**RICE GUARDIAN:**

*Xanthomonas Sensing Bacteria*

**Biological Binary Full Adder**

Bacterial leaf blight disease (BLD) is one of the prominent vascular diseases in irrigated rice. BLD in rice is caused by infection of bacteria known as *Xanthomonas oryzae*. It has been proven that bacterial RNA-genes (a-t, b, c, d, e, g, h, p, r, r) are responsible for BLD. Since Ax21 is a quorum-sensing molecule that signifies presence of *X. oryzae*, we decided to make a synthetic bacterial system that detects Ax21 and furthermore kills those pathogens. First, we designed Ax21 detecting E. coli by cloning rax21 and rax motifs which produce receptor of Ax21. When the receptor is activated by Ax21, signal is transmitted to rax promoter, and induces transcription of downstream RFP genes. Constructed synthetic circuit was tested by co-culturing with Ax21 producing *X. oryzae* genes which produce receptor of Ax21. When the receptor is activated by Ax21, signal is transmitted to rax promoter, and induces transcription of downstream RFP genes. Constructed synthetic circuit was tested by co-culturing with Ax21 producing E. coli. As a result, our Ax21 detecting E. coli showed more increased fluorescence than control in the presence of Ax21. We suggest that detecting quorum sensing of pathogen can potentially be applied to protecting plants from pathogenic diseases.

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**DESIGN & PARTS**

- **A. Ax21 Displaying Bacteria**
  - *pAT* is a vector to display any heterologous protein on cell surface of E. coli (ref. 1). pAT plasmid is activated by arabinose. By cloning ax21 gene into *pAT* vector, Ax21 is displayed on the membrane surface of E. coli in the presence of arabinose.

- **B. Ax21 Detecting Bacteria**
  - In this system, raxH and H which detect Ax21 protein are constitutively expressed under control of promoter BBA2_J23100. If there is no Ax21 protein outside Rice Guardian, basal level of mRFP is expressed.

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**RESULTS**

- **A. Ax21 display on the membrane of E. Coli**
  - Lanes:
    - M: Molecular weight standards;
    - 1 and 2: E. coli transformed with *pAT* empty vector;
    - 3 and 4: E. coli transformed with pAT-ax21 5 and 6: E. coli transformed with pAT-ax21 + induction* 1,3 and 5: soluble part 2, 4 and 6: insoluble part. *5% arabinose

- **B. Co-culture of Rice guardian with Ax21 displaying E. Coli**
  - Rice Guardian project was to build an engineered E. coli which detects *X. oryzae*. We made Ax21 producing E. coli to mimic *X. oryzae* secreting Ax21 protein. In order to find out whether Rice Guardian detects Ax21 and produces mRFP, co-culturing two cell types (Rice Guardian and Ax21 producing E. coli) was conducted. After cell density reached up to OD600 1.0, fluorescence level was measured (right).

The data show that when Ax21 gene is absent or gene expression is not induced, no promoter in rice guardian expresses only basal level of mRFP (2nd, 3rd, and 4th bar). Only when E. coli carrying Ax21 surface display system expresses Ax21, mRFP level in Rice Guardian cells doubles (5th bar). In these result is consistent with natural rax system in *X. oryzae*

- **C. Growth rate of individual cell kind**
  - Though initially inoculated at 1:1 ratio, there are possibilities that the growth rate of E. coli cells carrying different plasmids might be different. Also, L-arabinose, which works as an inducer for Ax21 protein expression, may affect the growth rate of each cell kind. We cultured each cell independently with and without arabinose inducement, and made each cell’s growth curve. It was proven that the growth rate of each cell kind was similar. So we confirmed that co-culture experiment is valid.

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**CONCLUSION**

Although interaction between Ax21 and two-component regulatory system by RaxH is not revealed completely, we found our synthetic genetic circuit properly functions to detect Ax21 outside cell membrane. Even though Rice Guardian we built can only determine the existence of *X. oryzae* at current state, we are planning to upgrade our design to both detect and kill *X. oryzae*, which eventually matches the title of our project. This method is eco-friendly and it will eventually increase crop productivity. Also in the long run, it can protect endangered plant species.

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**OUTREACH**

A. Participation in Creative Challenger Program (C*CPP*)
- Informing GEMS to Students
- Presentation Content
- British Columbia University Survey
- Highschool Observer

*C*CPP*- Korea University program that is dedicated to promote creativity of undergraduates by letting them form groups and engage in self-designed projects.

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**LOGIC GATE:**

**Biological Full Adder**

Logic circuit is an operation system consisting of sets of various logic gates such as AND, OR, XOR, and NOR, that interact with each other to draw certain outputs from input signals. We built a biological binary full adder by using those gates. C4 Acyl homoserine lactones (HSLs) were used as common signaling molecules, and 3OC6-OH (C6 HSL), 3OC12-OH (C12 HSL), and products of hflS and 5 genes were used as input signals. Our team used several bioinks and semi-permeable membranes to complete the circuit. Due to laboratory conditions and time limit, we were unable to do actual wet lab experiments. Thus, we made a mathematical model using Hill equation to predict the results. This in silico prediction properly reflected theoretical logic circuits in the microbial behaviors and showed a good result.

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**CONCLUSION**

In this research, we designed microbial logic gates and connected them to assemble binary full adder. We used AHLs as signaling molecules. However, quorum sensing experiment requires sophisticated conditions, so we designed the mathematical model using Hill equation which describes transcriptional regulation of specific gene. We plotted the result of simulation and it successfully reflected theoretical binary full adder. Based on the model, we are planning to perform this experiment for the further study.

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**REFERENCES**

- Nicola Radzulikut 2010 Froc of the AdvkIII Conference