, six serial subcultures were conducted with the anaerobic or aerobic samples. Each sample was subcultured after 96 h of incubation by transferring 0.2 ml of the culture to another vial containing 1 mg of vomitoxin and 0.8 ml of AIM or BMM. Three replicates per sample of inoculum were used at each incubation.

Vomitoxin transformation by soil microorganisms was not detected in the second subculture. In order to enrich the active microorganisms in soil for further isolation experiments, a soil perfusion column (15) which provided a continuous supply of vomitoxin solution (0.05%, wt/vol) to the soil was set up. The change in the concentration of vomitoxin in the recycling solution was monitored.

Determination of vomitoxin transformation by bacterial isolates obtained from chicken and soil samples. At the end of the sixth subculture of anaerobic vomitoxin-degrading microorganisms from CLIC, a portion of the incubation medium with microorganisms from CLIC was diluted with AIM and spread onto plates of AIM agar. Twenty colonies with different colony morphological characteristics, such as different color and shape, were isolated, and subcultures were conducted every week for 9 weeks in the anaerobic chamber. Each isolate was then incubated with 1,000 ppm of vomitoxin in AIM for 96 h. Vomitoxin recovery was measured at the end of incubation.

For the isolation of potential aerobic vomitoxin-degrading microorganisms, the soil in the aerobic circulation device was mixed with BMM and spread on BMM agar plates. Five colonies with different morphologies were obtained from streaks on agar medium from soil at the top of the column. After nine serial subcultures at 1-week intervals in the sample medium, the isolates were tested for vomitoxin transformation ability in BMM with 1,000 ppm of vomitoxin. The incubation time was 96 h, and the vomitoxin recovery was monitored.

The identity of each isolate before and after the incubation was confirmed by streaking each culture on agar plates and visually examining the colonies formed.

Vomitoxin analysis. The method of vomitoxin determination used in this study was a modification of that described by Visconti and Battalico (20). For analysis of grain, 50 g of moldy corn was extracted with 100 ml of methanol–1% saline (45:55, vol/vol). The sample was homogenized for 2 min at room temperature with a Polytron homogenizer (Brinkmann Instruments, Rexdale, Ontario, Canada) and then filtered through Whatman no. 4 filter paper. The filtrate was applied to an alumina-charcoal cleanup column (packed with 0.75 g of activated charcoal and 0.5 g of neutral alumina) and eluted with methanol. For analysis of vomitoxin in incubation medium, a sample of incubated mixture was directly added to the column and eluted with methanol. The total eluate was evaporated to dryness in a rotary evaporator at 60°C. The residues were then dissolved in 5 ml of methanol (high-pressure liquid chromatography [HPLC] grade) and filtered through a 0.45-μm-pore-size Millipore filter. The toxin concentration was measured by HPLC (model 5000; Varian Canada Inc., Georgetown, Ontario, Canada) equipped with a WISP 710B autoinjector, a C_{18} column (250 by 4.6 mm), and a UV-50 detector; the wavelength was 225 nm, and the split ratio was 1/100.

Identification of the metabolite. To obtain a sufficient quantity of the vomitoxin metabolite for gas chromatography-mass spectrometry (GC-MS) analysis, 2 mg of pure vomitoxin was incubated anaerobically with 4 g of CLIC in 2 ml of AIM for 120 h. This sample was purified by passage through an alumina-charcoal cleanup column as described above, and the eluate was collected and dried under N_2. The residue was first dissolved in 1 ml of acetonitrile plus 0.3 ml of distilled water. Next, 2 to 3 ml of distilled water was slowly added with shaking to complete the precipitation. The solution was then centrifuged at 550 × *g* for 15 min, and the supernatant was filtered through 0.45-μm-pore-size Millipore filter paper and dried under N_2. The residue was derivatized with 50 μl of TMS [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide, trichloromethylsilane, trimethylsilylimidazole (11:2:3)] and heated for 10 min at 60°C. The solution was then diluted with 50 μl of ethyl acetate. One milligram of pure vomitoxin was derivatized similarly and served as a control. An analysis was performed on a gas chromatograph (model 5160; Carlo Erba) coupled to a mass spectrometer (model MS 890; Kratos, Manchester, United Kingdom) and a Dadta generator (model 30; Kratos). A fused silica capillary column (model DB-5; 30 m by 0.25 mm [inner diameter]; J&W Scientific, Folsom, Calif.) was used for the analyses. The initial oven temperature of 60°C was maintained for 2 min and then increased to 200°C at 50°C/min. The injection port temperature was 320°C. The mass spectrometer was run at an ionization temperature of 200°C with the ionization energy at 70 eV, resolution at 1,000, emission current at 100 μA, and accelerating voltage at 4 kV.

