Gliadin degradation for assistance in celiac disease, gluten intolerance and sensitivities

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Gluten is the common name for a group of complex proteins found in grains such as wheat, barley, and rye. There are four conditions associated with negative reactions caused by gluten consumption, which affect 5-10% of the global population: celiac disease, wheat allergies, gluten sensitivity, and gluten intolerance. In our experiment, we used *Escherichia coli* as a microbial host to produce the PEP 2RA3 microbial enzyme, a bacterial isolate that has been cloned and expressed in *E. coli*. It breaks down gliadins, one of two main protein groups in gluten.

These enzymes will be used in a pill (similar behaviors to the common lactase pill) with the purpose of helping affected individuals safely ingest foods containing gluten, in a more effective manner. For patients with celiac disease, our pill treatment may not be as effective as previously presumed, due to the fact that even the smallest particles of gluten can set off an immunogenic response. This reaction can damage villi lining the small intestine as there is a certain threshold for the amount of gluten that can safely be in the system of a patient with celiac disease. If gluten content is above this amount of 10-50 mg (consumed per day) then the patient is at risk of an autoimmune response that can damage the lining of the small intestine (villi) or cause cytokine storms. For people with other conditions (wheat allergies, gluten sensitivity, and gluten intolerance), however, the effects of gluten have the potential to be greatly reduced, even if there are small particles of gluten remaining after degradation. This pill would allow users to find enjoyment in the taste, smell, and texture of gluten-containing foods, while avoiding the majority of debilitating symptoms that they would otherwise face.

Keywords Gluten, gluten degradation, gliadin, glutenin, celiac disease

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Watch a video introduction by the authors at https://youtu.be/JU7HrGKodeg

Background

There are four conditions associated with negative reactions caused by gluten consumption, that affect 5-10% of the global population: celiac disease, wheat allergies, gluten sensitivity, and gluten intolerance (Brazier 2020). In this project, we used *Escherichia coli* as a microbial host to produce the PEP 2RA3 microbial enzyme (Figure 1), which breaks down gliadins, one of two main protein groups in gluten (Amador, Arévalo-Rodríguez, Durán, et al. 2019). The *E. coli* will be used in a pill which helps individuals with gluten sensitivities safely ingest foods containing gluten. Even if small particles of gluten remain after degradation, this idea may be beneficial because it allows people to

enjoy gluten-containing foods, without the consequences they would otherwise face.

We created a basic DNA construct (See Figure 2), consisting of a promoter (start) sequence, a ribosome binding site (RBS), the enzyme coding sequence (CDS) for the PEP 2RA3, and a T7 terminator sequence (Figure 3). The terminator and promoter were both chosen from the iGEM parts registry (International Genetically Engineered Machine 2020). To convert the construct into a plasmid (Figure 1), we modified the ends of the restriction sites and put them together with ligase. To produce the enzyme, we would transform this plasmid into *E. coli*, and place the *E. coli* inside a pill to be taken orally. However, the enzyme needs to be purified before it is placed in the pill for consumption, and tested in conditions that mimic the human intestine. For this design, IPTG would not need to be present in order to activate transcription of the lac operon and express the enzyme as we are using an RBS sequence from the Anderson promoter library (Anderson 2006). We also need to test how the body will react to this enzyme, and how long it will take for it to destroy the protein for not having human similarity and glycosylation pattern. One way to test our design would be to see if *E. coli* can efficiently secrete the enzyme in the proper conditions and whether the enzyme degrades gluten in the typical range of pH and temperature of the human body, which might be measured with petri plates containing gliadins. We could also purify the enzyme from *E. coli* without secretion, and test the efficiency in-vitro without *E. coli*, just the enzyme.

