FOOD SAFETY AND TOXICOLOGY

TOWARDS UNDERSTANDING TRICHOTHECENE-MEDIATED DISRUPTION OF RIBOSOMAL FUNCTION AND HENCE ITS TOXICITY

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ABSTRACT

Trichothecene mycotoxins are macrocylcic fungal metabolites known to inhibit protein synthesis in eukaryotic ribosomes. These toxins contaminate kernels of wheat and barley as a result of infection with *Fusarium* species. The major trichothecenes associated with *Fusarium*-infected kernels belong to the Type B class of trichothecenes and include deoxynivalenol (DON) and its acetylated derivatives, 3-acetyl DON and 15-acetyl DON. However, Type A class of trichothecenes, including T-2 toxin, have also been found in *Fusarium*-infected kernels. Different degrees of toxicity have been observed among the different trichothecenes, and these differences are specific to the class of organism in question. For instance, DON is known to be more phytotoxic than T-2 toxin, whereas T-2 toxin is more harmful in mammalian systems than DON. While it is known that these toxins inhibit protein synthesis by disrupting peptidyl transferase activity, the exact mechanism of this inhibition is poorly understood. Furthermore, it is not known how differences in trichothecene structure can affect different levels of toxicity. Our long-term goals are to better understand the toxicity mechanisms of these compounds, and as an initial step towards this end, we have employed a series of solid-state and solution-state nuclear magnetic resonance (NMR) spectroscopy experiments to study the three-dimensional structures and hydrogen-bonding patterns of both Type A and B trichothecenes. In our study, the epoxide ring (essential for toxicity) seems to constrain the configuration of the other side of the molecule (specifically the tetrahydropyranyl pocket). This tetrahydropyranyl pocket appears to be conserved in ribosomal binding, as was observed in the recently published structure of the yeast ribosome co-crystallized with different trichothecenes (Loubresse et al. 2014). We propose that the epoxide ring is not directly involved in trichothecene toxicity, but rather is essential in stabilizing the three dimensional structure required for establishing the configuration necessary for inhibition of protein synthesis.

REFERENCES

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CHARACTERIZATION OF A DEOXYNIVALENOL-INACTIVATING UDP-GLUCOSYLTRANSFERASE AND ITS UTILIZATION FOR ENZYMATIC PRODUCTION OF DON- AND NIV-GLUCOSIDE

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ABSTRACT

Trichothecene mycotoxins such as the *Fusarium* metabolites deoxynivalenol (DON) and nivalenol (NIV) can be inactivated *in planta* by formation of glucose conjugates. Since such metabolites escape routine detection and the parental toxin can be reactivated by hydrolysis, they are termed "masked mycotoxins". To date, there is insufficient information concerning the toxicological relevance of glucosylated mycotoxins. It has been shown that DON-3-O-β-D-glucoside (D3G) can be efficiently cleaved by intestinal bacteria, and is indeed cleaved in the intestinal tract of rats and pigs. While currently the concentrations of mycotoxin glucosides are rather low in infected cereals ongoing attempts to increase *Fusarium* resistance by wheat breeding may increase the contribution of glucosides to the total mycotoxin load. We identified a plant glucosyltransferase 1 family member which could be successfully expressed in *Escherichia coli* and efficiently glucosylates DON and NIV. This enzyme was purified by affinity chromatography and its biochemical properties were characterized. Substrate inhibition occurs at higher DON concentrations, and the enzyme is strongly inhibited by the second reaction product UDP. The enzyme is able to glucosylate both DON and NIV regioselectively at the position C3-OH, as confirmed by NMR of the purified glucosides. Using UDP-glucose as co-substrate, the complete conversion of larger amounts of DON (50 mg range) is possible within short reaction times (< 4 hours) *in vitro*. This enzyme is therefore a suitable biocatalyst to efficiently produce relevant amounts of DON and NIV-glucosides for use as analytic standards and for toxicological risk assessment, e.g. in animal feeding trials.