Effect of ratio of CLIC to medium. Media consisting of CLIC and AIM in the ratios of 1:5, 1:1, and 5:1 (wt/vol) were prepared. Triplicate samples consisting of 1 ml of each working medium mixed with 1 mg of pure vomitoxin to give a 1,000-ppm vomitoxin concentration in the medium were prepared. Samples were taken for vomitoxin analysis at incubation time points of 0, 24, 48, and 72 h.

Effect of vomitoxin concentration. The CLIC were mixed with AIM at a ratio of 1:5 (wt/vol). Four levels of vomitoxin concentration were prepared by adding 0, 1, 10, and 100 μl of working solution (10,000 ppm of vomitoxin in AIM) to 1.0, 1.0, 0.99, and 0.9 ml of the working medium, respectively. The calculated vomitoxin concentrations were 0, 10, 100, and 1,000 ppm. The incubation time points at which samples for vomitoxin analysis were taken were 0, 48, and 96 h. Triplicate samples were prepared for each treatment.

Effect of initial pH level. To investigate the effect of pH on vomitoxin biotransformation, a phosphate buffer system was used. The pH values of the buffer media were adjusted to 5.36, 5.72, 6.08, 6.44 and 6.80 (digital pH/ion meter; model 425; Fisher Accumet) by mixing solution A (double the concentration of each component of AIM [described above]), solution B (0.4 M NaH_2PO_4), and solution C (0.4 M Na_2HPO_4) in ratios of 50:46:4, 50:38.8:11.2,50:25.5:24.5,50:11.5:38.5, and 50:2.65:47.35, respectively. The CLIC were added to each buffer medium at a ratio of 0.2 g/ml, and 4.0 ml of this solution was further mixed with 0.02 ml of working solution of vomitoxin (10,000 ppm in AIM). The calculated vomitoxin concentration was 50 ppm. Incubation time points at which samples were taken were 0, 24, and 48 h. The

vomitoxin concentrations and pHs of the samples were determined at the end of each incubation period.

Investigation of energy dependence of biotransformation. To determine the relationship between the deepoxidation of vomitoxin and electron transport in the bacterial cells, sodium azide was added to the vomitoxin incubation medium. The working medium was prepared by mixing CLIC with AIM at a ratio of 0.2 g of CLIC per ml of AIM, and 4.8 ml of the working medium was mixed with 0.1 ml of vomitoxin solution (10,000 ppm in AIM) and 0.1 ml of sodium azide solution (either 0.5% or 5%). The final concentrations of sodium azide in the medium were 0.01% and 0.1%, respectively, and the calculated vomitoxin concentration was 0.2 mg/ml. The incubation time points at which samples were taken were 0, 24, 48, and 72 h.

Contaminated corn. Vomitoxin-contaminated corn was produced in 1988 by inoculating developing cobs at the early silking stage by using toothpicks impregnated with *F. graminearum* (24). The infected corn cobs were machine harvested in the middle of October 1988, and the less moldy parts were removed by hand. The infected portions of the cob and kernels were ground through a 20-mesh screen, assayed for vomitoxin as described above, and screened for other mycotoxins by the Toxicology Laboratory, Ontario Ministry of Agriculture and Food, Guelph, Ontario, Canada, by the method described by Stoloff et al. (16) and Scott et al. (13).

The ground moldy corn contained 450 ppm of vomitoxin and 23.8 ppm of zearalenone (detection limits were 2.0 and 0.01 ppm, respectively). No other tested mycotoxins, such as aflatoxin, citrinin, ergot, ochratoxin A, penicillic acid, or sterigmatoxystin, were detected (detection limits were 0.01, 0.2, 0.01, 0.1, 3.0, and 0.05 ppm, respectively).

