Let's PLAY Project

Bioproduction of Poly-Lactic acid
Plastic waste – a worldwide problem

- 280 million tons
- 1000 years for a plastic bottle
- up to 450 years for a plastic bag

Figure 1. World Plastics Production 1950-2008. From The Compelling Facts about Plastic, PlasticsEurope (2009), p33.
Our team
SynBio for PLA Bioproduction

Poly-lactic acid
- Biodegradable polymer
- Thermoplastic

<table>
<thead>
<tr>
<th>Characteristic / Strain</th>
<th>E. Coli</th>
</tr>
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<tr>
<td>Genetic manipulation</td>
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<td>Lactate production</td>
<td></td>
</tr>
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<td>In vivo polymerization</td>
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</table>

- Biosynthesis less expensive than chemical synthesis
- Conducted by metabolic engineering in *E. coli* by

**Jung et al., 2010**

Poly-lactic acid

- Biodegradable polymer
- Thermoplastic

Our chassis: *Pseudomonas putida KT2440*

Table 1. Common chassis strains and their characteristics

<table>
<thead>
<tr>
<th>Characteristic / Strain</th>
<th>E. Coli</th>
<th>B. Subtilis</th>
<th>P. putida KT2440</th>
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High | Medium | Low
Implementation of pathway

• Enabling bioproduction of PLA in *P. putida*:
  
  - **Pct**
  
  ![Propionate CoA-transferase](image1)
  
  - **PhaC**
  
  ![Engineered PHA synthase](image2)

• Boosting the metabolic flux by increasing the quantity of precursor (an evolved lactate dehydrogenase (D-LDH*))
  
  - **D-LDH**
  
  ![Lactate dehydrogenase](image3)
Implementation of pathway

Situation of genes in plasmids pSEVA

- **Inducible devices** - Cyclohexanone and IPTG
- **Designed for** *Pseudomonas putida* - De Lorenzo’s lab (Spain)
- **Step-wised optimization for metabolic engineering applications**
  - Fermentation and Bio-polymerization
Results

- Different cloning strategies used (restriction enzymes, Gibson assembly)

**Improvements!**

- pSB1C3 + PhaC
- pSB1C3 + Pct

**New additions!**

- CH promoter
- IPTG-inducible promoter
Results

- Different cloning strategies used (restriction enzymes, Gibson assembly)

  - Biobricks
  - Expression in *Pseudomonas putida*

Improvements!

New additions!
Results

- Different cloning strategies used (restriction enzymes, Gibson assembly)

**Improvements!**

**New additions!**

**Pseudomonas putida in Glycerol**

$0.206 \text{ h}^{-1} = 0.0034 \text{ min}^{-1}$

**Pseudomonas putida in Glucose**

$0.212 \text{ h}^{-1} = 0.0035 \text{ min}^{-1}$

Test of the carbon source on *Pseudomonas putida* KT2440 growth rate
Modeling
In-silico improvements

Flux Balance Analysis (FBA)
In-silico improvements

Flux Balance Analysis (FBA)

Dynamic regulation system
  → Kappa-based modeling

  → Electronic circuit
Our implementation
Our Objective functions:

In blue: biomass
In red: PLA

Our implementation
Objective functions:
In blue: biomass
In red: PLA
Playing with FBA

Glucose

Fructose
Playing with FBA

Objective functions:
- **In blue**: biomass
- **In red**: PLA

Glucose
- **0.74**
- **0**
- **Biomass**

Fructose
- **0.77**
- **0**
- **Biomass**

Input:
- Glucose: 0.74
- Fructose: 0.77
### Playing with FBA

#### Objective functions:
- **In blue:** biomass
- **In red:** PLA

#### Glucose
- **Biomass:** 0.74 → 0
- **LactylCoA:** 17.38 → 0
- **PLA:**

#### Fructose
- **Biomass:** 0.77 → 0
- **LactylCoA:** 18.13 → 0
- **PLA:**
Playing with FBA

Objective functions:

