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

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Introduction

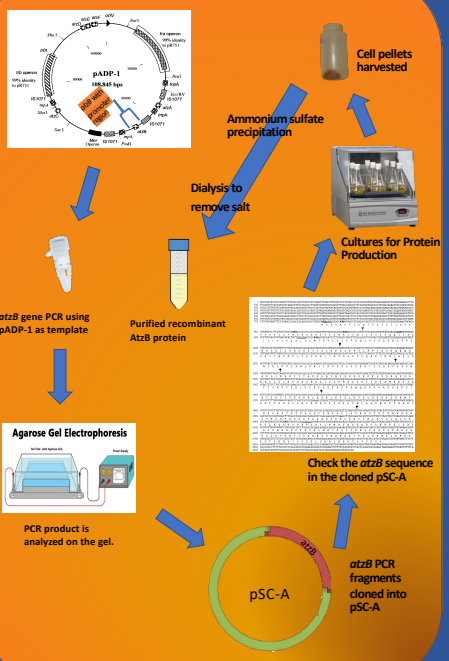
Hydroxyatrazine (HA) [2-(*N*-ethylamino)-4-hydroxy-6-(*N*-isopropylamino)-1,3,5-triazine] is a metabolite of Atrazine, a toxic herbicide of the triazine family frequently found in the soil and groundwater in the United States which could contaminate drinking water. This compound can cause kidney and 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 [1]. 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 [2]. 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 [3], and *N*-ethylaminohydrolase (AtzB) is the only known enzyme to catalyze hydroxyatrazine to *N*-isopropylammelide [5]. The goal of my study is to produce recombinant AtzB enzymes for the degradation of hydroxyatrazine (HA).

The promoter region and open reading frame of AtzB [4] will be cloned to a pSC-A and inserted to *Escherichia coli* cells for protein production. The enzymatic activity of recombinant AtzB protein will be determined using liquid chromatography and tandem mass spectrometry (LC-MS/MS). The recombinant AtzB enzymes will be utilized in comparison with immobilized AtzB enzymes on various platforms for efficiency. The results will help us to design a biofilter to remove the atrazine from contaminated drinking water.

Objectives

1. The *atzB* gene including the promoter region and the open reading frame from pADP-1 will be PCR-amplified and cloned into a pSC-A plasmid for protein production.
2. the *atzB* gene fragment cloned in the plasmid will be verified using restriction enzyme digestions and agarose gel electrophoresis by nucleotide size.
3. The sequence of *atzB* gene in the cloned pSC-A will be confirmed before culturing for protein production.
4. Recombinant AtzB protein will be expressed and isolated.
5. SDS-PAGE analysis with Coomassie blue staining will be performed to verified the production of recombinant AtzB protein.
6. the AtzB enzymatic activity will be demonstrated by the reactions with HA as substrate and the results will be determined using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis.

Methods



Results

Figure 1. *atzB* gene (1.64 Kb) was PCR-amplified using pADP-1 as template. The PCR products were analyzed by electrophoresis in 1% agarose gel. Lane 1: NEB 1kb DNA ladder; lane 2: *atzB* PCR product.

Summary

1. The *atzB* gene including the promoter region and open reading frame from pADP-1 was PCR-amplified and cloned into the pSC-A vector.
2. The *atzB* cloned vector was inserted in the SoloPack *E. coli* cells via transformation.

Future Directions

1. The sequence of the *atzB* gene in the cloned pSC-A will be verified before used in protein production.
2. The crude extract of protein culture will be collected and ammonium sulfate precipitation in 20% saturation will be applied to isolate recombinant AtzB protein.
3. The AtzB enzymatic activity will be determined in the reaction with hydroxyatrazine as substrate and the results will be analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis

References

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