Primary experiment on transformation of vomitoxin in moldy corn. Anaerobic incubation working media were prepared with CLIC and AIM at ratios of 0.2, 0.4 and 0.8 g of CLIC per ml of AIM. Two grams of ground moldy corn was mixed with 10 ml of each working medium to give an initial vomitoxin concentration of 90 ppm. The concentration changes of vomitoxin and DOM-1 were measured at 0, 48, and 96 h of incubation, and the pH was measured at 0 and 96 h of incubation.

Transformation of vomitoxin in moldy corn incubated in buffer media. To determine the optimum pH for the transformation of vomitoxin in moldy corn by microorganisms from CLIC, three pHs were tested. The working media were adjusted to pH values of 8.0, 7.0, and 6.0 by mixing solution A (described above), solution D (1.0 M NaH_2PO_4), and solution E (1.0 M NaOH) in ratios of 50:26:24, 50:31.6:18.4, and 50:45:5, respectively. The control medium consisted of solution A plus an equal volume of distilled water. One gram of ground moldy corn was mixed with 1 g of CLIC and 5 ml of working medium or control medium. The calculated vomitoxin concentration was 90 ppm. Triplicate samples were prepared for each treatment. Vomitoxin recoveries and pH were measured after 0, 96, and 192 h of incubation.

Vomitoxin transformation under different physical conditions. To investigate some physical conditions that may affect the transformation of vomitoxin in moldy corn, six treatments were incorporated in this experiment: 1, ground moldy corn plus CLIC; 2, whole moldy corn plus CLIC; 3, ground moldy corn plus CLIC and AIM; 4, ground moldy corn plus CLIC and AIM with agitation; 5, ground uninfected corn plus pure vomitoxin, CLIC, and AIM with agitation; and 6, pure vomitoxin plus CLIC and AIM with agitation. The vomitoxin concentrations in all six treatments were 150 ppm. Two grams of CLIC was added to each sample, and 10 ml of AIM was used in samples incubated with liquid. Vomitoxin recoveries were measured at 0, 96, and 192 h.

TABLE 1. Recovery of pure vomitoxin incubated with anaerobic or aerobic microorganisms during six serial subcultures

Subculture[a]	% of vomitoxin recovered from indicated inoculum			
	CLIC	Rumen fluid	SLIC	Soil
1	3.1	65.4	93.3	50.4
2	1.5	84.2	104.2	99.1
3	0.9	91.0	96.1	101.4
4	2.7	103.5	100.3	98.3
5	1.6	101.7	101.5	99.5
6	1.1	101.4	98.5	100.9
Avg (SE)	1.8 (0.9)	91.2 (14.7)	99.0 (3.9)	91.6 (20.2)

[a] Each culture time was 96 h.

Statistics. Measured vomitoxin concentrations were converted to percent recoveries based on zero time concentrations. The normal distribution of the data was confirmed and then analyzed by the General Linear Model procedure (14). Differences between treatments were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Screening of microorganisms for biotransformation of vomitoxin. Vomitoxin concentrations were unchanged in the media following incubation with SLIC, with an average vomitoxin recovery of 99%. Microorganisms from CLIC transformed over 98% of the vomitoxin in each of six subcultures (Table 1). Approximately 65% recovery of vomitoxin was detected in the first culture of rumen fluid, and the recovery increased in further subcultures. Complete recovery was obtained in the third and later subcultures of the rumen fluid sample. Vomitoxin recovery in the soil sample was about 50% in the first culture and approximately 100% in subsequent subcultures (Table 1). About 70% recovery of vomitoxin was obtained after 6 weeks of culturing soil with the recirculation device.

In all culture media where there was a decrease in the concentration of vomitoxin, a new compound was detected by HPLC. An increase in the amount of the new compound, which had a retention time of 20 min when analyzed by HPLC, occurred in parallel with a corresponding decrease in the amount of vomitoxin as the incubation time increased. None of the other chromatography peaks changed with the change in vomitoxin concentration.

