Droppy Coli : PHB Factory, Applications and Engineering

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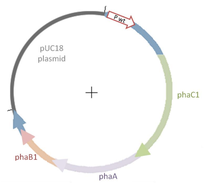
***Abstract* :** Polyhydroxyalcanoates granules (PHAs) are universal prokaryotic storage compounds of carbon and energy. We aim to control their production in *E. coli* thanks to a new part: a strong promoter sensitive to shaking speed, temperature and osmolarity. By controlling this production, our team focuses on two final purposes: the granule as a storage system for overproduced lipids with medical applications, such as DHA or EPA and the granule as self-cleaving micro-beads in order to purify a recombinant protein of interest. In bacteria, three separated monofunctional enzymes are required for PHA synthesis. In order to improve this pathway, we intend to model a single multifunctional enzyme based on the study of natural evolution of fatty acid synthesis in animals.

Production

phaCAB genes are localized on the 1st chromosome of the bacteria *Ralstonia eutropha*. They encode for three enzymes involved in the polyhydroxybutyrate (PHB) biosynthesis pathway: **Precursors** → PhaA (acetyltransferase) → PhaB (reductase) → PhaC (polymerase) → **PHB**

To our knowledge, the production of PHAs has never been observed in wild-type *E. coli* strains. But recombinant strains have been constructed by cloning the genes responsible for the production of PHB. Those strains produce PHB stored in granules.



Our part:

BBa_K342001	PhaC (poly-β-hydroxybutyrate polymerase)	Length: 1789

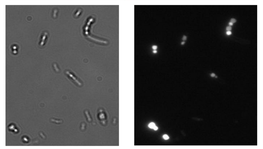
pILI1: a synthetic plasmid that makes granules

In pILI1, *phaCAB* native promoter responds to glucose.
PHB dropplets are fluorescent in presence of nile Red, with glucose.

Test on plates with nile red dye

Observation of stained lipid droplets in presence of glucose

Test by microscopy with nile red dye

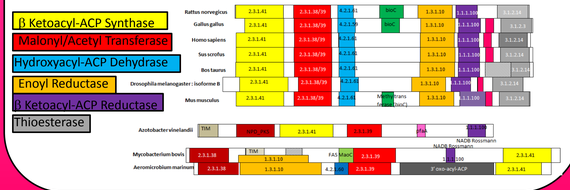


Visible light *Fluorescence (485nm/525nm)*

Design of synthetic multifunctional enzymes.

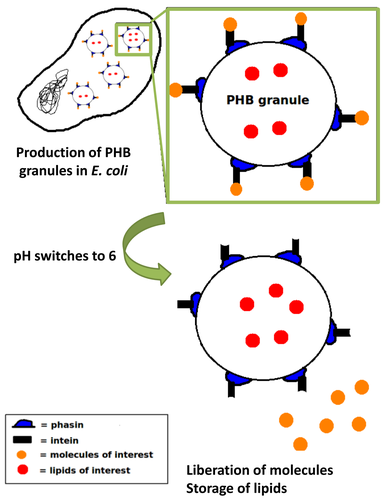
In bacteria, three separate monofunctional enzymes are required for PHB synthesis. In order to improve this pathway, we intend to design a single multifunctional enzyme based on the study of natural evolution of fatty acid synthase (FAS) in animals.

FAS enzymes contain conserved domains:



Uses

Many industrial and biomedical applications for PHB granules had been evocated:
- a storage system for overproduced lipids of interest
- self-cleaving micro-beads, in order to purify a recombinant protein of interest



▪ Because of their hydrophobic nature, lipids cannot exist freely in a cell. We assume that a cell producing granules would target lipids to them, rather than to another specific inclusion structure. To verify this hypothesis, we chose to test the localization of fluorescent lipids, as licopen, in cell producing granules. Unfortunately, fluorescence microscopy did not allow us to differentiate licopen from granules.

▪ Phasin-Phasin-Intein fusion is an easy way to purify proteins of interest by targeting them to a granule. We did not have enough time to develop such a technique of purification. But, a part corresponding to Phasin-Phasin-Intein silver fusion protein has been designed and sent to the registry.

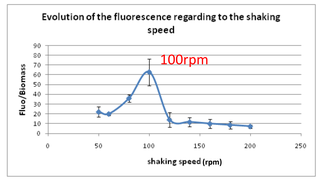
Our part :

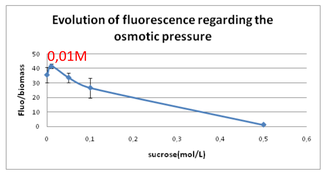
BBa_K342002	Phasin-Phasin-Intein (for silver standard fusion)	Length: 1751

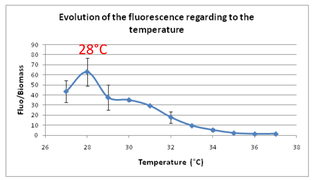
Regulation

Response of Curli promoter to various environmental factors.

We studied the Curli promoter thanks to the PHL1273 chassis, a genomic OmpR234 mutant, which contains a plasmid with an unstable GFP reporter gene under the Curli promoter control. This OmpR234 mutation allows a higher activation of the Curli promoter.







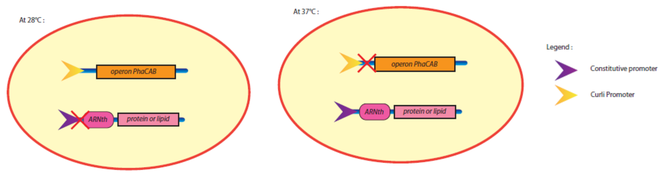
Optimal parameters values for Curli activation are indicated in red.

Our parts:

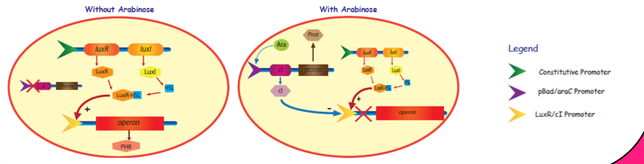
BBa_K342000	Curli promoter induced by temperature, shaking speed and osmolarity	Length: 70
BBa_K342003	OmpR234 protein, with higher effect on Curli promoter	Length: 739

How to sequentially produce granules and the proteins to purify ?

Thermoregulation



Chemoregulation



Further Directions

▪ ***Production***
Design & synthesis of a multifunctional enzyme for PHB granules production from the conserved global organization that we observed.
▪ ***Uses***
-Verify if the GFP fused to the Phasin-Phasin-Intein sequence is well addressed to the granule.
-Extract the granules and cleave the Intein sequence in order to collect the fused GFP.
-Prove the economic advantage of this purification way.
-Study the addressing of other lipids than PHB in bacteria producing PHB granules by fluorescent microscopy or HPLC analyze.
▪ ***Regulation***
Complete the Curli promoter and OmpR234 parts characterization.

Conclusion

Our project was based on our capacity to sequentially produce, thanks to the use of the Curli promoter, granules and then molecules to insert in those granules. During the study of this promoter, we realized that a lot of work had to be done to entirely characterize this system. However, our first results make us believe in its great potential.

Thus, we sent 4 parts:
- **all sequence checked by alignment**
- **documented in the registry**
- **phaC, a functional part**
- **Curli promoter and OmpR234 preliminary characterized**