FACULTY OF BIOSCIENCE ENGINEERING

UNRAVELING THE ZEARALENONE DEGRADATION PATHWAY USING A POLY-OMICS APPROACH

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Introduction

The microbial detoxification of mycotoxins is a promising tool to mitigate mycotoxins. However, the degradation pathway and metabolites need to be well defined to meet legislative requirements. Using a poly-omics approach, including a genomic and transcriptomic analysis, we aim to unravel the degradation pathway of the estrogenic mycotoxin zearalenone (ZEN) by Actinobacteria.

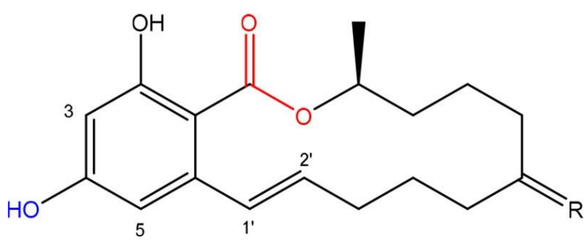


Figure 1: Chemical structure of zearalenone, indicating structures important for toxicity; lactone ring and C-4 hydroxyl group.

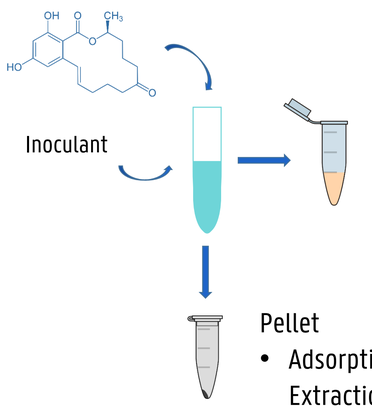
The toxicity of ZEN lies in its lactone ring and the C-4 hydroxyl group. Cleavage of the lactone ring followed by spontaneous decarboxylation as well as cleavage of the aromatic ring are shown to induce lowered toxicity. However, in multiple studies toxicity is not tested, no degradation products can be identified or degradation products show higher toxicity which is detrimental for the application[1]. **Can a poly-omics approach be the solution?**

Objectives

- Obtain **zearalenone-degrading Actinobacteria** and understand the circumstances wherein the degradation takes place
- Obtain a high-quality full genome sequence and conduct a transcriptomic RNAseq analysis to find **differentially expressed genes** and pinpoint important degradation steps and enzymes
- Find a gateway towards **practical application** in pre- and post harvest remediation

Workflow

Screening of the Actinobacteria strain collection



Supernatant
- Detoxification? BLYES/BLYR assay
- Degradation? HPLC-FLD and LC-MS/MS

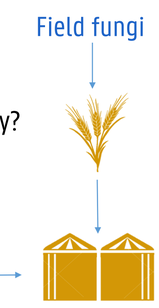
Pellet
- Adsorption? Extraction and HPLC-FLD

Poly-omics approach

- Genomic analysis of selected strains (PacBio SMRT)
- RNAseq analysis (Illumina)
- Differentially expressed genes and important enzymes
- Degradation products

Application in pre-and post-harvest remediation

Storage conditions
- Degradation of toxins?
- Biocontrol activity?



Field & pot trials
- Application while the plant is growing?

First results – Screening of Actinobacteria

18 Actinobacteria have been screened for the detoxification an degradation of 5 ppm ZEN, both in a rich medium and a minimal medium.

Detoxification

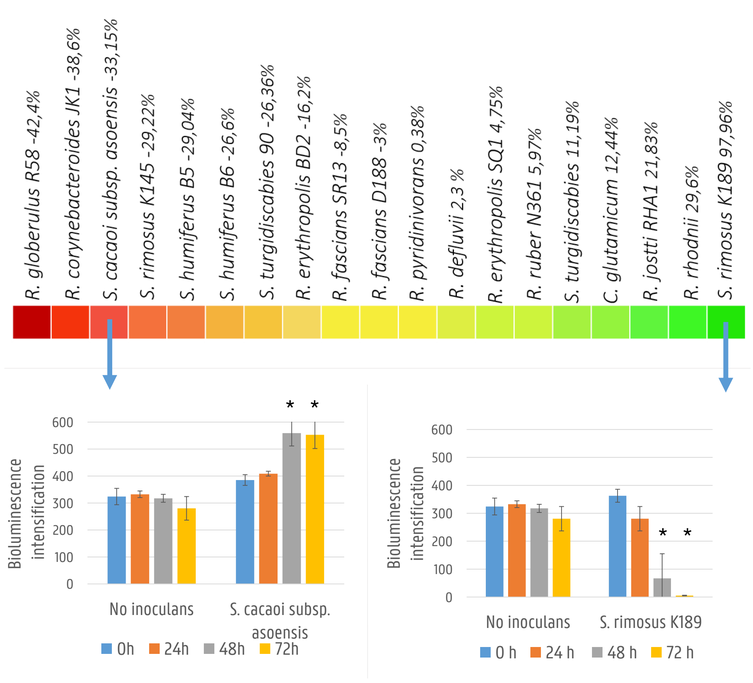


Figure 2: Overview of the first screening of 18 Actinobacteria for the detoxification of ZEN in rich growth medium. Detoxification is based on the BLYES/BLYR system, and percentages indicate the relative detoxification after 3 days. * p < 0,001. The figure highlights the importance of screening detoxification next to degradation.
For the same set of strains, no detoxification was observed when ZEN was present as the sole carbon source.

Degradation

Table 1: Degradation of ZEN by relevant strains in rich growth medium after 3 days. Percentages are calculated based on the initial concentration of 5 ppm ZEN. Results obtained via LC-MS/MS after QuEChERS extraction.

Strain	Degradation in 3 days	Adsorption by pellet in 3 days
S. rimosus K189	85,57%	0,37%
R. jostii RHA1	58,18%	7,24%
S. cacaoi subsp. asoensis	76,60%	4,42%
R. corynebacteroides JK1	86,82%	3,10%

- Degradation resulting in both higher and lower toxicity
- Degradation products can be more toxic
- Low adsorption by bacterial cell pellets

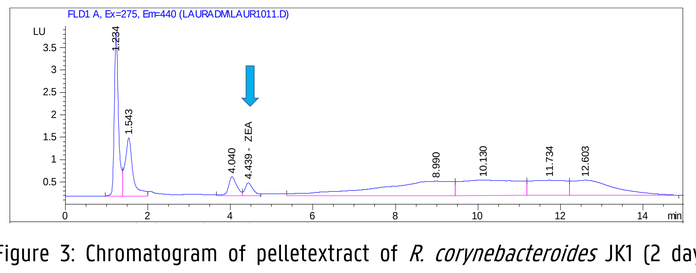


Figure 3: Chromatogram of pelletextract of *R. corynebacteroides* JK1 (2 days incubation in rich growth medium). The figure shows that not only a peak of ZEN, but also some possible degradation products, are found. (Obtained with HPLC-FLD.)

Conclusion

- **Degradation of mycotoxins by microorganisms** is a promising tool to be implemented in integrated crop management systems
- Degradation of zearalenone does not always entail **detoxification**
- *Rhodococcus* and *Streptomyces* strains show **divergent ZEN metabolism**
- The **poly-omics approach** will allow to identify biodegradation genes and enzymes, important for application in pre- and post-harvest remediation of grains.

References
1. Vanhoutte et al (2016). Doi: 10.3389/fmicb.2016.00561

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