Determination of vomitoxin transformation by bacterial isolates obtained from CLIC and soil samples. Of the 20 isolates from CLIC and 5 isolates from soil, none metabolized vomitoxin during 96 h of incubation.

Identification of the metabolite. By GC-MS, the fragment ions of vomitoxin-TMS were *m/z* 512 (M^+), 497, 422, 407, and 377, and those of the metabolite-TMS were *m/z* 496 (M^+), 481, 406, 391, and 361. Each fragment ion of the metabolite-TMS showed a 16-mass-unit shift compared with the corresponding ion of vomitoxin-TMS. These data were consistent with the loss of oxygen from the molecule of vomitoxin to give the metabolite.

Yoshizawa et al. (23) first described the deepoxidation of vomitoxin in rats. Wistar rats that received orally administered vomitoxin excreted a single metabolite, which was

named DOM-1, in the urine and feces. When the metabolite was converted to its trimethylsilyl derivative and subjected to GC-MS, its molecular ion was found at *m/z* 496, suggesting the elimination of 16 mass units from the parent molecule. Other fragments at *m/z* 496, 481, 406, and 391 were also shifted 16 mass units compared with corresponding ions in the trimethylsilyl derivative of vomitoxin (512, 497, 422, and 407). Moreover, a key feature of the metabolite was its proton magnetic resonance spectrum, which revealed that instead of a singlet resonance due to the epoxy ring, a doublet resonance appeared in the metabolite. On the basis of these data, the metabolite deepoxy vomitoxin (3α,7α,15-trihydroxytrichothecen-9,12-dien-8-one) was proposed (23). Later research (18, 22) confirmed the reduction of the epoxy group in vomitoxin by the same method. The trimethylsilyl derivative of the metabolite obtained in the present study from the vomitoxin media incubated with CLIC, rumen fluid, or soil and the trimethylsilyl derivative of pure vomitoxin displayed the same spectra by GC-MS. Therefore, the metabolite is believed to be DOM-1.

Transformation of vomitoxin to DOM-1 by rumen microorganisms was reported by other investigators (6, 18). Complete conversion of a high concentration of vomitoxin was not reported in any of these experiments. Without mention of the initial concentration of vomitoxin in the rumen fluid medium, Swanson et al. (18) indicated that although deepoxy vomitoxin was detected in the earliest sampling (12 h) and its concentration increased over time (36 h), the parent compound vomitoxin was the major species present at all time points. No change in vomitoxin concentration in rumen fluid (2.5 ppm) was detected after a 3-h incubation (5). King et al. (6) observed that with 10 ppm of vomitoxin in the rumen fluid, 89% was transformed to DOM-1 within 24 h. However, the transformation rate was only 37% in 24 h when the initial concentration of the toxin increased to 100 ppm. An average of 50% conversion was obtained in 7 days in a large-scale incubation with 1,000 ppm of vomitoxin (6). The vomitoxin concentration in the present study was set at 1,000 ppm. About 35% transformation was obtained after a 96-h incubation. The transformation rate decreased in the subsequent subcultures, and transformation ceased in the fourth subculture. This change may have resulted because the medium lacked nutrients essential for the growth of the metabolically active bacteria. In agreement with results reported here, it was found that the number of rumen microorganisms declined by 94% (4.9×10^7 to 2.8×10^6) in 48 h when incubated with trichothecenes (18).

When vomitoxin was incubated in vitro with SLIC in the present study, there was no decrease in vomitoxin concentration. It has been reported that vomitoxin orally administered to pigs was unchanged and that no DOM-1 was detected (10). Lack of metabolism of the toxin in the digestive tract may partially explain the high sensitivity of swine to vomitoxin in their diets. However, it was found that the fecal microorganisms of swine were able to reduce the epoxy group of diacetoxyscirpenol (DAS) (17). Whether the microorganisms in SLIC transform some trichothecenes but not others is not clear.

We have not found any reports regarding transformation of vomitoxin by microorganisms from soil. Degradation of mycotoxins by soil microorganisms may be expected. We detected about 50% reduction of vomitoxin in the first culture of soil microorganisms but not in later subcultures. This may have resulted because culture conditions did not favor the growth of the active bacteria. However, a longer incubation time in a recirculation device with a continuous supply of a lower concentration of vomitoxin solution in contact with the soil also induced some vomitoxin degradation. Since the bacterial population in the soil sample was not analyzed in this study, ascribing DOM-1 formation to microbial action requires further research.

