Production of Bioactive Isothiocyanates by Bacterial Myrosinase

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PRECS Phenotypic Plasticity Research Experience for Community College Students

Introduction

- The focus of the project is on the hydrolysis of glucosinolates (GSLs) into isothiocyanates (ITC) by gut microbial myrosinase.
- ITCs are bioactive products found in several plants belonging to the *Brassicaceae* family, and are produced upon disruption of the tissue by herbivores or pathogens.
- ITCs are normally formed within plants as a defense against herbivores but in humans they have shown anti-cancer and anti-inflammatory properties.[1]

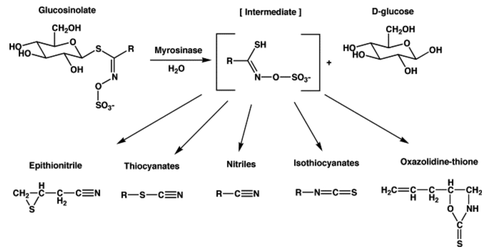


Figure 1: Glucosinolate hydrolysis and its various products.[3]

Background

- Although we know gut microbes can hydrolyze GSL into ITC, the mechanism is not known.
- Previous studies have shown that a probiotic strain of bacteria *Escherichia coli* Nissle 1917 is able to hydrolyze GSL without any detectable ITCs.[5]
- *E. coli* Nissle 1917, isolated from a German soldier during World War I, is now being used to treat gastrointestinal disorders.[4]
- *Citrobacter* Wye1 was isolated from a UK soil by enrichment using sinigrin.
- With improved detection methods, the aim of this experiment is to explore whether the bacterial myrosinase β-glucosidase found within the genome of *E. coli* Nissle 1917 can hydrolyze GSL into detectable ITCs.

Question

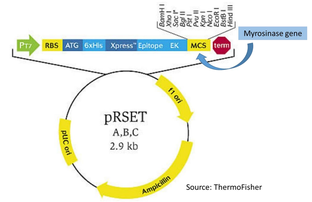
- Will the microbial myrosinase hydrolyze the GSL into bioactive ITC?

Hypothesis

- Microbial myrosinase is expected to produce bioactive ITCs but not as much as plant myrosinase.

Methods

- For this experiment we have chosen two candidate myrosinases from the gut microbe *E. coli* Nissle 1917 and the soil microbe *Citrobacter. Citrobacter* is the positive control in this experiment.
- We have the genes from these two candidate myrosinases synthesized (Thermofisher) and cloned into expression plasmids pRSET A.



- We transformed *E. coli* BL21(DE3) with the plasmids. We plated them on LB inoculated with Ampicillin, positive clones were picked and checked by DNA electrophoresis.
- The overexpression of microbial myrosinases was induced by IPTG. The myrosinases were purified by Ni-NTA column.
- The hydrolysis of GSL, sinigrin, was examined by cyclocondensation-HPLC.
- The hydrolysis of 4-nitrophenyl β-d-glucopyranoside (4NPG) was examined by A405nm

Results

Fig 1. DNA Gel Electrophoresis confirming the sizes of plasmids cloned into *E. coli* BL21(DE3)

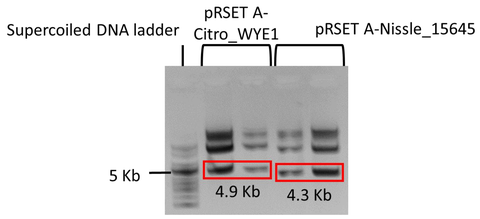


Fig 1. Transformation of plasmids with genes encoding myrosinase from Citrobacter sp. Wye1 myrosinase and 6-phospho-beta-glucosidase gene from E. coli Nissle 1917 into E. coli BL21(DE3).

- The overexpression of microbial myrosinases was induced by IPTG. The myrosinases were purified by Ni-NTA column.

Fig 2. Purification of Nissle & *Citrobacter* myrosinase by Ni-NTA columns and Amicon® Ultra Centrifugal Filters (30k MWCO)

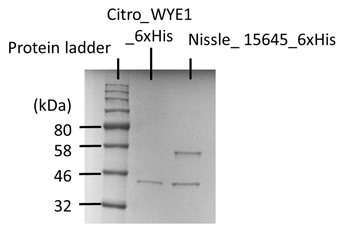


Fig 2. Purified Citro_WYE1 and Nissle_15645 (with 6x His-tag) visualized on 12% SDS-PAGE gel.

Results con't

- The hydrolysis of GSL, sinigrin, was examined by cyclocondensation-HPLC.

Fig 3. HPLC chromatogram showing the ITC production

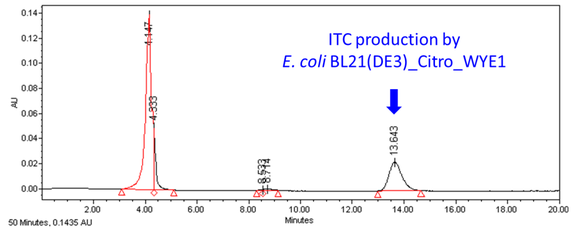


Fig 3. HPLC chromatogram showing the ITC production by E. coli BL21(DE3) transformed with pRESET_A plasmid expressing Citrobacter WYE1 myrosinase[2]

Fig 4. Sinigrin hydrolysis by purified microbial myrosinase candidates

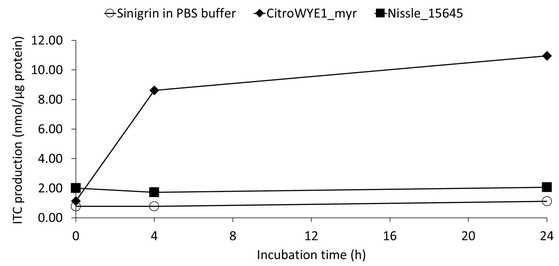


Fig 4. The myrosinase from Citrobacter produced detectable ITCs but not the myrosinase from E. coli Nissle.

- The hydrolysis of 4-nitrophenyl β-d-glucopyranoside (4NPG) was examined by A405nm

Fig 5. 4NPG hydrolysis by purified microbial myrosinase candidates

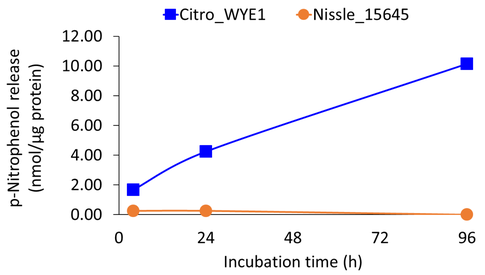


Fig 5. The myrosinase from Citrobacter produced detectable p-nitrophenol over time but not the myrosinase from E. coli Nissle.

Conclusions

- We have first-documented, direct evidence that a microbial myrosinase can produce detectable ITCs from GSLs.
- It is possible that the Nissle myrosinase is still a functioning enzyme, but only capable of hydrolyzing phosphorylated substrate, not GSLs/4NPG.

Future Work

- Use knock-out gene library strategy to identify the gene(s) encoding enzyme(s) that are responsible for the production of ITCs.

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Acknowledgments

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