**In blue:** biomass

**In red:** PLA

Glucose

- **Biomass**
  - 0.74
  - 0

- **LactylCoA**
  - 17.38
  - 0

- **PLA**

Fructose

- **Biomass**
  - 0.77
  - 0

- **LactylCoA**
  - 18.13
  - 0

**Oxygen**

- 29.5
  - 7.8

- 28.2
  - 5.6
Dynamic regulation – general design

Goal: optimization of cell metabolism
Kappa-based modeling
Kappa-based modeling

LDH gene → LDH mRNA → LDH → Pyr → Lac

PhaC+Pct mRNA → PhaC → PLA

Pct

Lactyl CoA

Lac

+ LidR prom.
Const. prom.
- McbR prom. RBS
Kappa-based modeling

LDH gene

LDH mRNA

LDH

Pyr

Lac

LldR gene

LldR mRNA

LldR

PhaC+Pct mRNA

PhaC+Pct operon

Pct

Lactyl CoA

Lac

PhaC

PLA

+ LldR prom.

Const. prom.

- McbR prom. RBS
Kappa-based modeling
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LDH gene

LDH mRNA

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Pyr

Lac

McbR

McbR mRNA

McbR gene

LldR

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PhaC+Pct mRNA

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RBS
Kappa-based modeling

LDH gene

LDH mRNA

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Lac

McbR gene

McbR mRNA

McbR

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PhaC+Pct mRNA

PhaC+Pct operon

LidR gene

LidR mRNA

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Lactyl CoA

PLA

31
Kappa-based modeling

LDH gene

LDH mRNA

LDH

Pyr

Lac

McbR

Lac

LidR

McbR mRNA

McbR gene

PhaC+Pct mRNA

LidR mRNA

LidR gene

PhaC

Pct

Lactyl CoA

PLA

+ LidR prom.

Const. prom.

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Kappa-based modeling

Lactate and PLA are being produced, but not all the lactate is directed towards PLA.

<table>
<thead>
<tr>
<th>Species</th>
<th>Synthesis rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>lldR mRNA</td>
<td>0.5</td>
</tr>
<tr>
<td>lldR protein</td>
<td>0.5</td>
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</tbody>
</table>

Strong promoter and RBS
Kappa-based modeling

The PLA production increased, but still a lot of lactate is being lost.

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<tr>
<th>Species</th>
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<tbody>
<tr>
<td>lldR mRNA</td>
<td>0.15</td>
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<td>lldR protein</td>
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Weak promoter and strong RBS
Kappa-based modeling

Species | Synthesis rate
--- | ---
IlidR mRNA | 0.15
IlidR protein | 0.15

Weak promoter and weak RBS
Dynamic circuit – general design

All the elements are stable over time, except PLA which is continuously increasing.
Modeling

Basic design
Modeling

Basic design

Dynamical modeling

FBA
Modeling

Basic design

Dynamical modeling

FBA

Improvement of growth conditions

Optimization by genetic elements of control
Modeling

Basic design

Dynamical modeling

FBA

Improvement of growth conditions

Improved design

Optimization by genetic elements of control
Human practices
Are people interested in PLA?
88.3% of respondents would choose bioplastics over petroleum-based equivalents.
Bioplastics acceptance

Bioplastics acceptance among participants with knowledge of both biology and synthetic biology (n = 187)

<table>
<thead>
<tr>
<th>Category</th>
<th>Non-pathogenic</th>
<th>Pathogenic</th>
</tr>
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<tbody>
<tr>
<td>Industrial material</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>Object for daily use</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>Medicine</td>
<td>95</td>
<td>5</td>
</tr>
<tr>
<td>Medical material</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>Toy</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
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Bioplastics acceptance among participants without any background in biology (n = 37)

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<td>30</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>100</td>
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Bioplastics acceptance

« Bacteria », « Pathogenic » = blurred knowledge

But

People are interested in bioplastics
1. Communicate efficiently on SynBio

2. Act towards more bioplastic consumption
Communicating efficiently on bioplastics
Meeting people with different backgrounds
Meeting people with different backgrounds
Meeting people with different backgrounds
Meeting people with different backgrounds
Physical meetings

Meeting people with different backgrounds
Uneven SynBio practice

http://2016.igem.org/Community/Map
How can we communicate on a wider scale?

