Abstract
To solve bees from Nosema ceranae infection, the culprit of colony collapse disorder (CCD), we have created the so-called from Escherichia coli MG1655, which naturally resides in bees. The Bee. coli is designed to work successively as follows:

1. Bee. coli continuously secretes mannosidase to inhibit the sprouting of N. ceranae spores.
2. If the bee is infected with N. ceranae, the fungus-killing circuit with a positive feedback design will be turned on to wake up N. ceranae.
3. If these defense weapons should fail to conquer N. ceranae, a bee-suicide-oopron will be activated to kill the infected bees but save their companions.

Besides, a light-inducible lysis system is created to ensure our Bee. coli only lives inside of the bee. In addition, we have used encapsulation as the method to send our Bee. coli into the bee. Since the capsule will only dissolve in a bee’s gut, our Bee. coli will not wantonly spread out.

Prohibiting Sprouting
To prohibit sprouting, we selected to use an enzyme, Mannosidase, to degrade the Mannose polymer, which composes the polar filament of N. ceranae spores. By simply cloned mannosidase coding sequence after a constitutive promoter in our Bee. coli to produce mannosidase, we can prohibit spores sprouting by interfering the polar filament assembly.

To test the function of mannosidase, we first conducted N. ceranae germination assay.

Sensing Infection and Killing N. ceranae
Taking advantage of immune reaction of bees, we can make our Bee. coli to sense N. ceranae.

Once the midgut epithelial cells are infected by Nosema, the cells will secrete reactive oxygen species (ROS). ROS functions as signalling molecules which can activate the transcription factors. OxyR and SoxR and transform them to their activated form.

Therefore we decided to use the ROS-induced promoters which are regulated by OxyR and SoxR. Based on literature, we found out four promoters which can be induced by ROS. They are Ikx3, IrhA, SoxA, and AhsCp.

The part AhsCp we used was improved by ourself from 2010 KIT-Tokyo GEM team.

Based on literature, antimicrobial peptide will significantly raise the survival rate of bees. We select defensin and abamecin as weapons for our Bee. coli to kill Nosema because they are peptides naturally produced by bees and the mechanism of them are well-studied. Additionally, their coding sequences are shorter and thus indicated to be more efficient.

Circuit Regulation
To regulate the production of the antimicrobial peptides, we designed a fungus-killing circuit with a positive feedback.

Sacrificing the Bees
Since N. ceranae can be consumed when other bees sipped the nectar digested by the infected bees, killing the infected bees will be the final choice to stop the infection. We chose ethylene to kill the bees. Ethanol is known as a kind of toxins of bees because of the toxicity to the nervous system. Besides, it could have influence on hydrostatical pressure, which is one of the essential factors for the germination of N. ceranae.

Safety
Our Bee. coli can express antimicrobial peptides to fight against N. ceranae. However, it is also possible that our Bee. coli can contaminate the natural environment and cause death to other species. Therefore, a light-induced lysis system was created to ensure our Bee. coli only lives inside of the bee.

We chose K592016 as our light sensing device. K592016 consists two parts: Y11 and Fsu. Y11 is a blue-light sensor protein. It works in conjunction with its response regulator, Fsu. When exposed to blue-light, they can activate K592016, the blue-light sensing promoter. The lysis device is composed of promoter K592066, the blue-light sensing promoter, and the lysis protein K592069, which is a lethal 51 amino acid membrane protein that induces lysis.

Product
The main purpose in this part is to send Bee. coli into bees and make sure it can survive in the midgut. Therefore, we chose Escherichia coli MG1655 as the chassis of our circuit, then incorporating MG1655 into alginate microcapsules, and led bees with sugar water containing microcapsule beads. According to our experiment, it could successfully transport the engineered Bee. coli into the bees’ midgut.

The alginate capsule can protect Bee. coli from the immune system of bees and ensure that Bee. coli will not spread out.

The result of transporting

The bee-saving powder

Diagnosis of Nosemiasis

Collaboration with NTU_Taida

Characterizing of their parts

Healthy bees
Nosemias

Healthy bees vs. infected guts
Nosemias vs. infected guts

Reference

SIDS PAGE result of defense expression

Outreach