TABLE 2. Recovery of pure vomitoxin in media incubated with different quantities of CLIC

Incubation time (h)	% of pure vomitoxin recovered with initial CLIC/AIM ratio (wt/vol) of [a]:			
	5:1	1:1	1:5	SE
24	21 A	24 A	20 A	3.7
48	9 B	8 B	11 B	2.1
72	1 C	1 C	2 C	1.1

[a] The initial vomitoxin concentration was 1,200 ppm, and the initial recoveries were treated as 100%. Each treatment was performed in triplicate. Vomitoxin recoveries for each treatment at different incubation times were compared, and values in the same column with different capital letters were significantly different ($P < 0.05$). At each time point, however, the differences between percent recoveries for the various CLIC/AIM ratios were not significant ($P < 0.05$).

Metabolism of vomitoxin by microorganisms from the chicken hindgut was suggested by Lun et al. (8). The toxin incubated with CLIC declined over time, and 0.5 ppm of the toxin in the medium was completely transformed in 24 h. Swanson et al. (17), who failed to observe deepoxidation of the trichothecene DAS incubated with chicken feces, suggested that chickens may lack the microflora necessary for epoxy reduction. Results from the present study with a high concentration of vomitoxin in the medium supported the observation of Lun et al. (8) and demonstrated the deepoxidation ability of microorganisms from chicken hindguts. In contrast to other microorganisms tested in this study, bacteria from CLIC caused the complete disappearance of the trichothecene vomitoxin during serial subcultures.

A number of investigators have reported the biotransformation of trichothecenes by pure cultures of bacteria. Ueno et al. (19) included T-2 toxin in the culture medium of *Curtobacterium* sp. strain 114-2, a soil bacterium, and found that the toxin was metabolized to HT-2 and T-2 triol. Others (21) obtained the same metabolites by incubating T-2 toxin with isolates of rumen bacteria. However, the deepoxy compound was not detected in either study. In the present study, we failed to detect any degradation of vomitoxin by the microbial isolates from soil or CLIC, but DOM-1 was detected when vomitoxin was incubated with CLIC and soil. The inability to isolate active vomitoxin-metabolizing bacteria was probably due to either screening too few colonies or the inability of metabolically active bacteria to grow in pure culture under the conditions provided.

Effect of concentration of CLIC in the medium. No significant difference was observed in vomitoxin recoveries among the treatments with different quantities of CLIC (Table 2). Vomitoxin concentration within each treatment containing the same amount of CLIC declined during the incubation time (72 h). The ratios of CLIC to vomitoxin used in the experiment of Lun et al. (8) were 25 g/20 ng and 25 g/50 ng, and the transformation rates in 24 h were 95% and 83%, respectively. When 1 g of animal feces was incubated with 1 mg of the trichothecene DAS, a complete transformation of the toxin was observed in swine, cattle, and rat fecal samples after 4 days of incubation (17). However, unless the species of active bacteria and enumeration of the viable population in the incubated mixture are specified, the comparison of

TABLE 3. Effects of initial concentration of pure vomitoxin and incubation time on vomitoxin recovery

Incubation time (h)	% of vomitoxin recovered at indicated initial concn (ppm)[a]				Effect[b]	
	14	143	1,419	SE (%)	Linear	Quadratic
24	44.3 A	31.0 A	29.9 A	1.4	S	S
48	10.4 B	2.3 B	2.0 B	0.9	S	S
72	3.0 C	2.0 C	1.0 C	0.4	S	NS
96	ND	0.9 C	0.9 C	0.6	NS	NS

[a] Zero time recoveries were treated as 100%. Each treatment was performed in triplicate. Vomitoxin recoveries for each treatment at different incubation times were compared, and values in the same column with different single letters were significantly different ($P < 0.05$). ND, not detectable.

