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Mycotoxin Production by *Fusarium avenaceum* Strains Isolated from Norwegian Grain and the Cytotoxicity of Rice Culture Extracts to Porcine Kidney Epithelial Cells†

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The secondary metabolites of 24 isolates of *Fusarium avenaceum* from Norwegian cereals and grown on rice have been characterized. Moniliformin (MON), enniatins (ENNs), and beauvericin (BEA) were analyzed by high-performance liquid chromatography. Porcine kidney epithelial cells (PK15, American Type Culture Collection) were used to study the cytotoxicity of MON in the extracts. The following metabolites were produced by all isolates, ranked by concentration in rice cultures: ENN-B, MON, ENN-B1, and ENN-A. BEA was produced by eight isolates. The productions of BEA and ENN-A were significantly correlated, as was the case with ENN-B and ENN-B1. MON production was correlated neither to any of the other toxins nor to toxicity.

KEYWORDS: Moniliformin; enniatin; beauvericin; MTT assay

INTRODUCTION

Fungi of the genus *Fusarium* contaminate crops, and infections are favored by prolonged surface wetness in specific periods of crop development (anthesis) (*1*). They are regarded as the most toxigenic fungi in the northern temperate regions, varying in species according to crop and geography. *Fusarium avenaceum* (*F. avenaceum*), however, is a ubiquitous fungus (*2*), causing head blight on wheat and pink ear rot or kernel rot on corn (*1*), and is found on maize from all parts of the world (*3*). *F. avenaceum* is predominant in Norway, found in abundance in grain samples from all agricultural districts (*4*−*6*).

Although extracts of crop naturally contaminated by *F. avenaceum* are found to be of low pathogenicity (*4*), the fungus produces secondary metabolites that are quite toxic in laboratory experiments. Moniliformin (MON) has been widely studied and causes significant serohematological alterations and impairs immune functions in broiler chicks, is acutely cardiotoxic to chicks and mink, and causes chromosomal aberrations (*7*−*12*). MON also has direct effects on the tricarboxylic acid cycle by potent inhibition of oxidation reactions, causing cellular energy depletion and reduced oxygen consumption (*13*−*15*). Beauvericin (BEA) and enniatins (ENNs) are cyclic hexadepsipeptides and selective cation ionophores (*16*−*18*). Both BEA and ENNs have antimicrobial properties, interfere with cholesterol storage in liver cells, and are found to be toxic in various bioassays (*19*−*23*). BEA facilitates apoptosis and interferes with smooth muscle contraction (*24*, *25*). Enniatins are also phytotoxic (*26*). Most strains of *F. avenaceum* do not produce trichothecenes or zearalenone (*5*, *27*).

Although there have been many studies of toxic effects of mycotoxins in naturally contaminated or spiked feed on animals, simpler bioassays, such as the methylthiazoletetrazolium (MTT) colorimetric cell culture assay, are better suited for preliminary and mechanistic toxicological studies (*28*−*31*). In previous studies of Norwegian *Fusarium* spp., samples of overwintered grain highly contaminated with *F. avenaceum* were quite toxic in a swine kidney cell (SKC) bioassay (*32*). Extracts of laboratory rice fermentations were somewhat toxic in the same SKC bioassay (*33*). This study was aimed at determining the profiles of the toxic secondary metabolites typical of Norwegian *F. avenaceum* isolates and comparing the cytotoxicity of their rice culture extracts to their content of moniliformin.

MATERIALS AND METHODS

Origin and Identification of Isolates. Twenty-four strains of *F. avenaceum* were isolated from cereal kernels gathered from various areas of Norway, identified according to the standards of Samson et al. (*34*) and Nelson et al. (*35*), and verified by Ulf Thrane, Department of Biotechnology, Technical University of Denmark. All isolates were characteristic of their species.

