Biphenyl Degradation by Pollutant Targeting, Biosurfactant Production, and Overexpression of Catabolic Enzymes
Introduction

- Clemson University’s inaugural iGEM team

- Problem:
  - Sangamo-Weston Inc. built capacitors with PCB containing dielectric fluid from 1955 to 1987.
  - The PCBs from improper disposal contaminated the water supply of Lake Hartwell and its tributaries.
  - Since PCBs have low water solubility, they remain mostly in the organic sediments and as particulate matter.
  - PCB sediment concentrations are within the range of injury for benthic macroinvertebrates and exceed healthy levels in the fish of Lake Hartwell.\textsuperscript{1}
Bioaccumulation

<table>
<thead>
<tr>
<th>WATERBODY</th>
<th>LOCATION</th>
<th>SPECIES OF FISH</th>
<th>ADVISORY</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Mile Creek</td>
<td>All Species of Fish</td>
<td>DO NOT EAT ANY</td>
<td></td>
</tr>
<tr>
<td>Seneca River Arm</td>
<td>All Species of Fish</td>
<td>DO NOT EAT ANY</td>
<td></td>
</tr>
<tr>
<td>Lake Hartwell</td>
<td>All remaining waters</td>
<td>Channel Catfish 1 meal a month</td>
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<td></td>
<td></td>
<td>Largemouth Bass 1 meal a month</td>
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<tr>
<td></td>
<td></td>
<td>Hybrid Bass/Striped Bass</td>
<td>DO NOT EAT ANY</td>
</tr>
<tr>
<td>PCB Advisory</td>
<td></td>
<td>Black Crappie No Restrictions</td>
<td></td>
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</tbody>
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| See page 10 to learn how to cook and clean fish from Lake Hartwell. | State of Georgia advisory for Lake Hartwell (Tugaloo Arm) |_mean| 2012 South Carolina Fish Consumption Advisory

http://www.epa.gov/greatlakes/atlas/images/chart402.gif
What do PCBs do?

- Known pollutant both locally and globally. This has been a problem with Lake Hartwell’s fish supply for 36 years, limiting the species and amount of fish that can be caught and consumed (the most common route of PCB toxicity for humans).

- PCBs have been proven to cause cancer by animal testing and there have been links to increased incidences of human cancers and developmental impairments in contaminated areas, in addition to normal toxicity symptoms: rashes, organ complications, among others.
What’s the solution?

- Bacteria! *Escherichia coli* to be exact.

- Multistep bacterial bioremediation system using two plasmids
  - Locate the PCBs in the sediment.
  - Goal is to expand on the 2011 iGEM UT-Tokyo Team’s guider system. By using a promoter specific for PCBs, the guider bacteria can signal the workers.²

- Two sets of workers: biosurfactant-producing and PCB-degrading bacteria.

- The second worker bacteria will breakdown the PCBs into less harmful byproducts, cleaning up Lake Hartwell!
Project Animation
Guider Bacterium

Biosurfactant-Producing Worker Bacterium

PCB-Catabolizing Worker Bacterium

PCB or Biphenyl

Aspartate

Mono- and Di-rhamnolipids

PCB-Catabolizing Enzymes
1. The *guider*, a magnetotactic bacterium naturally moves into the PCB containing sediment and send out aspartate that serves as a chemoattractant to the worker bacteria.

2. The two groups of *worker* bacteria follow the aspartate gradient towards the PCBs, and once there, a cell arrest system prevents them from migrating away.

3. The first group of worker bacteria produces a *bisurfactant—rhamnolipids* upon contact with PCBs. This helps to solubilize the PCBs that would otherwise not be bioavailable.

4. The second group of worker bacteria produces the *catabolic enzymes* that degrade the PCBs.
Guider System: UT-Tokyo 2011

- Replace the SOS promoter (activated by UV light) with a PCB specific promoter.

- Also, since PCBs are insoluble particles that remain in the sediments of Lake Hartwell, we need the workers to dive to the bottom.