[b] Effect of the initial concentration of vomitoxin in the medium on vomitoxin recovery at different sampling times. S, significant ($P > 0.05$); NS, not signficant ($P < 0.05$).

these ratio effects may not be very informative, except as an indication of the minimum effective ratio of CLIC to vomitoxin.

Effect of vomitoxin concentration. The initial vomitoxin concentrations measured at the start of the incubation time were 14, 143, and 1,419 ppm, respectively (Table 3). The concentration of vomitoxin decreased with increased incubation time. The transformation rate, however, was higher in the media with 143 and 1,419 ppm of vomitoxin than in that with 14 ppm at 24, 48, and 72 h of incubation, though the same percentage declines were observed in all samples by 96 h of incubation. It may be speculated that the higher substrate concentrations stimulated deepoxidation of vomitoxin by inducing greater microbial enzyme activity. A specific epoxy reductase in microorganisms responsible for the deepoxidation of vomitoxin has been suggested (6).

Effect of pH levels. In the pure vomitoxin incubation media, constant pH levels were maintained throughout the incubation period. The average pH values measured in the five treatments were 5.20, 5.72, 6.13, 6.54, and 6.91 (Table 4) with standard errors of 0.11, 0.10, 0.10, 0.08, and 0.09, respectively. Vomitoxin concentration was unchanged in the medium with a pH of 5.20 during 96 h of incubation, but at pHs of 5.72, 6.13, 6.54, and 6.91, there was low recovery of vomitoxin. CLIC range in pH from 5.7 to 8.4 in the ceca, 6.3 to 8.2 in the rectum, and 5.4 to 8.4 in the cloaca (4). Other incubation experiments with CLIC maintained a neutral pH (8, 17). The inhibition of vomitoxin transformation by low pH observed in this experiment may be due to either the inactivation of the microorganisms in the acidic conditions or a specific inhibitory effect on the deepoxidation process.

TABLE 4. Effects of pH on recovery of pure vomitoxin incubated with CLIC

Incubation time (h)	% of pure vomitoxin recovered at indicated pH[a]					
	5.20	5.72	6.13	6.54	6.91	SE
24	107 A	9 A	10 A	10 A	10 A	1.7
48	101 A	2 B	3 B	2 B	2 B	2.1
72	101 A	ND	ND	ND	ND	1.5
96	106 A	ND	ND	ND	ND	1.9

[a] The initial vomitoxin concentration was 60 ppm, and the zero time recoveries were treated as 100%. Each treatment was performed in triplicate. ND, not detectable. Vomitoxin recoveries for each pH treatment at different incubation times were compared, and values in the same column with different single letters were significantly different ($P < 0.05$). The linear and quadratic effects of pH on vomitoxin recovery were significant ($P < 0.05$) at each incubation time point.

TABLE 5. Effects of sodium azide on recovery of vomitoxin incubated with CLIC

Incubation time (h)	% of vomitoxin recovered at indicated % concn of NaN_3[a]			
	0	0.01	0.1	SE
24	16 A	20 A	101 A	4.7
48	6 B	8 B	99 A	2.8
72	1 C	2 C	98 A	1.3

[a] The initial vomitoxin concentration was 200 ppm, and the zero time recoveries were treated as 100%. Each treatment was performed in triplicate. Vomitoxin recoveries for each sodium azide concentration treatment at different incubation times were compared, and values in the same column with different capital letters were significantly different ($P < 0.05$). The linear and quadratic effects of sodium azide concentration on vomitoxin recovery were significant ($P < 0.05$) at each incubation time point.

Investigation of the energy dependence of biotransformation. Sodium azide at 0.1% (wt/vol) in the medium completely blocked the deepoxidation reaction (Table 5). Microorganisms with active electron transport are sensitive to the compound. The inhibitory effect of sodium azide on aerobic cellular electron transport reportedly is caused by azide binding to oxidized cytochrome α_3 so that no electron can be passed in the final step of the electron transport chain (2), but the site of action in anaerobic metabolism undoubtedly is different. Data in the present study suggested that the elimination of oxygen from the epoxy group of vomitoxin was related to the electron transport process in the bacteria, since no deepoxidation occurred in the microorganisms with inactive electron transport. The epoxy reductase activity may depend on the electron transport or the energy supply in the bacterial cells. A lower concentration of sodium azide (0.01%, wt/vol) in the medium may not completely suppress the electron transport activity in bacteria in the medium, possibly explaining why vomitoxin transformation was not affected under these conditions. However, more research is needed to reveal the relationship between deepoxidation and bacterial respiration.