Maintenance and Fermentation of Isolates. All *Fusarium* isolates are maintained on sterile soil and are obtainable from the *Fusarium*

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Culture Collection at the National Veterinary Institute of Norway. Fermentations of Norwegian isolates of *Fusarium* are carried out according to a standardized procedure that supports high growth rates while resembling the cooler conditions of the Norwegian climate. Briefly, isolates representative of their species and actively growing on Synthetischer Nährstoffarmer Agar (SNA) were transferred to malt extract agar (MEA) for subsequent inoculation of medium A (*36*). Mycel harvested from medium A was macerated, seeded onto 50 g of Uncle Ben's brand rice in triplicates, and incubated at 25 °C for 2 weeks followed by dark incubation at 10 °C for 2 weeks (*5*). Fungi growth was monitored during fermentation, and bacterial contamination was checked upon termination.

Chemical Analysis. *Sample Preparation and Analysis of Moniliformin and Ergosterol.* MON standard was purchased from Sigma (St. Louis, MO). HPLC-quality methanol, hexane, and acetonitrile were from Rathburn (Walkerburn, Scotland). Other chemicals were of pro analysis grade from Merck (Darmstadt, Germany). Tetrabutylammonium dihydrogenphosphate (TBAP), ≥97%, was purchased from Fluka (Buchs,Switzerland). Water was purified with Purite or Milli-Q. HPLC equipment for determination of MON and ergosterol consisted of a 250 binary LC pump and an ISS 200 autosampler coupled to either an LC-4 fluorescence detector or an LC-90 UV detector, all from Perkin-Elmer Instruments (Shelton, CT).

For chemical analysis of moniliformin, 1 g of rice culture was homogenized by blending and extracted with 50 mL of acetonitrile−water (84 + 46, v + v). An aliquot of 5 mL was filtered and defatted with an equal volume of hexane. The acetonitrile (AcN) phase was evaporated to dryness under N_2, resolved in 0.5 mL of mobile phase (AcN + H_2O, 8 + 92, v/v) and 1% ion pair solution (20% TBAP + 1.1 M KH_2PO_4, 50 + 50). Finally, the extract was filtered through a 0.22 μm membrane and transferred to an HPLC vial. MON was quantified by ion-pair chromatography on a C18 column with UV detection at 229 nm. Recovery, tested by spiking every fifth sample, was 98% for 66−266 μg of MON. The standard deviation of repeatability tests was 5.1%.

Ergosterol was determined according to a modified method of Young (*37*−*39*), in which 0.25 g of homogenized rice culture was dissolved in 4.0 mL of methanol prior to addition of 1.0 mL of 2 M NaOH and pumice. The mixture was heated cautiously for 2 × 20 s at 350 W by microwave. Intermittent pause was a minimum of 1 min. Following cooling to room temperature, 10 mL of hexane was added and the mixture shaken carefully for 2 min; 7 mL was aliquoted prior to evaporation to dryness at 40 °C under N_2, resolved in 1.0 mL of ethanol, and Spinnex filtered before high-performance liquid chromatography (HPLC) analysis. The separation was achieved on a Waters Nova-Pak C18 column [4 μm, 150 × 3.9 mm, with a mobile phase of methanol−water (98 + 2, v + v)]. Ergosterol was detected with a Perkin-Elmer detector at 282 nm, and the quantification limit was 10 ng/L.

Sample Preparations and Analysis of Beauvericin and Enniatins. Water for HPLC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA). Organic solvents (HPLC grade) were purchased from Merck. The standards for BEA (catalog no. B7510) and the enniatin mixture (catalog no. E3643) were purchased from Sigma Chemical Co. The enniatin mixture contained enniatin B, 19%; enniatin B1, 54%; enniatin A, 3%; and enniatin A1, 20%, according to Monti et al. (*40*).