- By implementing the UT-Tokyo System with our PCB promoter in a magnetotactic bacteria (microaerophile), our modified *E. coli* will come into contact with the PCBs much more quickly.
Guider Bacteria: Aspartate Production

- cheZ
- aspA
- SOS promoter
- Double Terminator
- Substrate-induced promoter
- Double Terminator
- cl
- Double Terminator

Cl-repressed promoter

Substrate-induced promoter

Biphenyls

Sediment
Biosurfactant Production Portion
Our biosurfactant pathway comes from the Gram-negative bacteria *Pseudomonas aeruginosa*, which produce their own surfactant molecules called rhamnolipids.

Significant properties include carboxylic acids and alcohol groups that can interact with lake water, and nonpolar regions that interact with the sediment layer.

Rhamnolipids in nature function to solubilize nonpolar compounds that resist interaction with water.

The pathway in *P. aeruginosa* uses several genes arranged on an operon to synthesize rhamnolipid components from both glucose and fatty acids.
Synthesis Pathway

This reaction combines the glycolysis and fatty acid pathways to create monorhamnolipids and dirhamnolipids.
Three Operons in one Plasmid

Biosurfactant - Rhamnolipid Worker Gene Construct

Three Operons in one Plasmid

RBS rmlB rmlD rmlA rmlC RBS RFP RBS rhlA rhlB RBS rhlG RBS GFP

T7 promoter with lac operator

Double Terminator

RBS rhlC MFST algC lacZα

T7 promoter with lac operator

Double Terminator

RBS lacZα

Sediment

Biphenyls
Plasmid Construction and Project Methods

1. Creation of electrocompetent *E. coli*
2. *P. aeruginosa* DNA extraction and purification
3. PCR of extracted DNA using forward and reverse primers
4. Gel electrophoresis to check the success of PCR
5. Purification of PCR samples
6. Ligation of genes into operons and operons into plasmids

A DNA gel showing the presence of genes contained within the operons
Chimeric PCR

- To combine the genes into several large operons, we designed new primers to fit on the 5’ end of the rhlAB gene attached to the rhlG forward primer.

- During the next rounds of PCR, the forward primer for rhlAB and the reverse primer for rhlG are mixed with the other components, creating a perfect strand of connected DNA.
The Degradation Pathway

- **BphA1a** – the large α subunit of the dioxygenase protein
- **BphA2, A3, & A4** – the small subunits of the dioxygenase protein that initially acts on the biphenyl
- **BphB** – a dehydrogenase protein
- **BphC** – an additional dioxygenase protein that serves to open one of the rings of the biphenyl molecule
- **BphD** – a hydrolase that forms the benzoic acid product
Operon Structure

- These genes were placed on two separate operons due to the size of the genes encoded and to increase transcription.

- Each operon received its own reporter gene with the activity of the genes responsible for the dioxygenase protein being indicated by RFP and the activity for the remaining biphenyl degradation genes being indicated by GFP.
Biphenyl Degradation Worker Gene Construct

T7 promoter with lac operator

RBS bphA1a RBS A2 RBS A3 RBS bphA4 RBS RFP

Double Terminator

RBS bphB RBS bphC RBS bphD RBS GFP

Double Terminator

Sediment

Biphenyls
Conclusion
Conclusion

- Successfully designed a model for enhanced PCB bioremediation
- Specifically designed primers for genomic DNA from *Pseudomonas* and *Sphingomonas*
- Successfully amplified 18 of 20 genes required for rhamnolipid production and biphenyl degradation
- Linking the genes into multiple operons on plasmids in progress
Future Work
Future Work

- Our T7 promoter will be replaced with a biphenyl-induced promoter that is more suitable for our system.
- The guider system will be transformed into a magnetotactic bacterium for increased targeting of sediment PCBs.
Future Work

- Verify expression of reporter genes: GFP, RFP, lacZ

- Protein expression will be analyzed by SDS-PAGE before and after induction by IPTG
Future Work

- Verify rhamnолipid production
- Solubilization of biphenyl by rhamnolipids will be measured
- Biosurfactant production will be checked by measuring surface tension and increased biphenyl solubility

http://www.astp.com/archives/1138
Future Work

- Verify biphenyl degradation

- Biphenyl degradation will be measured by gas chromatography (GC)
Team Members

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References

Markus Michael Muller and Rudolf Hausman, Appl Microbiol Biotechnol (2011) 91:251-264
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**Questions?**