ENGINEERING AN EDIBLE PROBIOTIC CONSORTIUM TO REGULATE APPETITE AND DIGESTION

Jacky Cheung, Samuel Magaziner, Suppawat Kongthong, Hudson Lee, Kenya Velez
THE RISE OF METABOLIC DISEASES

CHANGES IN CALORIC INTAKE PER CAPITA PER DAY OVER TIME IN THE U.S

COMPARISON OF AVERAGE CALORIC INTAKE PER CAPITA AROUND THE WORLD

Obesity & Diabetes

Medical Expenses

Quality of Life
FOOD AS THE SOLUTION – NOT THE PROBLEM

HISTORICAL AND PROJECT SALES OF YOGURT IN THE UNITED STATES

WHAT ARE PROBIOTICS?
Live Bacteria & Yeasts That Improve Health

DIGESTIVE FUNCTIONS

IMMUNE SYSTEM FUNCTIONS

SUPPORT FRIENDLY BACTERIA

“Let food be thy medicine and medicine be they food” - Hippocrates

GOAL: ENGINEER A PROBIOTIC FOOD THAT COUNTERS OBESITY AND DIABETES
METHOD TO COUNTER OBESITY AND DIABETES

GLUCAGON-LIKE PEPTIDE I (GLP-I)
- Insulin Levels
- Satiety Levels

Peptide YY (PYY)
- Appetite Levels
- GI Movement

Ghrelin
- Appetite Levels
- Cardiovascular Health
ENGINEERING AN EDIBLE PROBIOTIC TO SECRETE GUT PEPTIDES TO REGULATE APPETITE AND DIGESTION

E. Coli Nissle

Lactobacillus Reuteri

Signal Peptides

Signal Peptides (SPs)

Ghrelin

PYY

GLP-1

Gut Peptides (GPs)

phoA

pelB

Lp_3050

M6

X
EXPERIMENTAL SETUP: SIGNAL PEPTIDE SECRETION

1. **Anhydro-Tetracycline (or Nisin in Lactobacillus model)**
2. **pTet**
3. **SP**
4. **Hormone**
5. **His-Tag**

- Grow-up, spin down cell pellet, extract supernatant
- Concentrate supernatant + lyse cells
- Blot

**Lysate**
- $1 \times 10^1$
- $1 \times 10^{-1}$
- $1 \times 10^{-2}$

**Supernatant**
- $1 \times 10^2$
- $1 \times 10^1$
- $1 \times 10^{-1}$
## RESULTS: HORMONES (NO SIGNAL PEPTIDE)

<table>
<thead>
<tr>
<th>Lysate (Left) 1x10(^1)</th>
<th>Supernatant (Right) 1x10(^2)</th>
<th>Ghrelin</th>
<th>Glp-1</th>
<th>PYY</th>
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<td>Supernatant (Right) 1x10(^{-1})</td>
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Note: We expect to see no secretion
RESULTS: SUCCESS WITH PHOA-GLP1 (IMPORTANCE OF INDUCTION TIME)

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PhoA-Glp1 (12hr Induction) PhoA-Glp1 (36hr induction)

Note: Same construct, Wildly different Results; also improvement of previous biobrick BBa_K817000
RESULTS: SUCCESS WITH PELB

Here we demonstrate the generalizable nature of signal peptide secretion.
IMPLEMENTING A CONSORTIUM TO PROVIDE REGULATIONS AND SAFETY MECHANISM FEATURES

Our system is to be tightly regulated as a safety precaution.

Communication
Regulation
Safety
THE SYSTEM: SAFETY, REGULATION, COMMUNICATION, AND SECRETION MEET
THE SYSTEM BREAKDOWN: THE AHL FACTORY
THE SYSTEM BREAKDOWN: TARGET SECRETION
THE SYSTEM BREAKDOWN: TIME-DELAYED CELL LYSIS (BBA_K1848006)
RESULTS

• Timer-Lysis Cassette system submitted as biobrick (BBa_K1848006)
• System successfully cloned in
  • Time constraints prevented its testing
  • However…
Heat whole milk to 185°F

Cool to 115°F and add engineered L. reuteri + yogurt starter culture

Incubate at 37°C for 8 hr and refrigerate

Note: Yogurt was not intended for human consumption, nor did it leave the lab
SURVEYING THE PUBLIC

• Online survey
  • Polled public on GMOs and probiotics, to inform design of safety measures and yogurt products
  • Informed survey takers about probiotics and GMOs
• Twitter and blog

![Graph 1: Comfort level in consuming probiotics (On scale of 1-5) before informing the survey takers about probiotics](image1)

![Graph 2: Comfort level in consuming probiotics (On scale of 1-5) after informing the survey takers about probiotics](image2)
THE FUTURE: YOGHURT (OR LOOKING WHEY INTO THE FUTURE)

• Going Forward:
  1. Finish screen of submitted quorum sensing system
  2. Move system into L. reuteri
  3. Test active system’s ability to function within yogurt culture
  4. Screen more hormones

• The Foreseeable future:
  • Catalog of peptide secreting yogurt
  • Biotech branch based around personalized consumable medicines
IN SUMMATION…

• We managed to:

1. Submit 6 bio-bricks (5 of which are well-characterized) (BBa_K1848001-006)
2. Successfully secrete 3 gut hormones and 2 signal peptides in E. coli
3. Develop a promising system quorum sensing based secretion and lysis
4. Move an engineered L. reuteri system in yogurt
5. Poll on public opinion of probiotics and willingness to consume GMOs
6. Have an amazing summer and lay the foundation for future iGEM teams from our University

Student Team Members
ATTRIBUTIONS AND ACKNOWLEDGMENT

• Undergraduate Team Members: Suppawat Kongthong, Jacky Cheung, Kenya Velez, Hudson Lee, Samuel Magaziner

• Mentors: Nathan Johns, Sway Chen, and Sonja Billerbeck

• PIs: Harris Wang, Virginia Cornish, Ken Shepard, Dana Pe’er