To extract BEA and ENN, 10 g of each sample was ground and homogenized in a Ultraturrax model T25 basic from IKA Werke (Staufen, Germany) for 3 min at 13500 rpm with 50 mL of methanol of HPLC grade. Samples were filtered through Whatman No. 4 filter paper, and all methanol was removed under reduced pressure. Extracts were resuspended in 3 mL of methanol, prepurified once on a C18 column (500 mg, 3 mL, 40 μm) (Varian, Inc., Palo Alto, CA), concentrated to 1 mL, and filtered through an Acrodisk filter (pore size = 0.22 μm) (Millipore, Jonezawa, Japan) before HPLC analysis.

BEA and ENN (B, B1, and A1) analyses were performed according to the method of Monti et al. (*40*) with minor modifications. HPLC analyses were performed using LC-10AD pumps and a diode array detector from Shimadzu (Kyoto, Japan). A Shiseido Capcell Pak C18 (250 × 4.6 mm, 5 μm) column was used. HPLC conditions included a constant flow at 1.5 mL/min and AcN/water (65:35 v/v) as starting eluent system. The starting ratio was kept constant for 5 min and then linearly modified to 70% AcN in 10 min. After 1 min at 70% AcN, the mobile phase was returned to the starting conditions in 4 min. BEA and ENNs were detected at 205 nm. Mycotoxins were identified by comparing retention times and UV spectra of samples with authentic standards. Further confirmation was obtained by co-injecting pure standards with each sample. Mycotoxins were quantified by comparing peak areas from samples to standard calibration curves. Chemical structures were confirmed by liquid chromatography−mass spectrometry (LC-MS). A Perkin-Elmer LC series 200 connected to a 785A UV−vis detector was coupled with an API-100 single-quadrupole mass spectrometry (Perkin-Elmer Sciex Instruments, Ontario, Canada). The detection limits were 20, 1.3, 3.6, and 1.2 ng for BEA, ENN-A1, ENN-B1, and ENN-B, respectively. All analyses were run in triplicate, and the mean values are reported. The calculated standard deviation was always <5%.

Cell Cultures. *Cell Line*. Porcine kidney cells (PK15) were obtained from American Tissue Culture Collection (ATCC) and maintained in minimum essential medium, Eagles, with Earls basic salt solution (EMEM with EBSS) and L-glutamine (Bio Whittaker, Walkersville, MD) supplemented with 5% fetal bovine serum (FBS; Bio Whittaker) and 50 μg/mL gentamycin sulfate (Gibco BRL-Life Technologies, Paisley, U.K.) at 37 °C in a humidified atmosphere of 5% CO_2. Cells were reseeded by trypsination (trypsine−versene mixture, Bio Whittaker) and resuspended in complete medium as 1:2 or 1:4 dilutions. Cells were maintained at sub-confluence in large culture flasks (Costar, Corning Inc., Corning, NY) and reseeded frequently prior to cytotoxicity testing when hard to trypsinate to single cell suspensions.

MTT Cyctotoxicity Test. MTT (Sigma) was dissolved in phosphate-buffered saline (PBS; Bio Whittaker) at 5 mg/mL and filter sterilized before refrigeration. Sodium dodecyl lauryl sulfate (SDS), dimethyl sulfoxide (DMSO), both from Sigma, and 96% ethanol (Arcus, Oslo, Norway) were stored at room temperature. Ninety-six-well tissue culture plates were scanned with a Titertek Multiscan MS from Labsystems (Helsinki, Finland).

The MTT test measures metabolic activity of cells, namely, mitochondrial dehydrogenase reduction of a tetrazolium salt to formazan crystals (*41*−*43*). Following cell lysis and resuspension, formazan absorbance is measured with an ELISA reader. Pure toxins were included as positive controls and were dissolved in ethanol as stock solutions and stored at −20 °C. In the preparation of standards, aliquots were evaporated to dryness under N_2, resuspended in a 20 μL mixture of DMSO/ethanol (1:5.67), vortexed, and added to 980 μL of EMEM/gentamycin. Rice culture samples were prepared as for chemical analysis of MON. A 6 mL aliquot of purified extract was further evaporated to dryness in HPLC vials under N_2 and stored in −20 °C. Extracts were resolved in 20 μL of DMSO/ethanol (1:5.67) and vortexed prior to addition of 980 μL of EMEM/gentamycin. Each plate had eight wells of control cells with complete medium containing DMSO and ethanol. The samples were tested in duplicate and the tests run at least twice. Samples were diluted 2-fold in a series of 22 dilutions, according to a method revised from that of Hanelt et al. (*30*).