Primary experiment on transformation of vomitoxin in moldy corn. Approximately 79 and 47% of the initial vomitoxin were recovered after 48 and 96 h of incubation, respectively, and recovery was not influenced by CLIC/AIM ratios between 8:10 and 2:10 (wt/vol). The recovery of vomitoxin in the moldy corn was considerably greater than when pure vomitoxin was used in earlier experiments. The acidic condition in the medium could be one of the factors which affected transformation in moldy corn. The pH in the media decreased from 6.5 to 3.5 in the 96-h incubation

TABLE 6. Treatments used to test effects of different physical conditions on vomitoxin recovery

Physical condition	Presence of condition in treatment:					
	1	2	3	4	5	6
Moldy corn	+	+	+	+	−	−
Clean corn	−	−	−	−	+	+
Grinding	+	−	+	+	+	−
CLIC	+	+	+	+	+	+
AIM	−	−	+	+	+	+
Agitation	−	−	−	+	+	+
Pure vomitoxin	−	−	−	−	+	+

TABLE 7. Effect of different physical conditions on vomitoxin recovery in corn incubated with CLIC

Incubation time (h)	% of vomitoxin recovered after treatment[a]:						
	1	2	3	4	5	6	SE
96	98 A	100 A	48 B	50 B	46 B	ND	6.2
192	104 A	103 A	49 B	46 B	44 B	ND	7.5

[a] For specifications of treatments, see Table 6 or Materials and Methods. The initial vomitoxin concentration was 150 ppm, and zero time recoveries were treated as 100%. Each treatment was performed in triplicate. Vomitoxin recoveries were not significantly different ($P > 0.05$) between the 96- and 192-h incubations for each physical condition treatment. Vomitoxin recoveries for each treatment condition at each incubation time were compared, and values in the same row with different single letters were significantly different ($P < 0.05$). ND, not detectable.

period, presumably because of organic acids produced during corn fermentation. Previous data indicated that biotransformation of vomitoxin would be completely inhibited once the pH fell to 5.2, which presumably occurred between 48 and 96 h of incubation in this experiment. Other factors, such as improving the accessibility of microorganisms to the vomitoxin in moldy corn, should also be considered in attempts to improve transformation.

Transformation of vomitoxin in moldy corn incubated in buffered medium. The overall transformation rate of vomitoxin in moldy corn was not improved by the use of buffer solutions added to the media, since the buffer failed to neutralize the acid produced from corn fermentation. The pH decreased in the same pattern in all samples with or without buffer solutions. Vomitoxin transformation appeared to cease by 96 h of incubation, presumably because of the low pH (3.8) in the medium. According to previous results with pure vomitoxin, transformation of vomitoxin may stop when the pH reaches 5.20. Prolonging incubation to 192 h under the acidic conditions did not improve the results. There was very little transformation of vomitoxin after 96 h of incubation. This clearly indicated that control of the pH in the incubation medium is critical for further transformation. To improve the results, simply increasing the concentration of the base in the buffer may not be feasible, since the osmolality in the medium may also be increased to a level that cannot support living cells.

Vomitoxin transformation under different physical conditions. No change in the toxin concentrations was detected in the moldy corn, either ground or as whole corn grain, incubated with CLIC only (treatments 1 and 2) (Tables 6 and 7). With CLIC and liquid medium present, vomitoxin in ground moldy corn was converted at the same rate, with or without agitation (treatments 3 and 4). Pure vomitoxin added to ground clean corn was transformed at the same rate as that naturally present in the moldy corn (treatment 5). The data in this experiment suggested that the liquid medium was essential for the ability of microorganisms to access vomitoxin in the moldy corn. However, about 46% of the toxin remained in its original form, whether in the moldy corn or as an additive to clean corn, again indicating that acidic fermentation products inhibited the complete transformation of vomitoxin.

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