Our BioBrick Challenge: “The Biosurfactant”

The hydrocarbons can pollute soil, air, and water and alter ecosystems for years. The environmental cleanup of hydrocarbons has a long history using synthetic surfactants as the first weapon for remediation.

Here, we report the rhamnosyltransferase complex 1 (rhlAB) BioBrick as a biological solution that will enable us to a new ways to express, control and manipulate the Pseudomonas’s rhamnolipids biosynthesis into heterologous host like E.coli. The rhamnolipids are a well-known family of biosurfactants that are composed of a rhamnose sugar and lipids giving them an extraordinarily simple molecular form but with marvelous functions. Rhamnolipids are used in multiple industrial applications because its active properties and low toxicity make them an attractive alternative to synthetic surfactants used in the pharmaceutical industry, the petroleum industry, personal care products, agriculture, and other applications like in bio-medicine that has been experimenting it as a liposome for drug delivery.

rhlAB BioBrick, The Biosurfactant

In 2010, we had our first shot with trying to apply the Standardization of the Rhamnosyltransferase 1 gene complex (rhlAB) in E. coli. In our BioBrick Challenge 2011, we check our last year rhlAB BioBrick in order to continue with the second phase of our project that will consist in our BioBrick characterization, and the assembly of the expression platform for rhamnolipid production in E.coli. But we clash with reality and we realize that our last year rhlAB BioBrick part was denaturalized from the plasmid backbone. After this, we endeavor to the task of re-designing, re-constructing and assembling of the rhlAB BioBrick integrated with other composite BioBrick parts that we build in order to have an E.coli based-factory for rhamnolipid production.

How to make it

**Genome Extraction**

- **Pseudomonas aeruginosa**
- **R. aeruginosa genomic DNA**
- **Lysis**
- **Washing solution**

**Metabolic Pathway**

Rhamnolipid is composed of rhamnose sugar and β-hydroxyalkanoic acid. This marvelous molecule:
- Reduces the surface tension of water
- Has emulsifying properties
- Is a well known biosurfactant

The polar end of the surfactant molecule attaches to water, the non-polar end attaches to the ink particle. An emulsion is formed by the two components.

**Inconveniences**

- Procedure to prove which strain has chloramphenicol resistance:
  - We do not transform our strain.
  - In this assay nothing should grow, but it did grow. Then we did a partial extraction DNA and the results revealed that our competent cells has a plasmid that probably is the one that contains the resistance.

**Solution**

- After seeing bad results, we decided to ligate our BioBricks to plasmid backbone containing Ampicillin resistance, but we can’t send the part in that format, we must need new competent cell with no resistance to chloramphenicol at all so we can do it.

**Applications**

- 1. **Petroleum Recovery**, testing indicates that the surfactant and emulsifying properties of rhamnolipids can be used to recover and additional 40% of the remaining oil in a well. This Enhanced Oil Recovery (EOR) that may facilitate the doubling of the petroleum recoverable from an oil well or tar sands over traditional methods in a more environmentally friendly manner.
- 2. **Environmental Cleanup**, also known as bioremediation used rhamnolipids as a very safe and effective method of cleaning up oil and tar spills between other hydrocarbon pollutants.

**+ More Applications in:**

3. In **agriculture and farming** function as a wetting agent, sticker, and dispersal agent for the application of agricultural fungicides and pesticides.
4. **Pharmaceutical** having many beneficial uses medically, using it to heal bedsores, psoriasis, wound and burn healing, kill tuberculin bacteria, they are also good at fighting fungal and bacterial infections.
5. **Food Products** to enhance the volume of bakery products as well as stable dough and batter while protecting them from microbial attack. Rhamnolipids improve the properties of various pastry crannies.
6. **In cosmetics** will make the skin feel more moist and smooth. Used as a natural “green” compound to do its emulsifying capacity forming a mixture of ingredients and spreading them evenly.

**Genetic Engineering**

We cannot produce rhamnolipids industrially using *Pseudomonas aeruginosa* that is the bacterium that naturally produces rhamnolipids, because it’s pathogenic to humans and it has a very tightly regulated production of rhamnolipid. In view of a commercial production of rhamnolipid, there is still a huge potential for genetic optimization. Several attempts to produce Pseudomonas rhamnolipids in heterologous hosts have been reported. Yet, none produces rhamnolipids in comparable levels as the best *P. aeruginosa* strains.

Yet, using E. coli as a heterologous host for rhamnolipid production has been difficult and not produce competitive levels as the Pseudomonas heterologous host.

Genetic engineering reports using E. coli as a heterologous host for rhamnolipid production:

Ochsen et al. (1995) cloned the rhlAB rhamnosyltransferase gene into various hosts, *Pseudomonas fluorescens*, *Pseudomonas oleovorans*, *Pseudomonas putida*, and E. coli. The best rhamnolipid production was 60 mg/L and was achieved with *P. putida*, whereas no production was obtained with E. coli.

Cabrera-Valladares et al. (2006) succeeded in producing mono-rhamnolipids in E. coli. They found that the availability in E. coli of dTDP-L-rhamnose restricts the production of mono-rhamnolipids in this species.

**HUMAN PRACTICE**

**Petroleum Recovery**

Environmental Cleanup

Bioremediation

Petroleum recovery

**Environmental Cleanup**

- **Oil Spill**
- **Soil Remediation**

**Applications**

- **Petroleum Recovery**
- **Environmental Cleanup**

**Results**

- **Oil Spill**
- **Soil Remediation**

**HUMAN PRACTICE**

**Petroleum Recovery**

- **Oil Spill**
- **Soil Remediation**

**Results**

- **Oil Spill**
- **Soil Remediation**

**HUMAN PRACTICE**

**Petroleum Recovery**

- **Oil Spill**
- **Soil Remediation**

**Results**

- **Oil Spill**
- **Soil Remediation**