Cells were seeded on 96-well plates at 2×10^4 cells/mL, 100 μL/well, the day before exposure. Prior to exposure, the culture medium was aspirated from the plates, and 100 μL of fresh medium containing prepared dilutions of test compounds or controls wasadded to each well. After 24 h of incubation, 10 μL of 5 mg/mL MTT/PBS was added to each well and incubation was continued for a further 4 h. One hundred microliters of 10% SDS/0.01 M HCl was then added to each well, and the trays were incubated overnight. After thorough shaking, formazan absorbance was measured at 590 nm and at reference wavelength, 650 nm, to eliminate absorbance due to medium, cell debris, and plastic. The difference between absorbance (ΔA) at 590 nm and absorbance at 660 nm was automatically calculated for all wells. Cell count, given as percentage of control response, was calculated from mean $\Delta A = A_{590} - A_{650}$ for sample multiples and mean ΔA for control. Cytotoxicity is given as 100 − cell count (percent of control). All of the isolates of *F. avenaceum* were tested simultaneously in each run to ensure comparability of effects on cytotoxicity.

Mycology, chemical analysis, and cell culturing was carried out according to the National Veterinary Institute's quality assurance

Table 1. Identified Secondary Metabolites Found in Rice Culture Extracts of 24 Norwegian *F. avenaceum* Strains and Their Growth (Ergosterol)[a]

isolate	ERG	MON	BEA	ENN-A	ENN-B	ENN-B1
94.01.1049-3-6	497.3	0.711	0.00101	0.00007	3.093	0.206
94.01.1145-7	484.2	1.260	0.00103	0.00007	0.733	0.0002
94.01.1074-17	392.4	0.555	0.00127	0.00008	0.171	0.030
94.01.1155-7	518.5	0.707	0.00096	0.00006	0.606	0.177
94.01.1081-7	256.6	1.590	0.00195	0.00013	0.027	0.000
94.01.1198-7	400.7	0.772	0.00684	0.00008	0.629	0.069
94.01.1033-3	449.7	0.753	0.00111	0.00829	0.442	0.085
94.01.1212-5	560.6	0.324	0.00089	0.00006	0.779	0.072
94.01.1011-5	424.6	0.607	0.00577	0.01086	4.193	0.497
94.01.1035-18	290.9	1.018	0.05153	2.64170	0.133	1.745
94.01.1035-1	224.3	0.843	0.02617	0.00655	7.822	1.955
94.01.1066-14	461.7	0.294	0.00108	0.00007	0.052	0.020
94.01.1021-5	479.4	0.317	0.00104	0.06967	0.131	0.009
94.01.1018-3	400.0	0.637	0.00293	0.00008	0.162	0.011
94.01.1125-5	380.0	1.684	0.00655	0.01111	2.192	0.332
9401.1043-6	333.0	0.123	0.06907	0.02625	1.909	0.518
94.01.1020-3	392.9	0.375	0.00262	0.00629	0.811	0.049
94.01.1207-5	345.8	1.082	0.00145	0.00009	0.140	0.045
94.01.1209-16	339.2	0.175	0.00540	0.00010	0.251	0.324
94.01.1001-2	435.4	0.571	0.00115	0.02069	1.357	0.174
94.01.1002-4	484.0	0.551	0.00103	0.00007	0.395	0.179
94.01.1084-16	368.9	1.159	0.00136	0.03266	9.765	2.478
95.01.15056-5	315.0	0.585	0.00159	0.01924	1.189	0.424
95.01.15061-11	485.7	0.921	0.00103	0.04058	2.434	0.243
mean	405.0	0.734	0.00812	0.12062	1.642	0.402
median	400.4	0.672	0.00140	0.00321	0.681	0.176
SD	85.4	0.408	0.01709	0.53726	2.464	0.668
minimum	224.3	0.123	0.00089	0.00006	0.027	0.0002
maximum	560.6	1.684	0.06907	2.64170	9.765	2.478

