Team Information

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One Project Idea: Intervention for Celiac Disease

1 out of every 133 people in the United States suffers Celiac

Autoimmune disorder triggered by a reaction to gluten

SYMPTOMS

- Recurring bloating, gas, or abdominal pain
- Chronic diarrhea or constipation or both
- Unexplained weight loss or weight gain
- Pale, foul-smelling stool
- Unexplained anemia
- Bone or joint pain
- Behavior changes/depression/irritability
- Vitamin K Deficiency
- Fatigue, weakness or lack of energy
- Delayed growth or onset of puberty
- Failure to thrive (in infants)
- Missed menstrual periods
- Infertility male & female
- Spontaneous miscarriages
- Canker sores inside the mouth
- Tooth discoloration or loss of enamel

Source: http://www.celiac.org/
The Normal Intestine
Surface receptor protein HLA-DQ2 on antigen presenting cells *complexed* with gliadin peptide.
Current Treatments

Currently, the only accepted treatment for this disorder is adhering to a gluten-free diet.

This can be very difficult as great varieties of food contain gluten.
A Developing Strategy

Intervene HERE

NOT HERE!
Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease

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Running title: Newly identified prolyl endoprotease

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Abbreviations: TM-PEP = prolyl endopeptidase from Aspergillus niger absorption ionization time of flight mass spectrometer

AN-PEP = prolyl endopeptidase from Aspergillus niger

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WHAT IS REQUIRED?

1. Microbe that survives and produces protein at low pH
2. Persist in the stomach for about a month or longer
3. Recognize gliadin
4. Secrete a gliadin-degrading protein*
5. Detect pH change (low to high)
6. Cell death

We focused on step 4 and used *E. coli* as a model system
Project Purpose

Producing induced secretion of target protein

- Searched registry – none found
- A method of secreting target proteins synthesized in *E. coli* host
- Possibly serve as a generic secretion system for synthetic biology
General Construct

Cargo

Cleavage site

Anchor

Cell Membrane
Our Motivation

Stable Expression and Secretion of Polyhydroxybutyrate Depolymerase of *Paucimonas lemoignei* in *Escherichia coli*

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An efficient strategy for the expression and secretion of extracellular polyhydroxybutyrate depolymerase (PhaZ1) of *Paucimonas lemoignei* in *Escherichia coli* was developed by employing the signal peptide of PhaZ1 and a truncated ice nucleation protein anchoring motif (INPNC). Directly synthesized mature form of PhaZ1 was present in the cytoplasm of host cells as inclusion bodies, while a construct containing PhaZ1 and its own N-terminal signal peptide (PrePhaZ1) enabled the secretion of active PhaZ1 into the extracellular medium. However, the PrePhaZ1 construct was harmful to the host cell and resulted in atypical growth and instability of the plasmid during the cultivation. In contrast, INPNC-PhaZ1 and INPNC-PrePhaZ1 fusion constructs did not affect growth of host cells. INPNC-PhaZ1 was successfully displayed on the cell surface with its fusion form, but did not retain PhaZ1 activity. In the case of INPNC-PrePhaZ1, the initially synthesized fusion form was separated by precise cleavage of the signal peptide, and active PhaZ1 was consequently released into the culture medium. The amount of PhaZ1 derived from *E. coli* (INPNC-PrePhaZ1) was almost twice as great as that directly expressed from *E. coli* (PrePhaZ1), and was predominantly (approximately 85%) located in the periplasm when cultivated at 22°C but was efficiently secreted into the extracellular medium when cultivated at 37°C.
### Our Parts

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (kDa)</th>
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<tbody>
<tr>
<td>Myosin</td>
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<tr>
<td>β-Galactosidase</td>
<td>116.3</td>
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<tr>
<td>Phosphorylase b</td>
<td>97.4</td>
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<tr>
<td><strong>Luciferase</strong></td>
<td><strong>60.6</strong></td>
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<tr>
<td>Ovalbumin</td>
<td>45.0</td>
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<tr>
<td>Carbonic anhydrase</td>
<td>31.0</td>
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<tr>
<td><strong>GFP</strong></td>
<td><strong>26.4</strong></td>
</tr>
<tr>
<td>Aprotinin</td>
<td>6.5</td>
</tr>
<tr>
<td>Insulin, B chain, oxidized</td>
<td>3.5</td>
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</table>

**Cargo (reporters)**
- **GFP** – Green fluorescent protein (BBa_l746916) – 720 bp
- **Luciferase** – Luminescent reporter (BBa_l712019) – 1653 bp
Our Constructs

INPNC

Lucif

GFP

cell membrane

INPNC

cell membrane
Our Parts – The Specifics

INPNC (Ice Nucleation Protein, NC)
- *Pseudomonas syringae* use INP to nucleate ice onto plants
- Exogenous to *E. coli* as surface display protein
- We used a truncated version, INPNC
  *Truncated at the N-C terminals to use only as a secretion mechanism
  *Shortest possible length for cell surface display
Our Parts – The Specifics

**SS (Signal Sequence)**
- Allows protein fused to outer membrane proteins to be cleaved free
- A portion of PhaZI, the smallest polyhydroxybutyrate (PHB)
- Our part: just the SS portion (38 amino acids long)

**OmpA (Outer Membrane Protein A) - BBa_K103006**
- Normally found on the cell surface of *E. Coli*
- Test its efficacy versus INP when fused to SS
Testing Scheme

Cells → Gel → Western Blot with 6-histag

Cells in media → SPIN

Media → Gel → Western Blot or Fluorescence
Whole Cell

MW (kDa)

(-) Control
INP-SS:GFP (unind.)
INP-SS:GFP (ind.)
OmpA-SS:GFP (unind.)
OmpA-SS:GFP (ind.)
INP-SS:Lucif (unind.)
INP-SS:Lucif (ind.)
OmpA-SS:Lucif (unind.)
OmpA-SS:Lucif (ind.)

160
110
80
60
50
40
30
20
15
10
<table>
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<tr>
<th>Media</th>
<th>(-) Control</th>
<th>INP•SS•GFP (unind.)</th>
<th>INP•SS•GFP (ind.)</th>
<th>OmpA•SS•GFP (unind.)</th>
<th>OmpA•SS•GFP (ind.)</th>
<th>INP•SS•Lucif (unind.)</th>
<th>INP•SS•Lucif (ind.)</th>
<th>OmpA•SS•Lucif (unind.)</th>
<th>OmpA•SS•Lucif (ind.)</th>
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Conclusion and Future

- INPNC•SS constructs are producing protein
- The SS cleavage sequences is getting cleaved in the context of INPNC

- Would like to find out what the mystery band is
- Would like to see if “secreted” products are accumulating in periplasm
- INPNC may be an alternative cell surface display – need to test
New Parts Submitted to Registry

1. PhaZ1 Signal Sequence – cleavage tag
2. INPNC – small surface display protein
3. INPNC+SS
Safety Considerations

Researcher Safety
- General lab *E. coli* strains, parts from registry or already used before (DH5α, BL21, GFP, OMPA, INPNC etc.) – just need to consider regular lab safety

Public/Environmental Safety
- Will the probiotic disrupt native microorganisms?
- Can it be or easily become pathogenic?
- Transfer of DNA into other host organisms or environment?
Special Thanks

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