THE ZEARALENONE DETOXIFICATION PATHWAY OF *TRICHOSPORON MYCOTOXINIVORANS*: ELUCIDATION OF THE FIRST STEP

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ABSTRACT

Zearalenone (ZEN), a potent estrogenic mycotoxin produced by several *Fusarium* species, can be inactivated by the basidiomycete yeast *Trichosporon mycotoxinivorans*. ZEN is converted to a non-estrogenic metabolite [1], termed ZOM-1. We proposed a two-step mechanism involving a Baeyer-Villiger reaction leading to the formation of a hypothetical intermediate with a newly formed lactone bond, followed by lactone opening by an esterase. At this time the predicted intermediate could not be detected by LC-MS/MS in the cultures of wild-type *Trichosporon mycotoxinivorans* cells [1].

The gene encoding the first enzymatic step of the proposed pathway could be identified by a genetic approach. *Trichosporon mycotoxinivorans* was mutagenized by irradiation in a TRIGA Mark II nuclear reactor, followed by screening for mutants unable to degrade ZEN, which were detected using a *Saccharomyces cerevisiae* estrogen receptor bioassay. The determination of the genome sequences of the wild-type and mutant *Trichosporon mycotoxinivorans* strains and bioinformatical analysis revealed a large deletion containing a Baeyer-Villiger type monooxygenase candidate gene. Expression of a codon-optimized cDNA of this candidate gene in baker's yeast led to production of a metabolite of the mass of the expected hypothetical intermediate in ZEN treated transformants. After SPE pre-clean up and enrichment the metabolite was isolated by prep-HPLC and characterized by NMR. The estrogenicity of the new metabolite was tested using a yeast bioassay (based on induction of a *lacZ* reporter protein mediated by an expressed human estrogen receptor alpha fusion-gene). Nearly 100-fold higher concentrations of iZOM than ZEN are needed for activation of the reporter construct, demonstrating that the first step alone already leads to detoxification.

REFERENCE

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NEW TRICKS OF AN OLD ENEMY: ISOLATES OF *FUSARIUM GRAMINEARUM* PRODUCE A TYPE A TRICHOTHECENE MYCOTOXIN

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ABSTRACT

The ubiquitous filamentous fungus *Fusarium graminearum* causes the important disease Fusarium head blight on various species of cereals, leading to contamination of grains with mycotoxins. In a survey of *F. graminearum* (*sensu stricto*) on wheat in North America several novel strains were isolated, which produced none of the known trichothecene mycotoxins despite causing normal disease symptoms. In rice cultures a new trichothecene mycotoxin (named NX-2) was characterized by liquid-chromatography-tandem-mass spectrometry. NMR measurements identified NX-2 as 3α-acetoxy-7α,15-dihydroxy-12,13-epoxytrichothec-9-ene. Compared to the well-known 3-acetyl-deoxynivalenol it lacks the keto group at C-8 and hence is a type A trichothecene. Wheat ears inoculated with the isolated strains revealed a ten-fold higher contamination with its deacetylated form, named NX-3, (up to 540 mg kg^{-1}) compared to NX-2. The toxicities of the novel mycotoxins were evaluated utilizing two *in vitro* translation assays and the alga *Chlamydomonas reinhardtii*. NX-3 inhibits protein biosynthesis to almost the same extent as the prominent mycotoxin deoxynivalenol, while NX-2 is far less toxic, similar to 3-acetyl-deoxynivalenol. Genetic analysis revealed a different *TRI1* allele in the N-isolates which was verified to be responsible for the difference in hydroxylation at C-8.

The occurrence of isolates producing the new toxin raises the question whether such strains have a selective advantage, and in the worst case can counteract progress made by plant breeders in the last decade. We will discuss the hypothesis that production of a toxin with an acetylated C3-OH may be a response of the fungus to circumvent inactivation by glycosylation, while lacking the keto-group may prevent glutathione-mediated detoxification. Population genetic studies to determine whether the frequency of NX-producers is changing seem highly warranted.

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