[a] Ergosterol (ERG) is given as μg/g of rice. All other metabolite concentrations have been standardized by ergosterol levels and are given as g/g ergosterol. Secondary metabolites detected below the quantification limit were given the value of half the detection level. MON, moniliformin; BEA, beauvericin; ENN-A, enniatin A; ENN-B, enniatin B; ENN-B1, enniatin B1.

program, accredited according to the requirements of NS-EN-45001 (1989) and ISO/IEC Guide 25 (1990).

Statistics. Medians, means, and minimum and maximum values, as well as standard deviations (SD), were calculated by descriptive analysis. Metabolite concentrations found below the detection limits were included at half the detection limits in the subsequent statistical analysis. The relationship between production of the individual toxins was studied by factor analysis based on correlations. All strains were analyzed at identical concentrations, namely, the lowest extract concentration with high variance in toxicity among the individual isolates. Isolates were assumed to be random samples. Data analysis was performed with SAS (Raleigh, NC) and Minitab (Minitab Inc., State College, PA).

RESULTS

All fermentations were found to be satisfactory for further analysis, and ergosterol analysis showed that the species had a satisfactory growth rate. The results of the chemical analysis of 24 isolates of *F. avenaceum* isolated from Norwegian grain and grown on rice are given in **Table 1**. The individual toxin concentrations of the extracts have been standardized with their respective ergosterol contents to eliminate differences due to variable growth rates and permit direct comparison of isolates. The main secondary metabolites of rice fermentations at 25 °C were ENN-B and MON, followed by ENN-B1, ENN-A, and BEA, respectively. Only eight isolates produced BEA, namely, 9401.1198-7, 9401.1101-5, 9401.1035-18, 9401.1035-1, 9401.1018-3, 9401.1125-5, 9401.1020-3, and 9401.1209-16.

Table 2. Correlation Matrix of the Identified Secondary Metabolites Found in Rice Culture Extracts of 24 Norwegian *F. avenaceum*[a]

	MON	BEA	ENN-A	ENN-B
MON				
BEA	–0.335			
ENN-A	–0.002	**0.495**		
ENN-B	0.204	–0.024	–0.145	
ENN-B1	0.067	0.286	0.382	**0.775**

[a] Significant correlations ($P < 0.05$) are in bold.

Table 3. Factor Loadings of a Factor Analysis Based on Correlations of the Identified Secondary Metabolites Found in Rice Culture Extracts of 24 Norwegian *F. avenaceum*[a]

variable	factor 1	factor 2	factor 3	communality
MON	0.106	–0.023	**0.956**	0.926
BEA	0.121	**0.722**	–0.482	0.769
ENN-A	0.012	**0.946**	0.104	0.907
ENN-B	**0.965**	–0.174	0.089	0.97
ENN-B1	**0.904**	0.36	0.019	0.947
% variance	0.355	0.315	0.233	0.904

[a] High factor loadings are given in bold. Factor 1 accounts for 36% of the variance in our analysis. Likewise, factor 2 accounts for 32% and factor 3, 23%, of the variance.

