Reprogramming microbes to cater to or silence their hosts

Beta Carotene production and RNAi delivery

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• Established in 1969, but various colleges such as the Ontario Agricultural College and the Ontario Veterinary College date back to the 19th Century.
• U of G has a strong focus on agriculture, but is also known for innovation generally.
• U of G is also known for having the best cafeteria food among Canadian universities.
The University of Guelph

Johnston Hall in the fall, one of U of G’s most recognizable sights. (Also, team member Lisa’s freshman residence.)
U of G’s First iGEM Team
Where there’s a Problem, Synbio Might Help

- Food
- Materials
- Medicine

- Collaboration with Calgary Ethics
- Sharing with Minnesota Time Bomb and Edinburgh
Bacterial Software on Plasmid Floppy Disks:

- Food
- Materials
- Medicine
A Bacterially Expressed Host Plant RNAi Program

• Bacterially Induced Gene Silencing (BIGS) is a technique we hope to develop as an enabling technology for plant functional genomics
  • Potentially better than mutants, transgenic plants, and VIGS
• Will deliver RNAi to a plant via the use of endophytic or symbiotic bacteria transformed with a RNAi construct transcribing plasmid
Why Would BIGS Work?

- Bacteria lyse upon cell death, releasing their contents into the surrounding environment (MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, Sept. 2000, p. 503–514)
- Plants have systemic responses – the presence of RNAi in one region of the plant can induce signalling across the plant (Nature 431, 356-363 [16 September 2004])
- Plants are very sensitive to dsRNA – spraying lysed E. coli on plants protects them against virus (BMC Biotechnol. 2003; 3: 3)
BIGS Results:
Bacteria Induced Gene Silencing

- **Bba_K152009**
- **Bba_K152008**
- **Bba_K152010**

- Cut with *NdeI* & *PstI*
- **pDSK-GFPuv**
- **Electroporation**
- **Phenotype evaluation**
- **Stem injection**

**Klebsiella pneumoniae 342**
BIGS Results:

Black and white corn and tb1 mutant image reproduced from Nature Reviews Genetics 3, 11-21 (January 2002)
A Vitamin Synthesis Programme - Beta Carotene Production from a Synthetic Operon

Map and chemical structure obtained via WikiCommons
Why Should SynBiologists Care?

• Vitamin A deficiency causes 250,000 to 500,000 cases of blindness in children each year worldwide (WHO, 2008)

• Vitamin A increases resistance to measles (Chan, 1990), and in increases birth weights while decreasing antenatal complications of babies born to HIV positive mothers (Kumwenda et al, 2002).
Can Synbio Help?

- Golden Rice and Golden Rice II were engineered to offer a dietary source of beta carotene to deficient populations where rice is a staple food – this has yet to be realized.
- We believe that golden bacteria might fit a special niche – Might Calgary Ethics agree?
What?

Will it work?
How did we build it?

• The PCR primers for all of our genes of interest, as well as the promoter, were designed to add SpeI, XbaI and PstI restriction sites.
• The SpeI and XbaI generate complementary sticky ends.
• Iterative ligation steps involving cutting the vector with SpeI and the insert with XbaI result in the fusion of the restriction sites, with PstI left intact for future use.
How did we build it?

- Insertion of crt-E from *Erwinia uredevora*
- Insertion of crt-B from *Erwinia uedevora*
- Insertion of crt-I from *Erwinia uredevora*
- Insertion of crt-Y from *Erwinia uredevora*
- Insertion of GFP gene from BioBrick BBa_E0240
- Insertion of arabinose inducible or 250 bp promoter from pDSK-GFPuv
PSBA generates a strong GFP signal in the measurement device, pSB1A2-E0240.

Incomplete EcoRI / PstI digests of several different biobricks in pSB1A2. Which Crt gene or combination of genes is indicated over each lane. The ladder used was TriDye 2-log DNA Ladder by NEB.
Figure 2: Visual confirmation of synthetic operon function on 1% arabinose plates. On the left are cells containing Bba_K118005, while those on the right contain the same Biobrick with the arabinose inducible promoter prefix. Similar results were obtained with the PSBA promoter.
Results:

• The plasmid with the beta carotene ‘program’ was transferred to pDSK-GFPuv and electroporated into *E. coli* Nissle and Kp342, but we did not get a chance to test this in the intestinal model.

• Intestinal model was set up testing survival and plasmid maintenance of Nissle containing unmodified pDSK-GFPuv and DH5α containing Ara or PSBA promoters driving Bba_K118005 in pSB1A2.
Where Do We Go Next?

• **BIGS**
  – Wait for corn to grow for evaluating phenotype
  – Publish and promote BIGS to scientists working on corn functional genomics

• **Beta carotene production**
  – Test the beta-carotene cassette in pDSK-GFPuv in lab strains first, then in Nissle, Kp342
  – Switch to pTG262 and test in lactobacilli
  – Establish partnerships with institutes working in biofortification and international development
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- The Universities of Minnesota and Edinburgh, for *Erwinia urevedora* genes.
- The University of Calgary’s Ethics Team, for collaboration.
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How would we test BIGS?

- Define TB1 mutation phenotype – show picture
- The modification of a *Zea mays* intron construct to contain the BioBrick BBa_B1006’s strong transcription termination signal.
- Ligation of the modified intron into a plasmid vector
- The transformation of electroporated *Klebsiella pneumoniae*, a corn endophyte, with the plasmid vector.
- Innoculation of corn seedlings with *Klebsiella pneumoniae*.