Engineering the NADPH specificity of DepB, a novel aldo-keto reductase involved in the detoxification of the agroeconomic mycotoxin deoxynivalenol (DON)

Nadine Abraham[1,2], Jason Carere, Stephen Y.K. Seah[2] and Ting Zhou[1]

[1]*Guelph Research and Development Centre, Agriculture and Agri-Food Canada (AAFC)*

[2]*Department of Molecular and Cellular Biology (MCB), University of Guelph*

Agriculture and Agri-Food Canada / Agriculture et Agroalimentaire Canada

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Introduction

- Deoxynivalenol (DON), a member of the Type B trichothecene family of mycotoxins is a key virulence factor for *Fusarium graminearum*. The latter is the causative agent of Fusarium Head blight (FHB) and Pink Ear Rot disease which results in losses of millions of dollars annually for the cereal grain industry[(1-3)].
- Low doses of DON intake results in acute mycotoxicosis in humans and gastrointestinal and weight performance issues in livestock such as swine. DONs toxicity is attributed to the C-12,13 epoxide ring and the C-3 hydroxyl[1].
- There are currently no effective methods to reduce DON levels in cereal grain products. However enzymatic detoxification is an area of interest for its specificity and ability to completely transform DON to less toxic by-products.

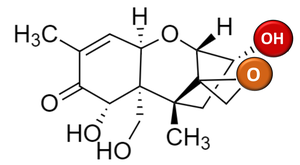


Figure 1. Structure of DON with toxin conferring groups: C3 hydroxyl (red) and C12/13 epoxide ring (orange)

Figure 2. Fusarium Head Blight (left) and Pink Ear Rot disease (right).

Microbial enzymatic detoxification : DON Epimerization

- *Devosia mutans* 17-2-E-8 detoxifies DON via epimerization[(2,3)].
- First, DepA, a pyroloquinolone quinone (PQQ) dependent alcohol dehydrogenase oxidizes DON at the toxicity conferring C3 hydroxyl position[2].
- Secondly, DepB, an NADPH dependent aldo-keto reductase(AKR) stereo-specifically reduces the intermediate, 3-keto-DON to 3-epi-DON (IC_{50}=1181 versus DON)[3].

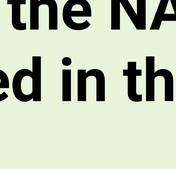


Figure 3. Scanning Electron Microscopy of *Devosia mutans* 17-2-E-8.

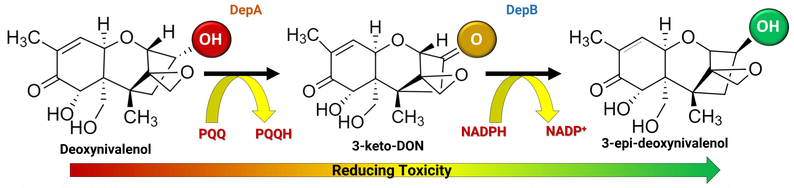


Figure 4. DON Epimerization involves two co-factor dependent oxidoreductases. 3-epi-deoxynivalenol is a diastereomer of deoxynivalenol and possess a reduced toxicity as evidenced by cytotoxicity assays.

Research Statement

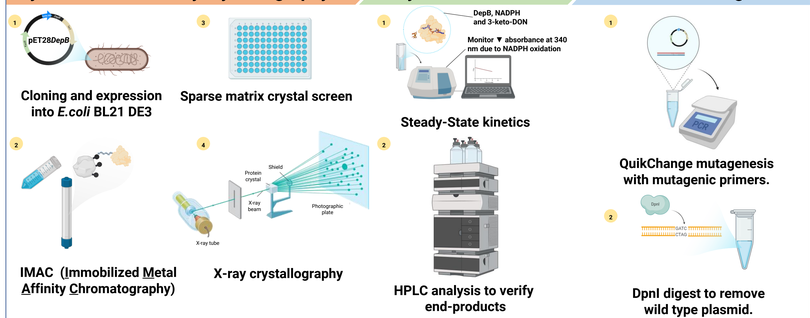
- **D**ON **Ep**imerization (Dep) enzymes could be employed in industrial processes, however the NADPH requirement does not make this feasible.
- AKRs such as DepB have a strong preference for NADPH[4] but may be engineered to utilize NADH which is a fraction of the cost and more stable. Uncovering residues involved in conferring NADPH specificity are instrumental to engineering DepB to utilize NADH instead.

Hypotheses and Objectives

- I hypothesize that the residues: Arg-289, Gln-293 and Lys-216 are involved in conferring co-factor specificity. Substituting these residues for acidic or hydrophobic residues should reduce DepB's affinity for NADPH while subsequently improving NADH binding.

Methodology

Crystal screens & X-ray crystallography → **Enzyme kinetics** → **Site directed mutagenesis**



- Large scale purification of DepB was conducted. Crystals obtained were optimized and sent to Canadian Light Source for x-ray diffraction studies.
- Steady state kinetics conducted spectrophotometrically at 340 nm. (Absorbance maxima for NADPH)
- Mutagenic primers for DepB NADH mutants designed & biochemically characterized.

Results 1. SDS-PAGE analysis of DepB

- The predicted molecular weight of DepB is 37kDa which corresponds to the position of the band.
- DepB was purified and the concentration determined to be 10.7mg/mL using a Bradford Assay.

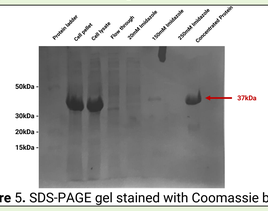


Figure 5. SDS-PAGE gel stained with Coomassie blue.

