Team ULB-Brussels

InteGreator

How to improve biological pathways!

Goals

A crucial issue in biological industry is the optimization of complex pathways. The goal of our project is to solve this problem using a natural tool: the integron. With this technique, it could be possible to alter the order of genes along an operon. As such, optimization of a pathway is possible if the natural order of genes is not the most productive. We used the production of two kind of microcins as a proof of concept.

Microcins

Microcins are antibacterial peptides produced by *Enterobacteriaceae.* They are active against closely related bacterial species.

We focused on two microcins:

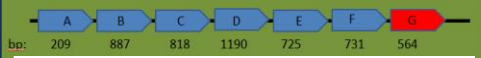
Microcin B17 :

•Propeptide: *mccB A*

•Maturation*: mccB B, C & D*

•Export and immunity*: mcc B E & F*

•Target: DNA-gyrase



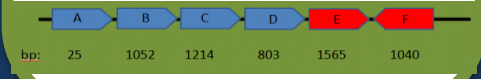
Microcin C7 :

•Heptapeptide: *mccC A*.

•Maturation: *mccC D & E*

•Export and immunity: *mccC E&F*

•Target: aminoacyl-tRNA synthetase



Biosafety

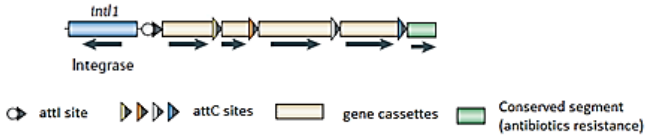
- Non pathogenic *E. coli* strains
- Integron plasmid can only replicate in specific *E. coli* strain (Pir)
- All biobricks and plasmids designed with bioreactor use in mind

Integron

What's an integron?

A natural optimization mechanism. It is composed of :

- Tyrosine recombinase (the integrase, IntI)
- Primary recombination site (*attI*)
- Promoter
- Gene cassettes composed of ORFs flanked by *attC* specific recombination sites

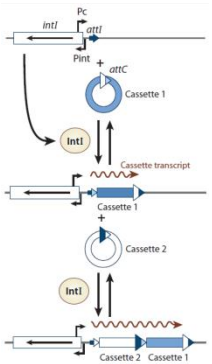


How does it work?

The integrase gene is expressed under stress and IntI allows recombination at *attC* sites. This leads to the excision of circular cassettes that can be reintegrated at the *attI* site.

Consequences?

The gene cassettes movements can lead to deletions, rearrangements or new genes capture by lateral gene transfer.



Perspectives

Consctruction of the two integrons

All genes of both microcins are flanked by *attc* site. Then, they are assembled in an iGEM plasmid provided by Mazel D. – pSWlib (compatible with RFC10) wich contain the *attI* site.

Competition

According to our model, we could select a subpopulation that produces the most microcin

1)MccB17 producers + integrase plasmid **Vs**. Bacteria with low immunity against MccB17

2)MccC7 producers with integrase plasmid **Vs.** Bacteria with low immunity against MccC7

Better than evolution?

A selection pressure more focused than in natural environment

⇒bacteria selected for their microcine production, not their fitness in nature

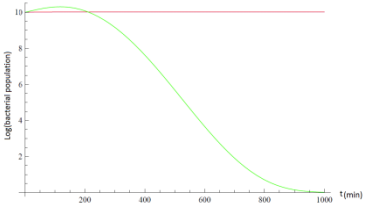
Bikard et al. (2010): Tryptophan integron ==> powerful tool to optimize pathways

Modeling

The selection of bacteria with optimal microcin production will be achieved via competition. At first we co-cultured populations of microcin B17 and microcin C7 producing bacteria, the model can be found below. Unfortunately, no selection of the best production amongst different subpopulations occurred in those conditions.

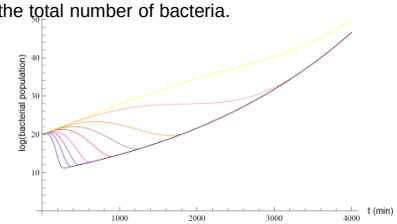
$$\begin{cases} \dot{N}_{Bi} = k\,\frac{D_{Bi}/N_{Bi}}{1+D_{Bi}/N_{Bi}}\,N_{Bi} \\ \dot{N}_{Ci} = kN_{Ci} - \alpha A_{Ci} \\ \dot{A}_{Ci} = \alpha^{B} N_{Ci}\,\frac{\gamma^{B}_{Ci} M^{B}_{Ci}/N_{Ci}}{1+\gamma^{B}_{Ci} M^{B}_{Ci}/N_{Ci}} - \delta A_{Ci} \\ \dot{D}_{Bi} = \alpha^{C} N_{Bi}\,\frac{1}{1+\gamma^{C}_{Bi} M^{C}_{Bi}/N_{Bi}} - \delta' D_{Bi} \\ \dot{M}^{Y}_{Xi} = \rho^{Y} N_{Xi}\,\frac{M^{Y}}{N} - (\delta^{Y} + \pi^{Y}_{Xi}) M^{Y}_{Xi} \quad (X \neq Y) \\ \dot{M}^{X} = \sum_{i} \pi^{X}_{Yi} M^{Y}_{Yi} - \rho^{X} M^{X} + \sum_{i} \tau_{Xi} N_{Xi} \quad (X \neq Y) \end{cases}$$

Differential equation system for a total competition.



Total number of bacteria populations respectively producing microcin B17 (yellow and green) and microcin C7 (red and blue) over time; both pairs of curves are superposed

However, testing the different subpopulations against bacteria of limited immunity could determine an optimal gene order. The production rate can be found, knowing only the total number of bacteria.



Total amount of bacteria over time for different production rates. A direct relation between amount and production can be observed