Malaria is a tropical disease that kills more than 1 million people each year and no effective cure or vaccine exists yet. The EPFL IGEM project aims to stop malaria propagation by acting on the vector: the mosquito. Asaia is a pink bacterium that naturally lives in the mosquito’s gut. We engineered it to express an immunotoxin that can prevent the malaria agent Plasmodium falciparum from infecting the mosquito, thereby eliminating the transmission of this parasite to humans. This would provide synthetic biology with a useful tool in the fight against malaria and other mosquito borne diseases.

**EPFL TEAM**

**MALARIA**
- > 1 million deaths
- no effective cure or vaccine
- transmitted to humans through mosquitoes

**HOW TO TARGET THE PARASITE ?**

Two different ways:
- An immunotoxin:
  - fusion protein of antibody fragment and porin.
  - specifically target and lyse the parasite.
- The p-proteins P25 and P28:
  - P. falciparum surface proteins.
  - need interaction with other proteins for plasmodium development.
  - competition between P25/28 and recombinant soluble proteins.

**ASIA : NEW CHASSIS**

Asaia is a gram-negative bacterium that naturally lives in the mosquito’s intestinal tract.

- easy to grow and genetically modify.
- high rate of transmission between mosquitoes, both horizontally (from one mosquito to another) and vertically (from parent to offspring).
- 100% prevalence in mosquitoes

These could assure the spread of the transformed agent through a host population.

**CHARACTERIZATION**

- Doubling time of 2 hours
- Optimal pH of 5
- Growth temperature of 30°C
- Origin of replication compatible with E.coli
- Antibiotics resistance:
  -kan
- :tetracycline
- :aminoglycosides
- :streptomycin
- :penicillin
- :neomycin
- :chloramphenicol

**HOSTS AND SAFETY**

We conducted different experiments with Drosophila Melanogaster to study its possible use as a model organism for Asaia.

- Asaia is not lethal for Drosophila.
- Asaia is not persistent in Drosophila.

These results confirm other studies with Asaia led us to think that this bacterium is really specific to mosquitoes. This would be helpful in case we want to release the modified organism in nature.

**REFERENCES**

- Favia et al., PNAS, 2007
- Yoshida et al., Mol. Micro and Biochemical Parasitology, 2001
- Tomas et al., EMBO, 2001

**CONCLUSION**

- Established Asaia as a new chassis by providing detailed tech-sheets
- Constructed biobricks that produce proteins against malaria infection and also Asaia-specific biobricks containing the Asaia origin.
- Expressed the immunotoxin in E.coli

**NEXT STEPS**

- Expression of the immunotoxin in Asaia in vitro and in Anopheles.
- Evaluate in vivo the immunotoxin’s efficiency.
- Plasmid insertion (without selective marker) inside the genomic DNA of Asaia.
- Modeling the transmission of the engineered bacteria within the mosquitoes’ population.
- Spreading of Asaia either by feeding or by transfecting the mosquitoes.