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Introduction

- Team: 7 students from Valencia
- Worktime

**PROBLEM**

Diseases transmitted by insects like malaria or sleeping sickness

**SOLUTION**

Creating a yeast capable of repelling these insects
Aim

Create a biological platform within common yeast to develop a device capable of producing several monoterpenoids acting like aromas and repellents.

- **AROMA 1**
  linalool synthase from *Clarkia breweri* (Dudareva et al., 1996) - M. Orejas

- **AROMA 2**
  geraniol synthase from *Ocimum basilicum*

- **REPELLENT**
  1,8-cineole synthase from *Arabidopsis thaliana* (Demissie et al., 2012)
Inducible promoter Cup1: linalool synthase

Inducible promoter H2O2: geraniol synthase
S-linalool synthase

- From *Clarkia breweri*
- GPP $\rightarrow$ S-linalool
- 2760 bp
- mRNA linear

- Restriction enzymes incompatibilities: EcoRI, XbaI, BglII, Xhol.
Materials and methods

WET LAB

- Raw material: LIS in ER85
- Amplification using PCR (TOPO vector to increase the concentration)
- Digestion of LIS by BamHI and Sall
- Ligation into pYEX-4T
- Transformation into S. cerevisiae ERG20 K197G

DRY LAB

- Genome-scale metabolic models to estimate the behavior of the organism.
- Organism metabolism modeled by a network of metabolites and enzymes that must integrate all biochemical reactions present in the organism.

- Flux Balance Analysis (FBA)
Results

Starting from the initial objectives, good results were not achieved in geraniol synthase and 1,8-cineole synthase. They could not be cloned in their respective plasmids neither expressed in yeast. In the first case, the cDNA could not be obtained; in the second one, the gene could not be amplified from the A. thaliana cDNA.

LIS was transformed into S. cerevisiae. No experiments were performed due to time limitation.
• GPP was excreted into the medium.
• Simulation in steady-state conditions → accumulation
• Decreasing yeast growth → excess of GPP
• Point of optimal growth → production value of GPP is zero (all is used to grow)
• The points near to the optimum have higher accuracy, since they are closer to the point validated experimentally

• Production of GPP by ERG20 higher than in the classical strain
• If the yeast was modified to diminish its growth → more GPP for our reaction
Conclusions

• *S. cerevisiae* ERG20 K197G is a good biologic device

• Extra experiments could have been performed:
  - Optimal concentration of copper for the expression
  - Time needed after the addition of copper to observe expression of the gene
  - Measuring how the expression varies depending on the concentration of copper
Human practices and others

- Survey about the impact of our project on society

Did you know that Saccharomyces is frequently used in research in the field of biology and health sciences?

General opinions about transgenic organisms.

Opinion about modified organisms.
• Lipdub to promote science
• Lab practices with teenagers


