

iGEM IGEM BS_United_China

Abstract:

During community communications, we often heard the fame of *Staphylococcus aureus*, a well-known pathogenic bacterium, which draws our great attention and interest. Our main goal here is to use protein proximity labeling to make *S. aureus* harmless within vivo. Through fused expression of TurboD and the interest protein inside the cells, TurboD catalyzes biotin and ATP it carried to produce Biotinyl-S-AMP, which is used for protein labeling. Such process can be accomplished within ten minutes in 25°C, which appears to be the most efficient and reachable method beyond us.

Because such kind of protein needs to be blocking modified, more ATP and biotin are required during the experiment, which is the reason why we choose to use engineered *E. coli* to produce what we need. Since there is already more than enough ATP in an organism, we only need to increase the production of biotin. In this stage, we will put the artificially modified plasmid back into *E. coli* to express the target product so as to increase the biotin synthase and increase the biotin production.

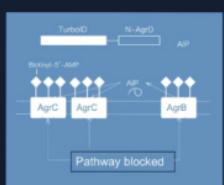
Background:

Saving food is considered a virtue for us Chinese who created our civilization out of agriculture. Often seen on the desks many Chinese families, unfinished foods will be persevered and return for the next meal after a night for few days. For the safety of these foods, the people who are more willing to risk their health in confronting the pathogenic bacterium in these foods. Moreover, the dispute of whether these foods should be consumed the next day is often seen in many families between the young and old. After conversing with communities, we noticed the *Staphylococcus aureus* that is omnipresent. They release virulence by quorum sensing and became one of the most popular pathogenic bacteria in food safety. Therefore, we decided to design a bio product to inhibit the virulence of *S. aureus*.



Technique:

Our main goal here is to use protein proximity labeling to make *S. aureus* harmless within vivo. We are planning to achieve this by using protein proximity labeling to block quorum sensing of bacteria hoping to kill off some *S. aureus* bacteria.



We are so fortunate that we could take part in the unbelievably dramatic science researches as senior high school students; furthermore, we believe that our findings could bring exiguity changes to the whole world.

As you can see in this slide, this is a natural process of an autoinducing peptide AIP production. As AIP enters the bacteria, it activates AgrB, which will then activate RNAPII and RNAPIII to produce P_{AIP}. This will result in two different paths. One is it will activate endonuclease genes to produce cytosine. One will begin the production of agrC. This then combine with AgrD to form this chained block which will then be cut by AgrB. After that the AIP could be sent out to begin a new cycle. What we plan to do is. We will first link AgrC and TurboD with AIP which will direct TurboD toward AgrC, while N-AgR-D will direct TurboD toward AgrB.



When AIP reacts with AgrC and N-AgR-D reacts with AgrB, TurboD catalyzes biotin and ATP it carried to produce Biotinyl-S-AMP, which is used for protein labeling. When a certain amount of Biotinyl-S-AMP is attached on AgrC and AgrB, the connection between the cell and the outside world will be completely cut off, the metabolic waste cannot be transported out of the cell, and the nutrients cannot enter the cell, which interferes with the biochemical reaction. Thus, achieving the goal of killing the bacteria. In the next level, we are hoping to use streptavidin combining with Florence discover the efficacy of our product or it can be used to show the intensity of proliferation of *S. aureus*. Another way we can increase the effect of TurboD by attaching a anti bacterial drug. At last our product could be used to inhibit different type of bacteria not just limiting to *S. aureus*.



Synthesis Biotin:

As for our basic materials, biotin and ATP. We thought we could use engineered *E. coli* to produce what we need. Since there are already more than enough ATP in an organism, we only need to increase the production of biotin. We have two ways to achieve this. One is by increasing the production of biotin in one specific metabolic pathway, and the other is that we can block the other channel. However, blocking other channels will lead to a decrease in the growth rate and productivity of the bacteria, so we chose to increase the amount of the catalyst, which in turn produces more biotin. We will be increasing the catalyst of these two catalyst as shown. In this stage, we will put the artificially modified plasmid back into *E. coli* to express the target protein so as to increase the biotin amount and increase the biotin production. After that, we will use D-easpr 9 technique to block the pathway after the biotin has formed to prevent it from continuing disintegration.



Human practices:

For the past few month, we tried to search for useful information by human practices. We designed our survey based on three perspectives: public consciousness of food safety, the level of knowledge about biotechnology used by the public, and the level with which the public can accept bio product. After sending out the survey and receiving data, we found out that the public does not have strong sense of knowledge in food safety and contamination. Most people believes that as long as the food does not change color, taste or smell, it would not be harmful to the human body. Because of this lack of knowledge, some foods that needs to be recycled or sanitized fails to the public's acceptance. Although continuing to increases of food infection and people health. This problem was also mentioned by the doctor we interviewed. Each year, a large number of people suffer from consuming foods that are contaminated resulting in cases of stomach food, foodborne illnesses or even deaths. Food contamination has been a popular illness that influence many people especially elders and children. From these areas, we determined our value and direction. The acceptance of the public and medical personnel has offered us great confidence in continuing working on our project.



Cooperation:

We have also contacted other teams such as a team from the University of Jilin, and we are working to facilitate the process of their Pbr detection. Furthermore, we made connection with a team, Denovocastrians from Australia and will be discussing the further details about bacterial inhibition and engineer the metabolic pathway of *E. coli*.