UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

NADINE ABRAHAM

on Friday, August 23rd, 2024 at 9:30 a.m. (SSC 2315)

Thesis Title: Structural and functional studies of oxidoreductases involved in the detoxification of mycotoxins

Examination Committee:

Dr. Jasmine Lalonde, Molecular and Cellular Biology (Exam Chair)
Dr. Stephen Seah, Dept. of Molecular and Cellular Biology
Dr. Matthew Kimber, Dept. of Molecular and Cellular Biology
Dr. Jennifer Geddes-McAlister, Dept. of Molecular and Cellular Biology
Dr. Christopher Garnham, Research Scientist, London Research Centre, Agriculture and Agri-Food Canada (External Examiner)

Advisory Committee:

Dr. Stephen Seah (Co-Adv)
Dr. Ting Zhou (Co-Adv)
Dr. Matthew Kimber
Dr. Tariq Akhtar

Abstract: Mycotoxins are toxic secondary metabolites synthesized by filamentous fungi that contaminate agricultural commodities destined for human and animal feed. Oxidoreductases that detoxify the agroeconomic mycotoxins deoxynivalenol (DON) and patulin have previously been identified. However, structural and functional studies for these enzymes are limited precluding their industrial application.

The two enzymes, DepA, a pyrroloquinoline quinone alcohol dehydrogenase and DepB an NADPH-dependent aldo-keto reductase (AKR) detoxify DON via epimerization. This process entails a stereoinversion of the C^3 OH group of DON to produce the less toxic diastereomer, *3-epi* DON. From an industry standpoint, the steep costs of NADPH supplementation impact the feasibility of epimerization. To facilitate future coenzyme engineering studies, the crystal structure of a DepB homolog from *Rhizobium leguminosarum* was solved. Using site-specific mutagenesis, the residues Lys^{217}, Arg^{290} and Gln^{294} were determined to confer NADPH specificity. As an alternative approach, an *E. coli* strain harbouring both wild-type DepA and DepB was engineered and employed as a whole-cell biocatalyst. The strain biotransformed 25 parts per million (ppm) of DON to *3-epi* DON in buffer within 2 hours, while in an animal feed matrix, corn steep water, the strain biotransformed an initial concentration of 15.9 ppm DON to *3-epi* DON within 24 hours. Patulin detoxification by an AKR, termed GOX1462 and a short-chain dehydrogenase (SDR) GOX0716 was assessed. Steady state assays were conducted to determine the substrate specificity, optimal pH, thermoactivity and thermal stability. Structural and phylogenetic analysis revealed that these oxidoreductases belong to evolutionarily divergent families with catalytic promiscuity towards other toxic aldehydes and ketones.

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Curriculum Vitae: Nadine obtained her Bachelor of Science (Honours) in Molecular Biology with a minor in Chemistry at the University of Toronto, Mississauga in 2016. In the Summer of 2019, she completed her Master of Biotechnology at the University of Guelph. In the Fall of 2019, she entered into the Ph.D. program under the joint supervision of Dr. Seah and Dr. Zhou (Agriculture and Agri-Food Canada).

Awards: Ontario Graduate Scholarship (2022- 2023)

Publications: Zhu, Y., Chan, E. T. S., **Abraham, N**., Li, X. Z., Wang, W., Mats, L., Zhu, H., Carere, J., & Zhou, T. (2024). Unveiling the Broad Substrate Specificity of Deoxynivalenol Oxidation Enzyme DepA and Its Role in Detoxifying Trichothecene Mycotoxins. Toxins, 16(3), 136.

Abraham, N., Chan, E. T. S., Zhou, T., & Seah, S. Y. K. (2022). Microbial detoxification of mycotoxins in food. Frontiers in Microbiology, 13, 957148.

Schroeter, K. L., **Abraham, N**., Rolfe, N., Barnshaw, R., Diamond, J., & Seah, S. Y. K. (2022). Bacterial Hydratases Involved in Steroid Side Chain Degradation Have Distinct Substrate Specificities. Journal of Bacteriology, 204(9), e0023622.

Abraham, N., Schroeter, K. L., Zhu, Y., Chan, J., Evans, N., Kimber, M. S., Carere, J., Zhou, T., & Seah, S. Y. K. (2022). Structure-function characterization of an aldo-keto reductase involved in detoxification of the mycotoxin, deoxynivalenol. Scientific Reports, 12(1), 14737.

Wang, W., Zhu, Y., **Abraham, N**., Li, X. Z., Kimber, M., & Zhou, T. (2021). The Ribosome-Binding Mode of Trichothecene Mycotoxins Rationalizes Their Structure-Activity Relationships. International Journal of Molecular Sciences, 22(4), 1604.

Bhatwa, A., Wang, W., Hassan, Y. I., **Abraham, N**., Li, X. Z., & Zhou, T. (2021). Challenges Associated With the Formation of Recombinant Protein Inclusion Bodies in Escherichia coli and Strategies to Address Them for Industrial Applications. Frontiers in Bioengineering and Biotechnology, 9, 630551.