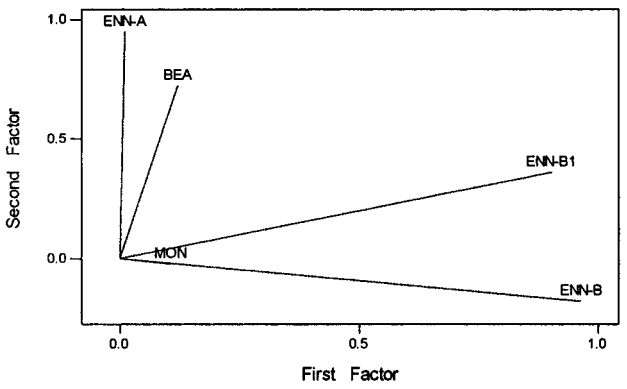

Figure 1. Loading plot of the factor analysis of the known secondary metabolites found in rice culture extracts of 24 Norwegian *F. avenaceum* strains, including three factors. The following labels identify the vectors: MON, moniliformin; BEA, beauvericin; ENN-A, enniatin A; ENN-B, enniatin B; ENN-B1, enniatin B1. The figure shows a three-dimensional plot drawn in the two-dimensional system defined by factors 1 and 2. The axis of factor 3 is orthogonal to the other two. The proximity of the vectors and their length indicate strength of correlations.

Fungal production of MON was not correlated to production of the other toxins. BEA was significantly correlated to ENN-A, whereas ENN-B and ENN-B1 were the most strongly correlated metabolites of these *F. avenaceum* isolates, as shown in **Table 2**. The factor loadings of a factor analysis involving three factors based on the correlations are given in **Table 3** and visualized in **Figure 1**. This analysis accounts for 90% of the variance in our results. The first factor affects production of ENN-B and ENN-B1 quite strongly and accounts for 36% of the variance. The second factor clearly separates ENN-A and BEA from the other ENNs and accounts for 32% of the variance. Factor 3 mainly affects the production of MON, although BEA is somewhat affected, too. In the corresponding loading plot (**Figure 1**), vectors ENN-B and ENN-B1 are in close proximity to each other and are of fairly equal length, whereas the vector of BEA is shorter and angled relative to ENN-A. The inter-

Table 4. Mean, Median, Standard Deviation (SD), Maximum, and Minimum Values of the Cytotoxicity of Rice Culture Extracts of 24 Norwegian *F. avenaceum* to PK15 Cells, in Percent of Control

extract concn, mg/mL	mean	median	SD	maximum	minimum
142.75	80	80	9.3	95	58
71.375	80	87	17.0	96	27
35.687	55	67	35.6	97	0
17.843	43	26	34.0	97	2
8.9218	37	29	25.9	98	10
4.4609	32	28	18.0	86	11
2.2304	22	20	9.8	47	10
1.1152	14	15	6.3	28	1
0.55761	10	11	7.4	26	0
0.27880	7	7	5.8	18	0
0.13940	5	5	5.7	17	0

proximity of the vectors and their length indicate the strength in correlation.

The mean cytotoxicity of the 24 rice culture extract dilutions is given in **Table 4**. We did not observe 100% toxicity (zero metabolic activity in the cells), even at very high extract concentrations. The linear phase of the individual dose−effect curves was very steep, and the concentration ranges spanning this phase varied among isolates. We found that MON was not correlated to toxicity (correlation coefficient, r, = 0.04). However, there was another compound in the HPLC chromatogram (UV detection at 229 nm) that eluted at 4.1 min relative to MON at 10.9 min. The area of this peak was significantly correlated to toxicity (r =0.44 and P = 0.044 for N = 22).

DISCUSSION

The isolates of *F. avenaceum* isolated from Norwegian grain grew well on rice with the applied laboratory conditions. Whereas it is probable that the initial inoculation of rice varied somewhat in colony-forming units, the standardization of the concentrations of secondary metabolites with ergosterol eliminates both variations in inoculation mixture and growth.

