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IRON COLI PROJECT

World Championship Jamboree 2013

What is the Iron coli project ?

Hemochromatosis is a genetic disease which causes **overabsorption of iron,** which in turn leads to chronic insufficiencies, heart failure, liver cancer or diabetes. It is the most prevalent genetic disease in Europe, and the only current treatment is **phlebotomy,** also known as **bloodletting.**

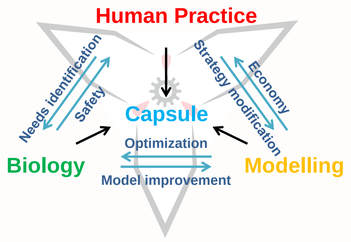


Fig. 1: Project overview.

Bacterial treatment

We constructed Iron coli by combining an **iron sensing device** based on the E. coli **Ferric Uptake regulation system** (FUR) with a **genetic inverter**. Together, these genetic parts activate **siderophore production** to chelate iron in response to elevated ambient iron. We designed a capsule that delivers Iron coli to the upper intestines and ensures it has time to act.

What could be the societal impact of our bacterial treatment ?

Survey

Would you take our treatment if it was allowed by the national drug safety agency ?

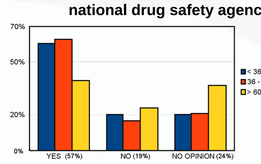


Fig. 2a: 270 answers to our French survey

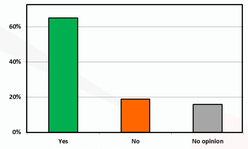


Fig. 2b: 185 answers to our international survey

Our survey shows that patients are interested in a bacterial treatment to supplement bloodletting. **This would especially help working patients**, for whom bloodletting is a real time constraint.

Fault tree

The fault tree shows the **possible consequences** of our bacterial treatment. It enables us to determine precisely the **safety issues** of our project and particularly which strategy is the safest.

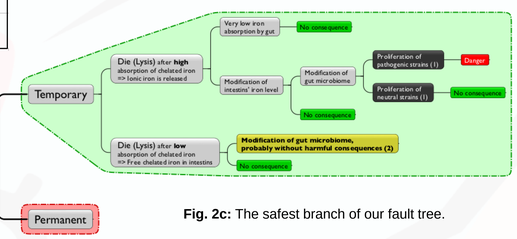


Fig. 2c: The safest branch of our fault tree.

How much iron can be chelated by Iron coli ?

Flush model (ODE model)

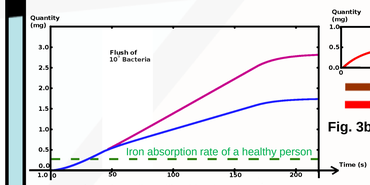


Fig. 3a: Total iron absorbed by the patient.

Assumptions:
- Instantaneous production of enterobactin
- Duodenum crossing = 42 sec

We showed that **iron quantity** in the duodenum and the **iron absorption** are **reduced two-fold**.

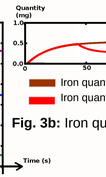


Fig. 3b: Iron quantity presents in the duodenum

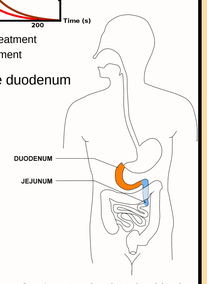


Fig. 3c: Iron is absorbed in the Duodenum (60%) and the Jejunum (40%)

How can Iron coli sense and respond to iron level ?

Sensor

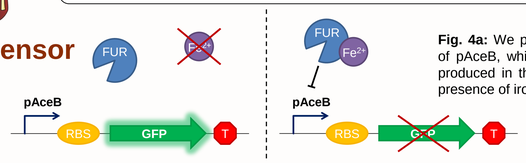


Fig. 4a: We placed a reporter gene (sfGFP) downstream of pAceB, which is regulated by the Fur protein. GFP is produced in the absence of iron and is repressed in the presence of iron.

Biology – Part characterisation

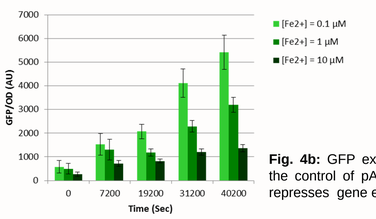


Fig. 4b: GFP expression under the control of pAceB. High iron represses gene expression.

Modelling – Promoter activation

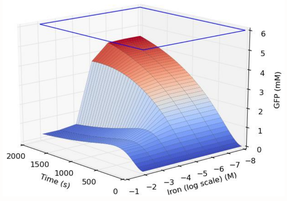


Fig. 4c: Our model show a significant variation in GFP production after 1000 seconds between 10^{-4}M of iron at 10^{-5}M.

Inverter

Fig. 4d: We combined our pAceB iron sensing device with an **inverter device.** The reporter (RFP) was, as expected, expressed at high iron concentrations and repressed in low concentration of iron.

Modelling – Plasmids' number optimisation

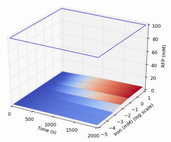


Fig. 4e: 300 plasmids with LacI + 15 plasmids with pLac

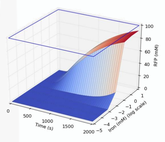


Fig. 4f: 15 plasmids with LacI + 300 plasmids with pLac

Based on our modelling results, we can **optimise** our inverter by expressing RFP on a **higher copy number plasmids** relative to the LacI plasmid.

Characterised BioBricks:
- W ♥ K1163102 – Sensor
- W ♥ K1163103 – Inverter

New !