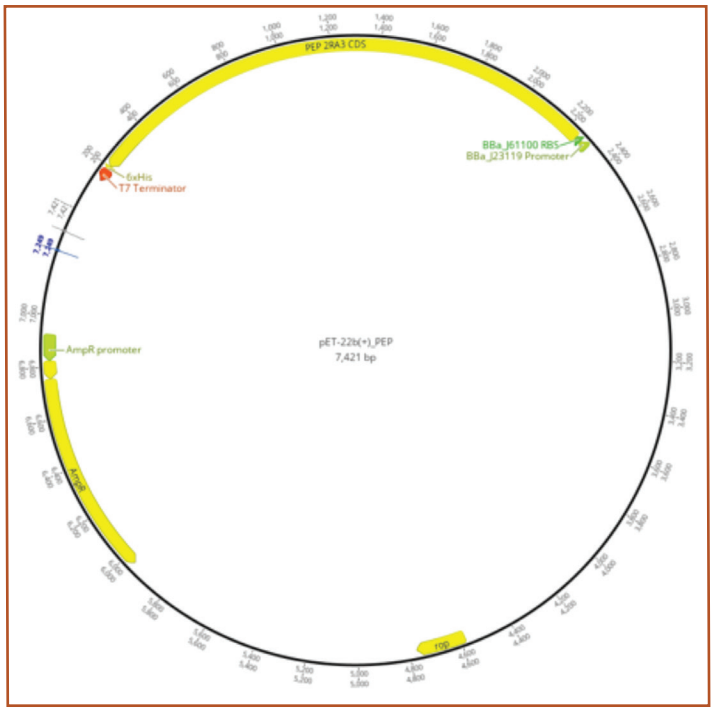

Figure 1. *A basic plasmid containing the enzyme, promoter, RBS, and terminator. Parts may be added as needed to purify the enzyme (International Genetically Engineered Machine 2020).*

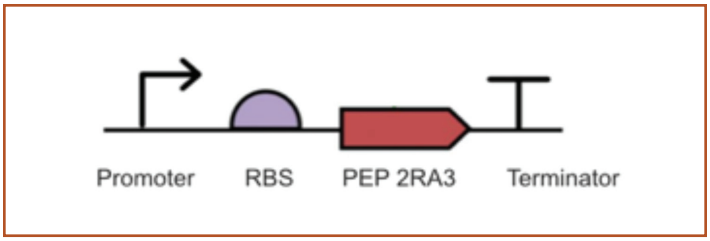

Figure 2. *A simple construct for the DNA. It consists of a promoter region, the ribosome binding sequence, the PEP 2RA3 enzyme, and a T7 terminator (International Genetically Engineered Machine 2020).*

Systems level

The PEP 2RA3 enzyme is purified and expressed in *E. coli*. The *E. coli* is placed in a pill to be ingested, upon which the enzymes break down gliadin proteins in the gut. The gluten content after degradation needs to be below a specific amount (about 10-50 mg, consumed per day) in order for the body's reaction to be reduced or relieved.

Device level

The key components of this design include the engineered *E. Coli*, the PEP 2RA3 enzyme, which will degrade gliadin proteins, and the pill which will transport the modified enzyme into the intestine to act upon the consumed gluten. It is uncertain how long it will take the body to destroy the ingested protein, as it does not have the human similarity or glycosylation pattern, and this needs to be tested- however, it is a reasonable conjecture to say that it should be enough time for much of the gluten in the system to degrade thanks to the embedded PEP 2RA3 enzyme. In order to ensure high gene expression of the enzyme and thus a greater amount of gluten degraded, we chose a strong constitutive promoter from the Anderson promoter library, located in the iGEM repository, as well as an RBS sequence from the Anderson RBS library to make sure translation is initiated properly.

Parts level

The plasmid containing the enzyme and necessary parts such as the promoter, RBS and terminator (International Genetically Engineered Machine 2020) will be put together in a pill and ingested. Upon arrival to the intestine, the enzyme will be activated and begin

Figure 3. *The sequence of DNA with a promoter, RBS, terminator, and the full PEP 2RA3 enzyme sequence (International Genetically Engineered Machine 2020).*

to digest gluten found in the gut (Amador, Arévalo-Rodríguez, Durán, et al. 2019).

Safety

This is a design brief, so we are not yet certain of the specific methods used to test this design. However, we are certain that testing is a crucial aspect of this idea and many clinical trials will need to be undergone in order to make sure that this pill is safe for human consumption.

Discussions

Additional testing will be needed to perceive the effectiveness of this enzyme in degrading gluten, and to be absolutely certain that this pill is ready and safe for consumption by humans.

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