The main metabolites of Norwegian *F. avenaceum* grown on rice at 25 °C were ENN-B and MON. ENN-A was detected in low amounts, whereas BEA was detected in only seven samples, corresponding with an Italian study in which only one of six tested *F. avenaceum* strains produced BEA (*44*). The ENNs and BEA are structurally related compounds with the same biosynthetic pathway (*45*−*47*). These cyclic depsipeptides vary in amino acid moiety and their aliphatic chain lengths. Growth conditions and nutrient levels are highly consequential for the production of secondary metabolites, and little is known about the selective induction of ENN and BEA biosynthesis. Inasmuch as a long fermentation toward nutrient depletion is expected to include synthesis toward end products, one may expect an activation of this biosynthesis to continue toward similar concentrations, unless there is competitive anabolism. The variation in rates of biosynthesis of the ENNs and BEA by *F. avenaceum* has, to our knowledge, not been investigated.

Of all of the five secondary metabolites that were identified, there were only two pairs of correlated toxins, namely, BEA to ENN-A and ENN-B to ENN-B1. These correlations are corroborated by the factor analysis, in which we determined that there were two underlying factors that clearly differentiate the production of BEA and ENNA from ENNB and ENN-B1. The factor analysis also determined a third underlying factor that was uniquely affected by MON production. This factor slightly affected BEA production while having no discernible effect on the production of the ENNs. This also confirms the absence of correlation of MON to the other metabolites in the analysis. These results may indicate secondary mutuality of anabolic pathways. To our knowledge, there are no similar studies of numerous isolates of the same species and their metabolite profiles.

In previous studies of secondary metabolite profiles of three other *Fusarium* spp. fermented at identical conditions (*48*, *49*), the correlations and factor analysis of these profiles, standardized by ergosterol levels, demonstrated relationships between trichothecenes and their known precursors; in factor analysis of *F. culmorum* and *F. graminearum*, the vectors of 3-acetyldeoxynivalenol and deoxynivalenol were in close proximity to each other and of similar lengths (*48*). Furthermore, a similar study of the *F. equiseti* metabolites resulted in loading plots of vectors representing 5-acetylnivalenol and nivalenol in close proximity and likewise of diacetoxyscirpenol and scirpentriol (*49*). All of the above examples of factor analysis were based on strong correlations as well. Although metabolite profiles are dependent on fermentation conditions, factor analysis may well be a useful tool in the studies of biosynthetic pathways.

In this study, MON was not correlated to cytotoxicity. In the older literature, MON was the only known metabolite of *F. avenaceum*, and there have been conclusions that the fungus was not very pathogenic. Conversely, MON is reported to be genotoxic to rat hepatocytes and lens cells (*12*, *15*) and is toxic to chicken splenocytes and cardiac and skeletal myocytes (*50*, *51*) at concentrations corresponding to the exposure levels in this study (5−91 μg/mL MON to PK15 cells). It is possible that the PK15 bioassay established for screening *Fusarium* spp. and their toxicity at The National Veterinary Institute is not suitable for testing of MON, although the rice culture extracts were toxic in this bioassay. Because the defatting of the crude extracts also extracts BEA and ENNs from our mixture, there must be unknown toxic compounds in the rice culture extracts. The HPLC chromatogram revealed several peaks of low peak height in addition to MON. The area of the largest peak was correlated to toxicity but not correlated to MON. The peak was much smaller than that of MON, but it is probable that this compound has another maximum detection wavelength.

SAFETY

Extracts of rice culture fermentations were prepared and handled in ventilation hoods. MTT is carcinogenic, and gloves should be used in all handling of rice culture extracts and MTT.

ABBREVIATIONS USED

AcN, acetonitrile; BEA, beauvericin; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; EMEM, minimum Eagles medium with Earl's basic salt solution; ENN, enniatin; *F. avenaceum*, *Fusarium avenaceum*; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; LC-MS, liquid choramtography−mass spectrometry; MON, moniliformin; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NaOH, sodium hydroxide; KH_2PO_4, potassium dihydrogenphosphate; PBS, phosphate-buffered saline; PK15, porcine kidney cell line; SDS, sodium dodecyl sulfate; SNA, Synthetischer Nährstoffarmer agar; TBAP, tetrabutylammonium dihydrogenphosphate; UV, ultraviolet; VIS, visible.

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