Mycotoxin inactivating enzymes as feed additives

Science Flash
ID15

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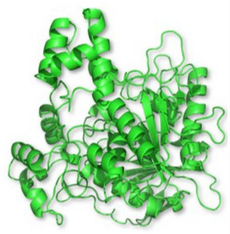
Introduction

Molds that colonize crops can produce mycotoxins, which reduce the value of the crop and can be harmful to both humans and animals. About a quarter of the dietary grains produced in the world are contaminated with mycotoxins [1]. One of the most common mycotoxin families is fumonisins, which are produced by various *Fusarium* fungi. Their most significant representative is Fumonisin B_1 (FB_1, see below), which is the number one cause of pulmonary edema in pigs and has a toxic effect on the liver and kidneys, among others [2]. In addition, it negatively affects sphingolipid biosynthesis through the inhibition of ceramide synthase [3]. Classical grain processing methods do not significantly reduce the FB_1 content of the contaminated crop [4], however, enzymatic degradation methods are known (see below), which can be used to transform FB_1 into less toxic metabolites. The two ester bonds of the molecule can be cleaved with the help of the fumonisin esterase (FE), which results in the less toxic hydrolyzed Fumonisin B_1 (HFB_1), through the partially hydrolyzed Fumonisin B_1 ($pHFB_1$) intermediate compounds [5].

Aim of the study

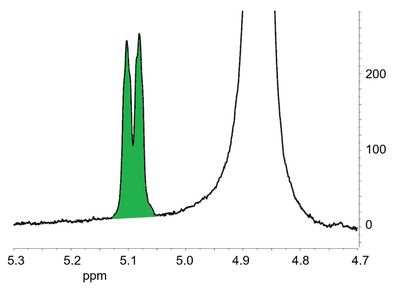
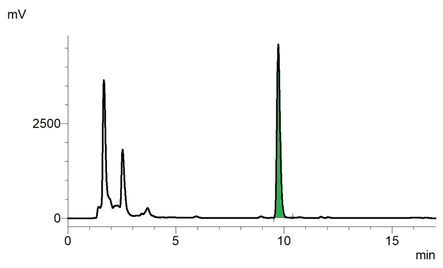
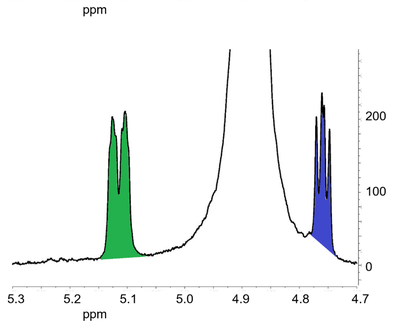
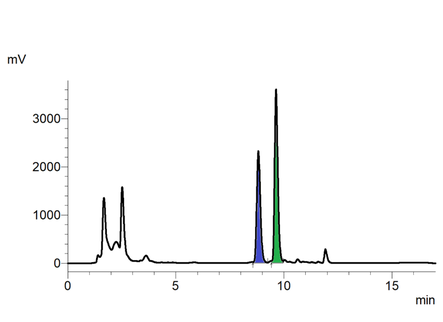


The kinetics of FEs and the details of the full hydrolytic process remains unknown, despite the successful practical application of FEs as feed additives. The study aims to reveal the details of the complete hydrolysis of FB_1 by the biodegradation of the naturally occurring quantities of FB_1 leading to unambiguous structural characterization of the two regioisomer partial hydrolysis products and detailed kinetics characterization of two FEs. Since the monoester metabolites are not available commercially, the development of novel synthesis methods allowing the production and analytical characterization of these metabolites for further studies were also aimed.

