Engineering bacteria to help fight soil erosion
The problem: Desertification

31,000 hectares of arable land are degraded every day
1.3 x the area of Boston lost per day!
Our solution

*Lateral root growth enhances soil stability*
Engineering bacteria to move towards roots
Engineering bacteria to produce auxin
Human Practices: Informing design

Gene Guard

Chassis Choice

Royal College of Art
Postgraduate Art and Design

BIOS

syngenta

GREENPEACE

incotec
involved in seeds
“Horizontal gene transfer happens, and at high frequencies; it is the greatest, most underestimated hazard from GMOs released into the environment” (Dr. Mae-Wan Ho)
Human Practices: Informing design

Gene Guard: Containing our genetic constructs

GMO

Gene transfer

Soil bacterium

Gene Guard

Chassis Choice
Human Practices: Informing design

Chassis choice

Gene Guard

Chassis Choice

B. subtilis  OR  E. coli

We chose E. coli
Gene Guard

Chassis Choice

Human Practices: Informing design

Chassis choice

E. coli
Human Practices: Informing design

*E. coli* K12 survive in soil for more than 7 weeks
Module 1: Phyto-Route

Rewiring chemotaxis

Movement towards roots
PA2652, malate-responsive chemoreceptor

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 1: Phyto-Route

Optimal malate receptor expression level?

Sensitivity and saturation analysis for malate receptor

\[
\frac{dT_2}{dt} = \frac{-Lk_5T_2 + k_{-5}LT_2}{Ligand \ binding/release} - \frac{k_8T_2}{Phosphorylation} + \frac{B_p k_{-1}T_3}{Demethylation} \\
+ \frac{k_y T_{2p}(Y_0 - Y_p) + k_b T_{2p}(B_0 - B_p)}{Phosphotransfer} - \frac{V_{max}}{K_R + T_2} \frac{T_2}{methylation}
\]

\[
\frac{dY_p}{dt} = k_y P(Y_0 - Y_p) - k_{-y} ZY_p
\]

\[
\frac{dB_p}{dt} = k_b P(B_0 - B_p) - k_{-b} B_p
\]

Concentration of chemoreceptors can be variable
Module 1: Phyto-Route

Phyto-Route construct

- BBa_J23100
- PA2652
- BBa_K515102
Module 1: Phyto-Route

Behaviour of bacteria upon chemoattractant saturation

- PA2652 cells in 10 mM malate
- PA2652 cells in 10 mM serine
- Negative control (cells without construct) in 10 mM malate

E. coli senses malate
Module 1: Phyto-Route

Specifications & Design
Modelling
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Status

Capillary assay

Seal

Capillary with chemoattractant inside

Well with bacterial suspension
Module 1: Phyto-Route

Optimum experiment duration

3D Simulation

% population in capillary

Time (s)

60 mins
Chemotaxis dependence on malate concentration

The greatest response was observed at 1mM of chemoattractant.

E. coli chemotaxes towards malate
Module 1: Phyto-Route

Specifications & Design

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Status

Bacteria in the root
Module 1: Phyto-Route

Status so far

Bacteria chemotax towards root exudate
Module 1: Phyto-Route

Status so far

- Bacteria chemotax towards root exudate
- Bacteria are uptaken by the root
Module 2: Auxin Xpress

Release of auxin

A simple IAA producing pathway \(\rightarrow\) IAM pathway

- **Trp** \(\rightarrow\) **IAM** \(\rightarrow\) **IAA**
- **IaaM** \(\rightarrow\) **IaaH**
- **BBa_K515010** \(\rightarrow\) **IaaM** \(\rightarrow\) **IaaH**
- **BBa_K515100**
Module 2: Auxin Xpress

How much bacterial auxin can we produce?

Intracellular IAA concentration of 72 µM
Module 2: Auxin Xpress

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 2: Auxin Xpress

What is the effect of the IAA on the root?

The optimum is 0.1 nM
Simulated *Arabidopsis* root growth after 25 days

- \([\text{IAA}] = 10^{-6} \text{ mol/L}\)
- \([\text{IAA}] = 10^{-10} \text{ mol/L}\)
- \([\text{IAA}] = 10^{-14} \text{ mol/L}\)

**Parameters:** Branching, root growth rate, gravity, twisting
Module 2: Auxin Xpress

Specifications & Design

Modelling

Assembly

Testing & Results

Status

Auxin Xpress construct

BBa_K515010 → laaM → laaH → BBa_K515100
Module 2: Auxin Xpress

Salkowski assay standards

Colourimetric auxin detection
Module 2: Auxin Xpress

Successful auxin production

Auxin production levels determined by Salkowski assay

Extracellular auxin yield of 52 µM
Module 2: Auxin Xpress

IAA peak confirmed by Liquid Chromatography Mass Spectrometry (LCMS)

IAA peak
Mass 130, 176

Specifications & Design
Modelling
Assembly
Testing & Results
Status
Module 2: Auxin Xpress

DR5 Venus Construct

Construct kindly provided by Dr Darren Wells (University of Nottingham)
Auxin Xpress
Engineered E. coli

Control
Wild type E. coli
Can Arabidopsis detect bacterial auxin?

