Pine Beetle Epidemic

Over 17.5 million hectares of pine trees in British Columbia, Canada have been killed by Mountain Pine Beetles (MPB) with serious socioeconomic and environmental consequences such as:
1. The loss of forestry jobs in small communities
2. Reduction of trade and economic revenue
3. Depletion of the pine forest carbon sink
4. Increased carbon emissions from dead trees

This epidemic has spread to American states such as California, Arizona, Montana and Colorado, even reaching the pine forests in Mexico.

Terpene Factory

In the environment

released into the environment
development of synthetic endo-
geneous mevalonate pathway essential to terpene production.

The Blue Stain Fungus lives symbiotically with the MPB attacks. The Blue Stain Fungus is a well-studied model organism that possesses an endogenous defenses.

4. Increased carbon emissions from dead trees
3. Depletion of the pine forest carbon sink
2. Reduction of trade and economic revenue
1. The loss of forestry jobs in small communities

We used our synthetic yeast to inhibit blue stain fungus growth.

Co-culture of S. cervesiae and G. clavigera:

By co-culturing the spores of the blue stain fungus with yeast spores, we found mutual inhibition. This further supports the potential use of terpene-producing yeast as a biological agent to slow fungal growth. We also co-cultured yeast with blue stain fungus mycelium and found mutual inhibition, as well as stress signs (pigmentation) in the blue stain fungus.

Control Plates: a) 200 G. clavigera spores plated on 530 S. cervesiae spores plated on 1% Malt extract agar (OMEA) for 4 days. b) Reduced numbers of spores of both G. clavigera and S. cervesiae 24h. 100 G. clavigera & 330 S. cervesiae spores plated on 1% OMEA for 4 days. d) G. clavigera mycelium growing from the centre and outwards towards the diterpenoid LAS-producing S. cervesiae (see also GC-MS data above) on 1% OMEA.

Metabolic Model

Objective: To model monoterpane output by an engineered mevalonate pathway in S. cervesiae.

Monoterpane Data: We purified alpha-pinene, beta-pinene and limonene synthases from E. coli and incubated with GPP substrate. Products were analysed by GC-MS, confirming that the expected monoterpenes were produced for each of the synthases.

Diterpene Data: We expressed levopimaradiene/abietadiene synthase (LAS) in yeast. The resultant diterpene products were analysed by GC-MS. This in vivo experiment confirms that yeast can produce terpenes.

Epidemic Model

Objectives:
1) To simulate the spread of MPB in British Columbia from the years 2011 to 2020
2) Derive a strategy for containment of the MPB outbreak by implementation of trap boxes at sub-population centres

Release Strategy

The aim of our project is to over-produce monoterpenes known to confer resistance in trees to beetles. We chose Saccharomyces cervesiae, a well-studied model organism that possesses an endogenous mevalonate pathway essential to terpene production. Our strategy incorporates monoterpenes synthases and variants of metabolic enzymes in the mevalonate pathway to boost production. This serves as a proof-of-concept for future development of synthetic organisms that can be feasibly released into the environment to tackle the MPB epidemic.

Beetle Vector Experiment

Our tests to see if the MPB can act as a vector for genetically modified yeast clearly showed that these beetles are able to transport yeast from one place to another.

Furthermore, our yeast can survive on the MPB’s exoskeleton (containing a host of microflora such as fungi, yeast and bacteria) for at least 36 hours in an sterile empty plate prior to transfer to the next media plate.

Monoterpane Production In Yeast

This is the simplified version of the mevalonate pathway with targeted metabolic genes highlighted in red. The erg20-2 and K6R-hmg2 are variants of endogenous enzymes.

Developed using Simbiology MatLab, our model predicts that introduction of targeted metabolic genes results in a roughly two fold increase in monoterpane production when all [enzymes] are the same. However, sensitivity analysis showed that tripling [K6R-hmg2] increased [GPP] by 8.7 times and increased beta-pinene production by 7.3 fold. Similarly, tripling [ID1] increased beta-piene production by 1.3 fold.

Synthetic Biology in the Wild

We interviewed experts in various fields to garner attitudes toward releasing synthetic organisms in the wild. We discussed the standards of release, the challenge of public acceptance and what future directions synthetic biology may have. Their answers have shaped how we thought about safety and informed our synthetic biology strategy against the beetle epidemic.

“There must be clear unequivocal benefits to the public with no alternative solutions.” — Dr. Andrew Riseman

Achievements

Submitted and characterized 4 new standard biobrick parts:
- BBa_K517003
- Functionally characterized the 4 parts above using GC-MS and FACS.
- Functionally characterized BBa_K118025 Limonene Generator in the Registry
- Characterized BBa_J63006 GAL1 promoter and found it to be non-functional. We submitted the characterized BBa_K517003 GAL1 promoter.
- Outlined and detailed new human practices approaches: (i) Guide for How to Start a iGEM High School team; (ii) Gathered public perceptions on synthetic biology; (iii) Interviewed experts concerning “Synthetic Biology in the Wild”

Co-culture experiments

Our model was developed using ArcGIS, Python and R. By predicting the impact of our trap box strategy, this model can inform future conservation policies.

3D Synthase Structures

Using homology-based modeling, we predicted the 3D structures of alpha-pinene, beta-pinene, and limonene synthases. These structures allowed us to identify amino acid residues that are targets for mutagenesis to optimize efficiency of terpene synthesis.