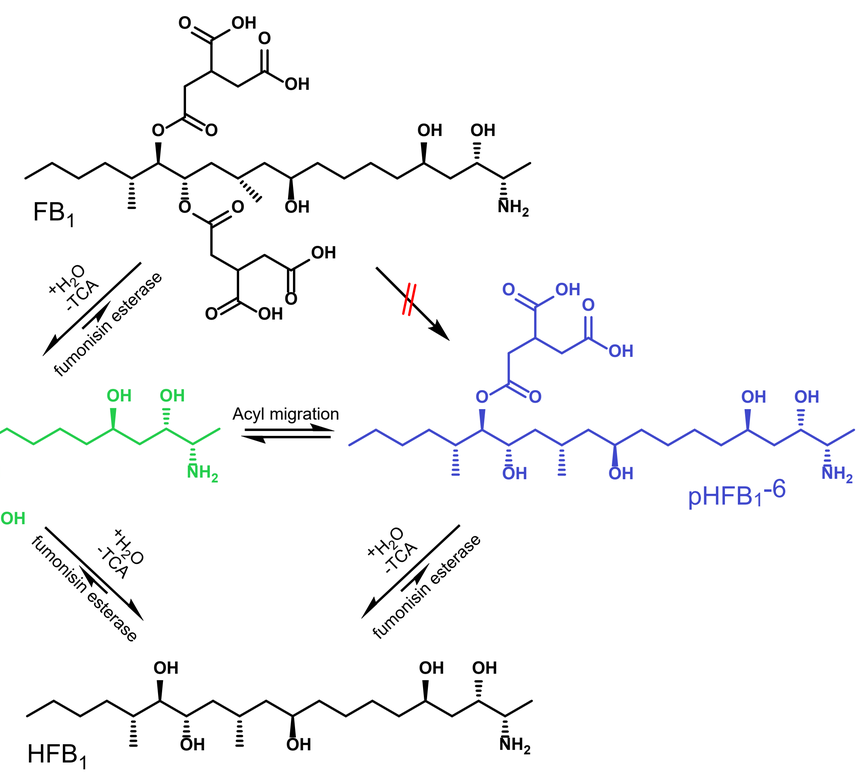
Methods

Two fumonisin esterases (FE1 and FE2), and FB_1 were produced by fermentation of *Pichia pastoris* and *Fusarium verticillioides*, respectively. FB_1 was purified in several extraction and chromatographic steps [6]. Pure FB_1 was partially or completely degraded by FE1 and FE2, and the reactions were terminated by the addition of methanol to the reaction. The reaction intermediate and final products were isolated with preparative chromatography and were identified with ^{1}H-NMR. The initial reaction rates were determined for both enzymes at 37°C and p*H*=6, with both FB_1 and $pHFB_1$ as substrates at different concentrations, and the kinetic parameters (K_M, k_{cat}, k_{cat}/K_M) of the FEs were calculated. Furthermore, the kinetics of the spontaneous acyl migration reaction between the two $pHFB_1$s were studied at different temperatures and in various solvents.

Results

Compounds	^{1}H-NMR	HPLC-FLD
$pHFB_1$_7		
$pHFB_1$_6:$pHFB_1$_7 38:62		

The enzymatic reaction selectively produces $pHFB_1$_7



Enzyme	Substrate	K_M (µM)	k_{cat} (1/s)	k_{cat}/K_M (1/(s*M))
FE1	FB_1	8.37 ± 0.55	140	$1.67*10^7$
FE1	$pHFB_1$_7	45.1 ± 2.2	121	$2.69*10^6$
FE2	FB_1	6.03 ± 0.39	115	$1.91*10^7$
FE2	$pHFB_1$_7	4.76 ± 0.61	124	$2.60*10^7$

FE1 and FE2 have exceptionally high catalytic efficiency

FE2 has a 10-times higher affinity towards $pHFB_1$_7 than FE1

Medium	E_T^{N*} (-)	Temperature (°C)	$t_{1/2}$($pHFB_1$_7) (h)
DMSO	0.444	20	80.44 ± 2.12
MeOH	0.762	20	108.8 ± 1.3
MeOH:H_2O, 3:1	N/A	20	166.1 ± 4.1
H_2O	1	20	213.3 ± 2.8
H_2O	N/A	37	26.76 ± 1.23
Simulated Gastric Fluid (pH= 3.0)	N/A	37	31.99 ± 0.95
Simulated Intestinal Fluid (pH= 8.5)	N/A	37	5.562 ± 0.174

*Empirical parameter of solvent polarity, data were taken from Reichardt et al. [7]

$pHFB_1$_7 can transform into $pHFB_1$_6 via acyl migration

Elevated temperature, increasing solvent polarity, and alkaline conditions facilitate the acyl migration

Conclusion

The present study showed that the enzymatic degradation of FB_1 mediated by fumonisin esterases produces $pHFB_1$_7 selectively as an intermediate. These enzymes are potent candidates for the decontamination of FB_1, and FE2 is proposed to be a more efficient enzyme in the total hydrolyzation of FB_1. These results could support the development of new enzyme products against various fumonisin-contaminated raw materials and processed products. The novel preparation and isolation method of $pHFB_1$_7 described here yields isomerically pure $pHFB_1$_7 on the preparative scale. This enables future *in vivo* and *in vitro* tests about the biological effects of this less-studied mycotoxin metabolite. Furthermore, the kinetic results of the acyl migration promote the correct interpretation of the measured $pHFB_1$ ratios of environmental or experimental samples. Acyl migration of $pHFB_1$_7 is catalyzed by heat, alkaline conditions, and weakly polar solvents. Therefore, samples containing FB_1 and its degradation products must be stored and manipulated at reduced temperatures, in a slightly acidic aqueous solvent, if the study intends to quantify the partially hydrolyzed compounds, to conserve the original ratio of the partially hydrolyzed metabolites.

Literature

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