Fluorescence intensity

- No bacteria
- Auxin-producing *E. coli*
- Non-modified *E. coli*

Yes, it can.
Module 2: Auxin Xpress

Dendra2 photoconversion

Irreversible Photoconversion

Excitation 486 nm

405 nm

Excitation 558 nm
Module 2: Auxin Xpress

E. coli remain metabolically active inside roots

Dendra2-expressing E. coli imaged in an Arabidopsis root
Module 2: Auxin Xpress

*E. coli* remain metabolically active inside roots

Dendra2 photoconverted after 4 days
Module 2: Auxin Xpress

**E. coli** remain metabolically active inside roots

Root re-imaged after 24 hours
Module 2: Auxin Xpress

Mass of soil eroded vs. exposure to different concentrations of IAA

Specifications & Design
Modelling
Assembly
Testing & Results
Status
Module 2: Auxin Xpress

Status so far

Bacteria produce auxin
Module 2: Auxin Xpress

Status so far

- Bacteria produce auxin
- Plants respond to bacterial auxin
Module 2: Auxin Xpress

Status so far

- Bacteria produce auxin
- Plants respond to bacterial auxin
- Determined optimal auxin concentrations
Module 2: Auxin Xpress

Status so far

- Bacteria produce auxin
- Plants respond to bacterial auxin
- Determined optimal auxin concentrations
- Dendra2: A new platform for imaging gene expression in root
Module 2: Auxin Xpress

Status so far

- Bacteria produce auxin
- Plants respond to bacterial auxin
- Determined optimal auxin concentrations
- Dendra2: A new platform for imaging gene expression in root
- Auxin improves soil stability
Phyto-Route

Auxin Xpress

Gene Guard
Module 3: Gene Guard

Preventing horizontal gene transfer

1. Prevent horizontal gene transfer
   - Holin/endolysin

2. Without harming our GMO
   - Anti-holin on the genome

**Diagram:**
- **GENOME**
  - **Bba_K515104** (Anti-holin)
  - **Bba_K515105** (sfGFP)
  - **Bba_J23103**
  - **BBa_E1010** (RFP)
  - **BBa_K515106**
  - **pSB1C3**
  - **BBa_K112805** (Holin)
  - **BBa_K112806** (Endolysin)
Module 3: Gene Guard

What is the best promoter and RBS strength ratio?

If the ratio $m$ is larger than 300, then the concentration of Holin in the GM cells drops to 0.

$$m = \frac{P_{\text{anti-holin}} \times K_{\text{anti-holin}}}{P_{\text{holin}} \times K_{\text{holin}}}$$

At $m \geq 300$, $[\text{Holin}] = 0$

**A promoter strength ratio $\geq 300$**
Module 3: Gene Guard

Will it work?

Yes, it will

Gene Guard bacteria

Wild type cell

number of holin molecules / cell

Time (s)
Module 3: Gene Guard

Stage 1

- PCR
  - RBS
  - Antiholin

- PCR
  - J23100
  - B0012
  - B0010

- In-Fusion
  - Antiholin
  - B0012
  - B0010

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 3: Gene Guard

Stage 2

Cell Transformation
Transformed into pir+ cells to replicate.
Co-transformed into cells with accessory plasmid.

Integration into Genome

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 3: Gene Guard

Stage 3

- Ligation of J23103 into RFP
- Ligation into pSB1C3
- Digestion
- PCR
- Ligation + Transformation

Specifications & Design
Modelling
Assembly
Testing & Results
Status
Module 3: Gene Guard

Horizontal Gene Transfer Experiment

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 3: Gene Guard

Horizontal Gene Transfer Experiment

GFP & RFP

Cell lysis - no fluorescence

GFP & RFP

Specifications & Design

Modelling

Assembly

Testing & Results

Status
Module 3: Gene Guard

Status so far

Anti-holin construct is completed
Module 3: Gene Guard

Status so far

- Anti-holin construct is completed
- Anti-holin has been inserted into the genome
Module 3: Gene Guard

Status so far

- Anti-holin construct is completed
- Anti-holin has been inserted into the genome

Troubleshooting toxin construct
Future applications

- Technological
- Practical
Future applications

How can we best implement our project?

Technological

Practical

Implementation using seed coat

Use a seed coat!

Leaders in seed technology

A world leading agri-business
Future applications

Image provided by Dr Frans Tetteroo from Incotec
Future applications

THE BERKELEY REAFFORESTATION TRUST

Technological

Practical
Future applications

Technological

Practical

Effect of auxin on plant biomass

Biomass (g)

IAA concentration (nM)
Outreach

Event at Natural History Museum

Broadcast show Radio iGEM

Script writing

Artistic representation of our project by college intern
Summary

Experimental work → Human practices → Modelling

Each influenced the others to produce a compelling proof of concept.
Achievements

- Rewired chemotaxis
- Got bacteria in the root
- Tracked cell viability with Dendra2 inside *Arabidopsis* roots
- Produced auxin in our chassis
- Got *Arabidopsis* to express venus in response to bacterial auxin
- IAA increases *Arabidopsis* biomass and soil stability
- Integrated anti-holin into the genome
- 6 new working BioBricks submitted to the Registry
- Re-characterised two fluorescent proteins
- Collaborated with WITS-CSIR team from South Africa
- Designed a joint-codon optimising software
- Incorporated human practices and modelling into our design and implementation
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