This sphere represents all the water on Earth. Its **diameter** is about **860 miles**.

Wide range of toxic compounds
Introduction

- Cadmium
- Phenol
- Lead
- Nitrite
- PCB
Introduction

- Cadmium
- Phenol
- Lead
- PCB
- Spongia officinalis
- Cadmium
- Nitrite
Our goal

Combine the sponge filtration capacity and its microbiome to detect toxic compounds

Filtrates
20 000 L of water/day/kg

Epibiosis

*Spongia officinalis*

Pseudovibrio denitrificans

Are you allowed to modify my DNA?
Why *P. denitrificans*?

- *Pseudovibrio* is the main genus present in sponge microbiome.
- *Pseudovibrio denitrificans* naturally has a denitrifying metabolism.

Filtrates
20 000 L of water/day/kg

Epibiosis

*Spongia officinalis*

*Pseudovibrio denitrificans*

Oh ok! Not me!
Ethic

Should our *sponge* be considered as a *GMO*?

≠ GMO

GMMO

Is modifying my microbiome any different than modifying my DNA?
Safety
Epibiosis: a biological containment?

- Lack of competitiveness
- Strict Epibiosis
- Physical containment

Much better!
Engineering a new chassis

KLUDGE

Klumsy Lame Ugly Dumb but Good Enough

Engineering biology

Unexpected results

Are you going to kludge me?
Are you going to kludge me?

Our goal: Engineering approach

- Genome assembly of *P. denitrificans*
- Transcriptomic analysis
- Sponge physiology model
Main problems:
- Complexity of sponge shape
- Variability of sponge characteristics (number of ostia, oscula)
- Bacteria location
Virtual Sponge

Internal VS external concentration

Model 1: fluxes

\[ \phi_{\text{in}} \quad \text{Sponge} \quad V \times C \quad Q \]
- Water inflow
- Filtered compound flow
- Sponge Volume
- Ext. compound conc.

Model 2: 2D Diffusion

\[ \frac{\partial \phi(r, t)}{\partial t} = D \nabla^2 \phi(r, t) \]

Compound quantity

Diffusion coefficient

Compound accumulation caused by geometry

Diagram showing:
- External Compound Concentration (mol/L) vs. Q (micro mol/s)
- Lines for min, mean, and max param.
- Graph indicating compound accumulation caused by geometry
**P. denitrificans** in the literature

*Timeline of articles* mentioning *Pseudovibrio denitrificans*

- 2004: Shieh WY
- 2006: Enticknap JJ
- 2007: Sertan-de Guzman AA
- 2008: Muscholl-Silberhorn A
- 2010: Santos OC
- iGEM Evry team 2014

- Not very well documented
- No transformation protocol
- No genome assembly

- ✔ Genome sequencing
- ✔ Genome assembly
- ✔ First transformation
Nitrate/Nitrite reduction
Cadmium resistance
Phenol degradation
Exogen DNA digestion
Antibiotic resistance (Amp&Tet)

De novo genome assembly
Predicted pathway
Annotation transfer

Chassis
Velvet
Prokka
EcoKI
TetR
AmpR

Annotation transfer
Predicted pathway

FOBEG1 CDS
(5456 sequences)

> 90%
> 95%
> 99%

Sequence similarity (blastp)

86%
81%
46%
Antibiotic tests

Growth on MB after 72 hours at 30°C with different antibiotics

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicilin</td>
<td>resistant</td>
</tr>
<tr>
<td>Tetracycllin</td>
<td>resistant</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>sensible</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>sensible</td>
</tr>
</tbody>
</table>

A: P. denitrificans
B: Double selection
C: E. coli
D: Transformed E. coli
According to the Genome Assembly:

- **EcoKI**
- **Electroporation + Transposon**

Chemical + Replicating plasmid

Transformation
- Tn10 = transposase
- IS10 = repeat inverse
- RFC10 compatible
**Transposons**

**PCR products of kanamycin resistance gene**

1, 2, 3 and 4: clones of *P. denitrificans* transformed

NT: No transformed *P. denitrificans*
Biosensors

Cadmium
Lead
Nitrite
PCB
Phenol

No working BioBricks

Existing BioBricks

RNA Sequencing

Existing BioBricks

(BBa_K1031211) (BBa_K1031222)
According to the image, the Phenol Biosensor comprises two constructs:

1. **BBa K1413001**
   - With GFP’s RBS BBa_B0032

2. **BBa K1413002**
   - With mutated GFP’s RBS
   - According to the Shine Dalgarno motif

The processes involved in the biosensor include:

- **GFP** (Green Fluorescent Protein) is regulated by the RBS (Ribosome Binding Site) and the constitutive promoter Pr.
- **DmpR** (Differential Modulation Protein Response) interacts with ATP and binds to the GFP.
- Phenol interacts with DmpR, indicating a potential interaction between the phenol and the biosensor.

The diagram illustrates the interplay between these elements, highlighting the key components and their interactions.
**Phenol Biosensor**

**Fluorescence INTENSITY PER CELL**

- **BBa_K1413001 vs BBa_K1413002**

**Fluorescence INDUCTION RATIO**

- **BBa_K1413001 vs Bba_K1413002**

**BBa_k1413002** (mutated GFP’s RBS):
- Better **strength**
- Better **sensitivity**
Phenol Model

Hypothesis 1

Hypothesis 2

No phenol degradation

Phenol degradation

DmpR

Phenol dimer

ATP

Kappa Stimulator

Phenol degradation model

No phenol degradation model
• Characterized required promoters (I020260, J23101 and K823012 )
• 8 characterized out of 18 promoters tested in the Anderson Library
Achievements

- *Pseudovibrio denitrificans:*
  - First marine bacteria in iGEM
  - First transformation
  - Genome sequencing/assembly

- Kludge

- Virtual sponge model

- 6 BioBricks submitted
  - 2 Improvements (❤)
  - 6 news

- Compounds:
  - PCB and phenol biosensors
  - Phenol Model

- Safety

- Interlab study: 8 promoters characterized
• Lab facilities: Institute of Systems & Synthetic Biology (iSSB, Evry)

• DNA /RNAs sequencing facilities: CEA (Genoscope, Evry)

• Sponge microbiome advices: MDCEM team of the French National Museum of Natural History (MNHN, Paris)

• Conjugation plasmid: Institute of Molecular Enzyme Technology, Group of Bacterial Photobiotechnology (Heinrich-Heine-Universität, Düsseldorf)

• Sponge expertise: Mediterranean Institute of Biodiversity and Ecology (IMBE, Marseille)
Thank you for your attention

- 9 biologists
- 3 bioinformaticians
- 1 philosopher
- 4 advisors
- 2 supervisors