

## Degradation of Hydroxyatrazine using AtzB from *Pseudomonas* sp. strain ADP

Bond Life Sciences Center<sup>1</sup>, Department of Biological Sciences<sup>2</sup>, Department of Veterinary Pathobiology<sup>3</sup>, Center for Agroforestry<sup>4</sup>, University of Missouri, Columbia, MO 65211

### Abstract:

Hydroxyatrazine (HA) is a metabolite of Atrazine, a toxic herbicide of the triazine family frequently found in the soil and groundwater in the United States which could contaminate drinking water. This compound can cause kidney and neuroendocrine problems in humans and animals. Atrazine is currently banned in Europe because of its toxicity to human health. However, there are not many restrictions on its usage in the United States resulting in it to contaminate drinking water [WHO, 2010]. A catabolic pathway of atrazine degradation had been well defined in the *Pseudomonas* sp. Strain ADP that was isolated from a heavily atrazine-contaminated site [Mandelbaum *et al.*, 1995]. There are six enzymes (AtzA-F) required to break down atrazine to carbon dioxide and ammonia in the pathway and encoded in the plasmid pADP-1 of the strain [Martinez-Vaz *et al.*, 2001]. The goal of my study is to produce recombinant AtzB enzymes for the degradation of hydroxyatrazine (HA).

The *N*-ethylaminohydrolase (AtzB) gene in the plasmid pADP-1 was amplified by PCR, cloned into the vector pSC-A and later inserted to *Escherichia coli* cells. The gene sequence in the clones were checked for no mutation in the promoter region and open reading frame of AtzB [Boundy-Mills *et al.*, 1997]. The correct cloned strain will be utilized for recombinant AtzB protein production. The AtzB enzymatic reaction using hydroxyatrazine as substrate will be performed to evaluate the enzymatic activity of recombinant AtzB protein [Seffernick *et al.*, 2007]. The results of the enzymatic reactions will be analyzed with Liquid Chromatography and tandem mass spectrometry (LC-MS/MS).

## References

- “Atrazine and Its Metabolites in Drinking-Water.” *World Health Organization*, World Health Organization, 29 Aug. 2016, [https://www.who.int/water\\_sanitation\\_health/dwq/chemicals/dwq\\_background\\_20100701\\_en.pdf](https://www.who.int/water_sanitation_health/dwq/chemicals/dwq_background_20100701_en.pdf)
- Mandelbaum, RT, Allan, DL, Wackett, LP. 1995. “Isolation and Characterization of a *Pseudomonas* sp. That Mineralizes the *s*-Triazine Herbicide Atrazine” 61(4):1451-1457.
- Martinez-Vaz, B., Tomkins, J., Wackett, L., Wing, R., & Sadowsky, Michael. 2001. “Complete Nucleotide Sequence and Organization of the Atrazine Catabolic Plasmid pADP-1 from *Pseudomonas* Sp. Strain ADP.” *Journal of Bacteriology*, 183(19): 5684–5697.
- Boundy-Mills, K. L., de Souza, M. L., Mandelbaum, R. T., Wackett, L. P., & Sadowsky, M. J. “The *atzB* gene of *Pseudomonas* sp. strain ADP encodes the second enzyme of a novel atrazine degradation pathway.” *Applied and environmental microbiology* vol. 63,3 (1997): 916-23.
- Seffernick, JL, Aleem, A, Osborne, JP, Johnson, G, Sadowsky, MJ, Wackett, LP. 2007. “Hydroxyatrazine *N*-Ethylaminohydrolase (*AtzB*): an Amidohydrolase Superfamily Enzyme Catalyzing Deamination and Dechlorination” *Journal of Bacteriology*, 189(19):6989-6997.