An enhanced yeast-based system for detection and decontamination of organophosphate neurotoxins.

University of Chicago

IGEM 2009
OCTOBER 31, 2009
Motivation
Motivation

Organophosphorus Insecticides

Carbamate Insecticides

Sarin

Soman

VX agent

Nitrogen Mustard

Phosgene Oxime

Lewisite
Acetylcholinesterase Inhibitor

Acetylcholine $\rightarrow$ Acetic Acid + Choline

Bond is now strengthened
Can't be hydrolyzed
Biological solutions

Certain soil bacterium are able to degrade paraoxon and similar compounds….

Still other organisms are capable of degrading the byproducts…

Goal:
Engineer an recombinant organism that can both detect and fully degrade organophosphate neurotoxins
Improvement on previous models

- *S. cerevisiae*
- Highly characterized
- Storage
- Broad response to genotoxic agents
- Genomic integration
“Recombineering”

Genetic engineering through homologous recombination
Project Overview

- Biosensor
- Degradation
- Standardization
- Human Practices
## Biosensor: Promoters

### Paraoxon-sensitive

<table>
<thead>
<tr>
<th>ORF</th>
<th>Gene</th>
<th>Fold expression</th>
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<tbody>
<tr>
<td>YGR035C</td>
<td>-</td>
<td>1789.2</td>
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<tr>
<td>YHR139C</td>
<td><em>SPS100</em></td>
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<tr>
<td>YOR049C</td>
<td><em>RSB1</em></td>
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<tr>
<td>YLR346C</td>
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### Hydrolysis-sensitive

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<td>YGL205W</td>
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<td>YHL012W</td>
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Biosensor: Design

Paraoxon

P-Nitrophenol

Diethyl-phosphate

GFP

TadH
Genomic Integration

Paraoxon Promoters

Hydrolysis Promoters
Induced GFP expression

~1hr after induction with 1mM paraoxon

YGR035C

YLR346C
Overview of degradation

Degradation

Genes amplified from and p62pnpADEC-pCALJF and pMM11
Donated by Matthew Mattozzi
Considerations

- Toxicity
- Accessibility
- Sensitivity
- PNP kills
- Low permeability
- Use ADH1 promoter
- Gene deletion
- C-terminal tagging/truncation
- N-terminal tagging/truncation
- Up-regulation
  - +tagging/truncation
Our modules

- 5’-F*- [Gene specific region]
- 5’-R*- [Gene specific region]

- F1:
  - GTACTGAGAGTGCACCATATG
- F2:
  - TGA CTGGAC GC ACTTCTAAA
- R1:
  - CCGCGGATCTGCGGGTTTCC

 xảy ra với các dấu hiệu:

- Marker 1
- ADH1 terminator
- PGAL1 promoter
Human Practices

“Registry of Parts” for presentations

Topics
- Genetically modified foods
- Bioengineering in medicine
- Bioethics

Deposit slides online
- Comment. Success?
- Experience

Calgary, UIUC
Summary

- Paraoxon and hydrolysis reporter constructs
  - Testing

- Degradation genes
  - Constructs made
    - Testing

- Standardization
  - Biobrick design
    - Complete construction

Human Practices
- Online registry
- Collaboration
Future Directions

- Test degradation constructs
  - How well will *S. cerevisiae* express PdeA, pnpA?
- Finish and test integration constructs
- Refine GFP assay
- Genomic integration of degradation
Thank You!

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