**iGEM Bee Gut Microbiome**

**Team:** IIT-Chicago

**Summary**

Bees are the most important pollinators. They transfer pollen among crops, facilitating seed formation and fruit production. The economic value of pollinators, including honeybees, in global crop production is estimated between **$235 billion to $577 billion annually**.

As the variety and area of subtropical crops reliant on pollinators continue to grow, the role of pollinators becomes increasingly critical. Honeybees significantly impact **11 out of the 17 United Nations Sustainable Development Goals (SDGs)**.

**Values of Bees**

A troubling phenomenon has been observed in recent years: the **mass disappearance of honeybees**.

* **Winter 2021:** 7.8 billion honeybees disappeared due to **Colony Collapse Disorder (CCD)**.
* **Sept–Nov 2022:** 10 billion more bees vanished.
* **Early 2023:** An estimated 14 billion additional bees disappeared.

**Causes include:**

* Climate change
* Parasitic mites
* Loss of habitat
* Agricultural chemical use

This trend poses a severe threat to the beekeeping industry and global food security.

**Colony Collapse Disorder (CCD)**

CCD is driven by several major stressors:

* **Pesticides and Biocides**
  + Acute and chronic toxicity from herbicides and insecticides.
* **Loss of Genetic Diversity**
  + Resulting from prolonged artificial breeding.
* **Malnutrition**
  + Caused by environmental changes and reliance on monoculture crops.
* **Pests and Diseases**
  + Includes *Varroa destructor* mites, *Tropilaelaps* mites, Asian hornets, and viral infections.

**Pesticides**

Even post-DDT, harmful pesticides remain in use. Of particular concern are **organophosphate pesticides** (OPs):

* **Composition:** Phosphorus combined with oxygen or sulfur.
* **Mechanism:** Inhibit acetylcholine breakdown → disrupt nerve function → cause paralysis and death.
* **Toxicity:** Highly toxic to humans, livestock, and especially bees.

**Why Target Organophosphates?**

* **Still Present Globally:**
  + Despite bans, OPs are widely used.
  + Residues are found in soil, pollen, wax, and nectar.
  + Bees are frequently exposed during foraging.
* **Toxicity to Bees:**
  + Extremely low LD₅₀ values (e.g., **chlorpyrifos: 70 ng/bee**).
  + Rapid paralysis and death upon exposure.
  + Immediate threat to colony survival.
* **Genetic Tools Available:**
  + OP-degrading genes like **opd**, **opdA**, and **mpd** have been functionally characterized.
  + These genes are known to work well in microbial expression systems.
  + Enzyme pathways are well-understood and simplified.

**Our Proposed Solution**

**Goal:**  
Engineer *Snodgrassella alvi*, a key gut microbe in honeybees, by inserting a gene that degrades organophosphate pesticides.

**Expected Outcome:**  
Pesticide-resistant bees, reducing the impact of CCD and supporting honeybee survival.

**Method: Genetic Engineering**

**Golden Gate Assembly + BTK Toolkit:**

* BTK (Bee ToolKit) is designed for *S. alvi* genetic modification.
* Utilizes **Type II restriction enzymes** for modular genetic assembly.
* Components are categorized:
  + Type 1: Connectors
  + Type 2: Promoters, ribosome binding sites
  + Type 3: Coding sequences (CDS)

**Method: Genome Integration**

Since plasmids may not remain stable in the bee gut, the gene will be integrated **directly into the *S. alvi* genome** via **homologous recombination**.

* Employ **RecA** and other recombination proteins.
* Requires plasmids to contain homologous arms flanking the target gene.
* BTK toolkit supports design and insertion of these homologous arms.

**Simplified Vector Design**

Vector components:

* **opd** – Organophosphate degradation gene
* **Promoter** – Drives gene expression
* **Terminator** – Ends transcription
* **Selector** – Selectable marker
* **H1 and H2 arms** – Homologous recombination arms
* **Backbone** – Plasmid structure

**Major Project Milestones**

1. Clone OPH gene into a homologous recombination vector.
2. Transfect into *S. alvi* using electroporation.
3. Screen recombinants using a selectable marker.
4. Confirm OPH gene activity in *S. alvi*.
5. Verify that engineered *S. alvi* can colonize the bee gut.
6. Demonstrate OPH activity in vivo (bee gut).
7. Evaluate pesticide impact on bees with vs. without OPH-*S. alvi*.