Results 2. 3D Crystal structure of DepB

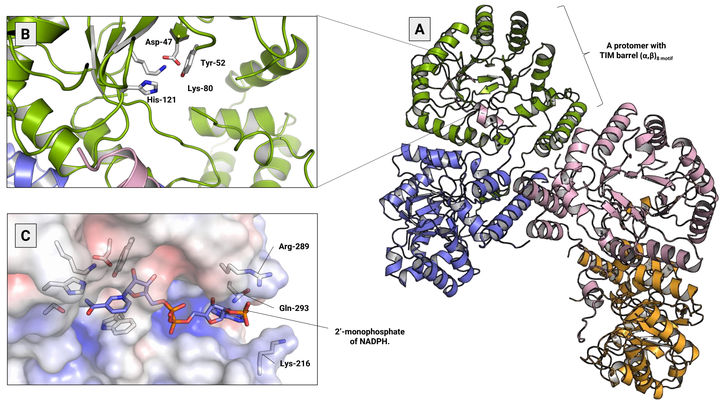
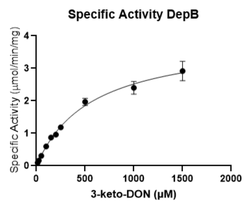


Figure 6.(A) Asymmetric unit of Apo-DepB. (B) Close up of substrate binding pocket of DepB with catalytic residues. (C) Electrostatic potential map surface representation with NADPH modeled into the co-factor binding pocket. Blue regions are indicative of positive, basic regions and red of electronegative rich regions. Residues interacting with the 2'monophosphate of NADPH are indicated in the diagram. Figures generated using PyMOL version 2.3.3

- Apo-DepB crystals diffracted to a resolution of 2.1Å (unpublished). DepB possesses the classical TIM barrel $(\alpha,\beta)_8$ motif present in AKRs.
- AKRs possess a series of conserved residues interacting with the 2'monophosphate of the adenine ring of NADPH.

Results 3. DepB Kinetics Assay

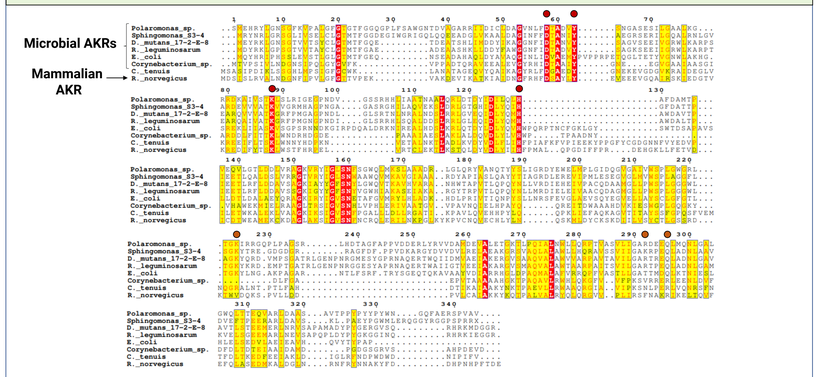
- The Km of DepB for 3-keto-DON is 563.9µM and dissociation constant (Kd) for NADPH is 44 µM.
- Preliminary kinetics with DepB, 3-keto-DON and NADH show some activity (data not shown), but less than NADPH indicating DepB's preference for NADPH.



Michaelis Menten Parameters
Km for 3-keto-DON = 563.9 µM
Kd for NADPH = 44 µM
Vmax = 3.942umol/min/mg
Product turn over = 2.61s^{-1}
Catalytic Efficiency = 4.63 x 10^3 $M^{-1}s^{-1}$

Figure 7. Steady state kinetics for wild type DepB with the cofactor NADPH.

Rationale for residue selection



- Multiple sequence alignments of DepB with other aldo-keto reductases indicate strict conservation of catalytic residues (red dots). Residues involved in interactions with the 2'-monophosphate of NADPH are partially conserved (orange dots).
- The following DepB NADH variants have been designed: R289E, R289L, R289G K216M, K216G. Mutants Q293E and Q293L will be designed in the future.

Conclusions and future work

- The following residues were identified as potential candidates for altering cofactor specificity: R289, Q293 and K216.
- DepB NADH variants will be tested for their activity towards 3-keto-DON using NADH. The catalytic efficiencies will be determined to evaluate the best mutants.
- Crystallization screens are also underway to obtain the binary complex of DepB with NADPH in the event the computational approach method fails to yield results.

Acknowledgments

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References

1. Karlovsky P. 2011. Biological detoxification of the mycotoxin deoxynivalenol and its use in genetically engineered crops and feed additives. Appl Microbiol Biotechnol 91:491–504.
2. Carere J, Hassan YI, Lepp D, Zhou T. 2018. The enzymatic detoxification of the mycotoxin deoxynivalenol: identification of DepA from the DON epimerization pathway. Microb Biotechnol 11:1106–1111.
3. Carere J, Hassan YI, Lepp D, Zhou T. 2018. The identification of DepB: An enzyme responsible for the final detoxification step in the deoxynivalenol epimerization pathway in devosia mutans 17-2-E-8. Front Microbiol 9:1–9.
4. Jez JM, Bennett MJ, Schlegel BP, Lewis M, Penning TM. 1997. Comparative anatomy of the aldo-keto reductase superfamily. Biochem J 326:625–636.