Gluc-o-Gone

uOttawa
iGEM 2009
Overview

• Background
• Organisms we used
• Genetic Circuitry
• Device Construction
Sugar, Sugar

- One gram of glucose contains 3.75 kcal, or 16 kJ of energy\(^1\)
- It is the preferred energy source for the brain\(^2\)

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The Problem With Sugar

• In the past 20 years, consumption in the US has increased from 26 to 135 lbs per person\(^1\).

• Leading cause of:
  – Obesity
  – Diabetes Mellitus

Obesity

• Viewed as one of the most serious health concerns of the 21\textsuperscript{st} century
• A leading cause of preventable death in developed countries\textsuperscript{3}
• Childhood obesity rates in Canada have increased from 11\% in the 1980s to 30\% in the 1990s!\textsuperscript{4}


Obesity

Source: Statistics Canada, 2004 Canadian Community Health Survey

Data sources: 2004 Canadian Community Health Survey: Nutrition 1978/79 Canada Health Survey
* Significantly higher than estimate for 1978/79 (p < 0.05)
E Coefficient of variation 15.6% to 33.3% (interpret with caution)
Diabetes Mellitus

• Between 2005-2006, 1.9 million Canadians were diagnosed with diabetes.
• By 2016, the annual cost of diabetes is expected to surpass $8 billion in Canada
• 7.8% of the American population suffers from a form of diabetes

Gluc-o-Gone!

• When you just can’t say no to sugar – Gluc-o-Gone fights back!
Cellulose

- An indigestible polymer of β-glucose, it locks up sugar in a form your body can’t access!
Dietary Fiber

• Cellulose is an *insoluble fiber*, which resists acid hydrolysis and fermentation in the human GI tract

• The US Academy of Sciences suggests a daily intake of 20-35g of dietary fiber, while most Americans consume only 12-18g per day

The Joys of Fiber

1) Fiber promotes the proliferation of the intestinal flora by providing nutrients
2) Cellulose has been specifically shown to augment metabolism of mutagens
3) By absorbing water, fiber eases the passage of food
4) Water-mediated attenuation of glucose in the GI tract

Goal: A Probiotic Bacterium That Converts Sugar Into Cellulose

Specifications:

1. Target organism must be present in the gut flora

2. Cellulose precursor must be part of target organism metabolism
Lactobacillus plantarum

• Gram-positive strain used in milk production
• Commonly used as a probiotic due to its ability to attach to the gut wall
Gut Presence
Acetobacter xylinum

Figure 1: Glucose to cellulose pathway in A. xylinum

Cellulose Synthase

• Codon comprised of 4 genes
• Well-characterized by Brown, Saxena et al.
• 10 kb total

Figure 2: Cellulose synthesis in vitro

Figure 3: acs operon in A. xylinum

What’s Important

Glucose-6-Phosphate → Glucose-1-Phosphate

Glucose → UDP-Glucose

Cellulose
Figure 4: Proposed model of Gluc-o-Gone function\(^1\)

• P45 is a strong, constitutive promoter
• *NisI*, RepA as described by Takala *et al*
• RFP for expression testing
• “Cut-and-paste” of acs operon under P45 expression
• Prophage integrase used to integrate acs operon into genome
• More stable expression over generations
Biobricks

- **Submitted parts:**
  - CMV promoter
  - CYC1 terminator
  - Gal 1 / 10 divergent promoter

- **Characterization:** Gal 10 promoter by dose response experiment
Modelling

• Jones and Smith predict spatial separation of strains in their two-strain plug-flow model

• Desirable for our strain; takeover by Gluc-o-Gone would present many issues

• Simulation of the genetic network is difficult without preliminary expression data
Challenges

• Two new organisms; microbiological techniques and non-standard plasmids
• Optimization of PCR for cellulose synthase size
• Difficult to BioBrick cellulose synthase
• Cross-species ribosome binding site issues
Results

• Development of *A. xylinum* media recipe and growth protocol, an optimized miniprep protocol for *A. xylinum* and protocols for transformation into *Lactobacillus plantarum*.
• Successful amplification of the cellulose synthase codon (~10 kb).
• RFP construct was also completed and confirmed, although transformation was unsuccessful.
**Figure 4.** PCR of the Cellulose Synthase operon from *Acetobacter xylinum*. Top: lane 1, lambda Hind III digest marker; lane 2-8, PCR unsuccessful. Bottom: lane 1, Hind lambda III digest marker; lane 2, First sample of cellulose synthase operon at ~10 Kb; lane 3, Second sample of cellulose synthase operon at ~10 Kb; lane 4-7, PCR unsuccessful; lane 8, Positive control. Each PCR product was amplified from a miniprep sample of wildtype *Acetobacter xylinum* genomic DNA.
Future Directions

• Construct optimization
• Expression testing
• Gut flora competition testing
• Testing in mice
Bi-Regulatory System
Fimbriae and Injectisomes
Safety and Ethics

• *Lactobacillus* is generally recognized as a safe probiotic organism
  – Genomic integration to eliminate lateral transfer
  – Cellulose synthase decreases fitness of bacteria
• Proposed biofilms and mice testing
• Please consult our wiki for our assessment of ethics and human practices
Thank You!

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