- J04450 – pLac-RFP → **Improved !**

Biology – Part characterisation

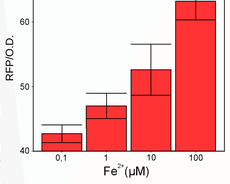


Fig. 4g: RFP expression, under the control of pAceB-inverter, is expressed at high iron concentrations.

What is the impact of our circuit in E. coli metabolism ?

Flux Balance Analysis (FBA)

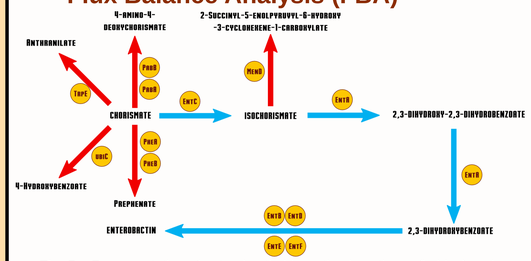


Fig. 5a: Representation of enterobactin biosynthesis pathway (in blue) and side metabolic reactions (in red).

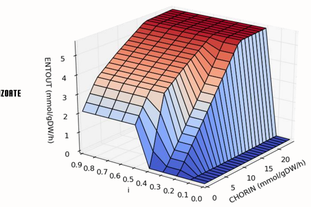


Fig. 5b: Enterobactin production depending on the chorismate input (CHORIN) and on the number of side reactions (i).

FBA results allowed us to identify that **chorismate** input is a **limiting factor** for enterobactin production. Chorismate could therefore be added in later versions of the delivery capsule.

How much time does Iron coli need to produce enterobactins ?

Enterobactin production dynamics

Fe - FUR complex:

$$\frac{d[FeFUR]}{dt} = K_{FeFUR}\cdot[FUR]\cdot[Fe] - diff\cdot[FeFUR] - \frac{1}{N_a\cdot V}\cdot\frac{dLacI}{dt}$$

LacI / LacO:

$$\frac{dLacI}{d[FeFUR]} = \frac{K_g}{K_f}\cdot LacI([FeFUR])\cdot\left(1 - \frac{LacI([FeFUR])}{N_{plsd}}\right)$$

$$\frac{dLacO}{dLacI} = K_g\cdot LacO(LacI)\cdot\left(1 - \frac{LacO(LacI)}{N_{plsd}}\right)$$

Enzymes:

$$\frac{d[mRNA]}{dt} = K_r\cdot LacO - D_{mRNA}\cdot[mRNA]$$

$$\frac{d[Enz]}{dt} = K_p\cdot[mRNA] - D_{Enz}\cdot[mRNA]$$

Fig. 6a: Cloning strategy of the enterobactin pathway

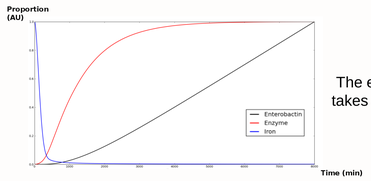


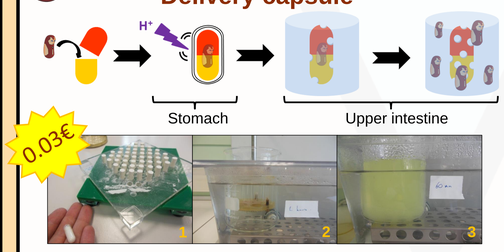
Fig. 6b: Enzyme and Enterobactin production in response to Iron input

The enterobactin production takes approximately **5 hours**.

This model led us to improve our approach by using a **delivery capsule** increases the residence time of E. coli in the intestine.

How to give Iron coli enough time to produce enterobactins ?

Delivery capsule



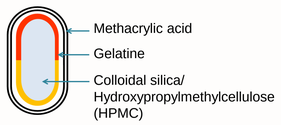


Fig. 7a: We produced and tested the delivery capsule (1). At stomach pH, the capsule stays intact (2). When the capsule encounters the higher pH of the intestine, it releases Iron coli and provides a protective matrix which allows Iron coli to settle and produce Enterobactin (3).

Population scale model

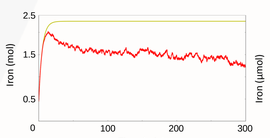


Fig. 7b: Iron dissolved in jejunum.

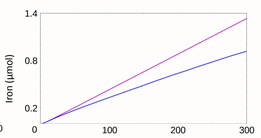


Fig. 7c: Iron absorbed by the organism.

Taking into consideration **bacterial growth** and the time of **enterobactin production**, our population scale model showed that **Iron coli can reduce** the intestinal iron absorption.

What is the best approach to create a bacterial treatment ?

Philosophical approach

- Synthetic Biology: Science or Technique ?
- How could we ethically integrate the patients into our project ?

We discussed philosophical issues of synthetic biology as a technique. Then we focused on the question of ends and means that is recurrent in the **philosophy of technique**. The question of **patients' status** in the project then became the main ethical question.

Seminar

We gathered patients, scientific experts and our team to discuss the **scientific and medical perspectives** of our bacterial treatment.

Expert's consultation

Dr Gaël Nicolas – PHD, *Cochin institute*

"It is always very enriching to meet patients, to hear their difficulties, to understand how they live, accept or refuse some aspects of their disease."

Mr Omar Jbilo – *Sanofi*

"Asking directly the patients, meeting with researchers and physicians is a very pertinent approach to identify the true needs of patients."

International survey of 455 answers !

Collaborations

- Workshop with Paris Saclay team at iSBB on 19th August .
- Scientific collaboration with Edinburgh team which was working with Fur and iron chelator (FbpA).

Attributions

- Plasmids were provided by our advisor Cyrille Pauthenier.
- Patients associations help us to spread our survey to the patients.
- The scientific conference was organised by member of the team with the help of Julien Picot and Veronique le Boulch.