Massive Online Open Course
How can we communicate on a wider scale?

Learn → Interact

Cross-border
MOOC Experiments

High Schools
Not an easy public

University students
More reachable
Discussing on MOOCs

Souleymane Bachir Diagne
French and Philosophy Professor @ Columbia University
Former Professor at Cheikh-Anta-Diop University (Senegal)
Digital divide: gaps in access to the Internet
Discussing on MOOCs

Digital divide: gaps in access to the Internet

http://www.youandjerrycan.org/
Acting for more bioplastic consumption
**European Directive:** reducing plastic bag consumption

**French legislation:** a progressive ban on petroleum-based plastic bags
Plastic bags – French and European legislations

**European Directive**: reducing plastic bag consumption

**French legislation**: a progressive ban on petroleum-based plastic bags

Could banning petroleum-based plastic affect bioplastic prices?
Stakeholders point of view

Paris City Council
Cabinet of deputy Mayor for sustainable development

Artaxerkes
A bioplastic factory Liaison operating in Africa and Europe

Biofutura
Importer, wholesaler and online shop of sustainable products.
Reducing prices

Increase of demand + increase of competition

Economies of scales - Bioprocess
Reducing prices

Increase of demand + increase of competition

Economies of scales - Bioprocess
Bioprocess
Bioprocess - the whole picture
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Bioprocess – DIY Continuous Pump

Incompressible flow equation

\[ \frac{P}{\rho g} + H + \frac{V^2}{2g} = K \]

Fluid velocity in the drip chamber

\[ V = (2gH)^{\frac{1}{2}} \]

Steady & Continuous flow rate:
- Fluid velocity
- Area of the tube

\[ F = V \cdot A \]
Bioprocess – DIY Continuous Bioreactor(s)

**PARAMETERS FOR THE BIOREACTOR**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Max growth rate* ($\mu_{\text{max}}$) (min⁻¹)</th>
<th>Max flow (F) (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioreactor 1 (1 - LDH)</td>
<td>0.0035</td>
<td>&lt; 0.17</td>
</tr>
<tr>
<td>Bioreactor 2 (PCT &amp; PhaC)</td>
<td>0.0035</td>
<td>&lt; 0.17</td>
</tr>
<tr>
<td>Auxiliar Reactor 3 (Extension)</td>
<td>-</td>
<td>-</td>
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Bioprocess – DIY PLA Extruder & Roller

DIY-PLA-Extruder
- Piston
- Cylinder
- 1.5 mm hole
- Heater

Made out of INOX material
- Heat-resistant
- Durable
- Standard
- Easy to use
Collaborations

- HPLC and MS (collab. with Bettencourt’s team)

- Plotting growth rates – iGEM Imperial College London
The European Experience
Quick summary – take home message

**The plastic problem**

- Designed a bioproduct using variety of SynBio tools
- Investigated about plastic beyond the lab and provided tools for raising awareness
- **BioBricks:**
  - 2 improved coding seq. (Pct, PhaC)
  - 2 new inducible promoters
- Improved and integrated by developing a fully DIY bioprocess
Acknowledgements

**Students:**

![Student images]

**Advisors:**

![Advisor images]

and to all other previous members and PIs!
Acknowledgements

Host institutions:

To all researchers and members from host institutions that helped

To all external people or associations that contributed to the project development, human practices, and the supporters of our crowdfunding campaign

find them in Attributions in our wiki!
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