Tunable Systems for Protein Display on Bacterial Spore Surface

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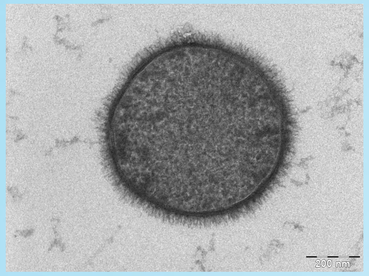
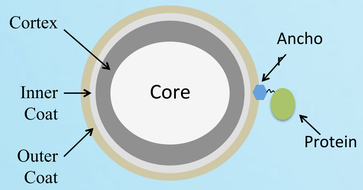
Abstract



Bacterial spores have been used for the display of various proteins including enzymes and vaccines. These proteins become an integral part of the spore coat and benefit from the robustness of the spore. The resulted spores with the displayed proteins provide excellent biocatalysts and drug carriers that are resistant to harsh environments. However, due to the complexity of the spore coat layer, currently protein display on spore surface relies on a few anchor proteins and their expressions are all under native regulatory elements, which means there are no tools to modulate the abundance of the proteins displayed on the spore surface. In this work, tunable expression systems are designed and built using the natural regulatory elements for the anchor proteins and binding sites for non-sporulation transcription factors such as LacI. Using GFP and mCherry as the reporters, we plan to map the display pattern of the commonly used anchor proteins and explore the regulatory limits of these hybrid promoters. This work will provide valuable tools for protein display on spore surface as well as a better understanding of how the composition of the spore surface layer affects the spore integrity.

Introduction

- Spores from bacteria have a rigid surface layer composed of over 70 proteins to protect them from harsh conditions such as extreme temperatures, pH, radiation, and toxic solvent.
- By genetically fused to an surface anchor protein, a foreign protein can be displayed on the spore surface.
- Using spores as a protein display platform has many advantages:
  - Enhanced robustness
  - No need for protein purification
  - Reusability

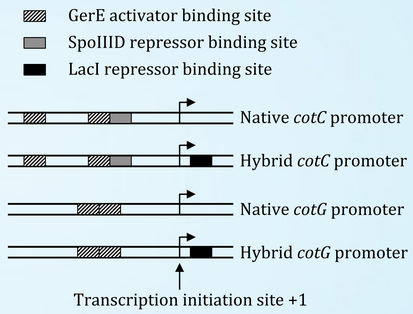


- Problem of current spore surface expression and display methods:
  - The organization of spore surface proteins is unknown.
  - No tunable expression system is available.

Experiment Design

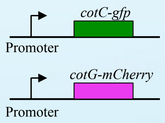
Hybrid promoters:

- Hybrid promoters composed of the native regulatory sequences and a LacI binding site (lacO) were constructed.
- LacO is located either between the native regulator binding sites, or before +1, or after +1.



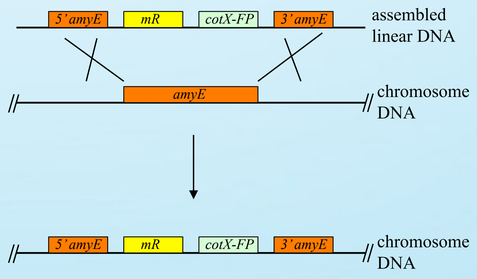
Reporters:

- GFP and mCherry are used for CotC and CotG respectively to study their abundance and co-localization.



Integration:

- Instead of using integration vectors which needs cloning in E. coli first, DNA fragments are directly assembled after PCR amplification.



Results and Future Work

- A method has been designed to directly assemble linear DNA fragments to integrate the target *cotX-FP* into the chromosome. Compared with commonly used integration vectors, this method could reduce a typical cloning procedures from 3-4 weeks to about 1 week.
- However, the efficiency of transformation/ integration is extremely low, which is likely due to the small size of the linear fragment. New homologous fragments have been designed to improve the efficiency.
- Collect spores displaying CotC-GFP and/or CotG-mCherry either with their native promoters or hybrid promoters at different concentrations of the inducer IPTG.
- Measure the fluorescence intensity at the bulk level using fluorescence microplate reader and at the individual level using fluorescence microscopy and flow cytometry.
- Design hybrid promoters using other regulators, such as TetR and PbrR.

References

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