Silencing of functional genes circuit in *Bacillus subtilis* based on automated and reactive flow cytometry

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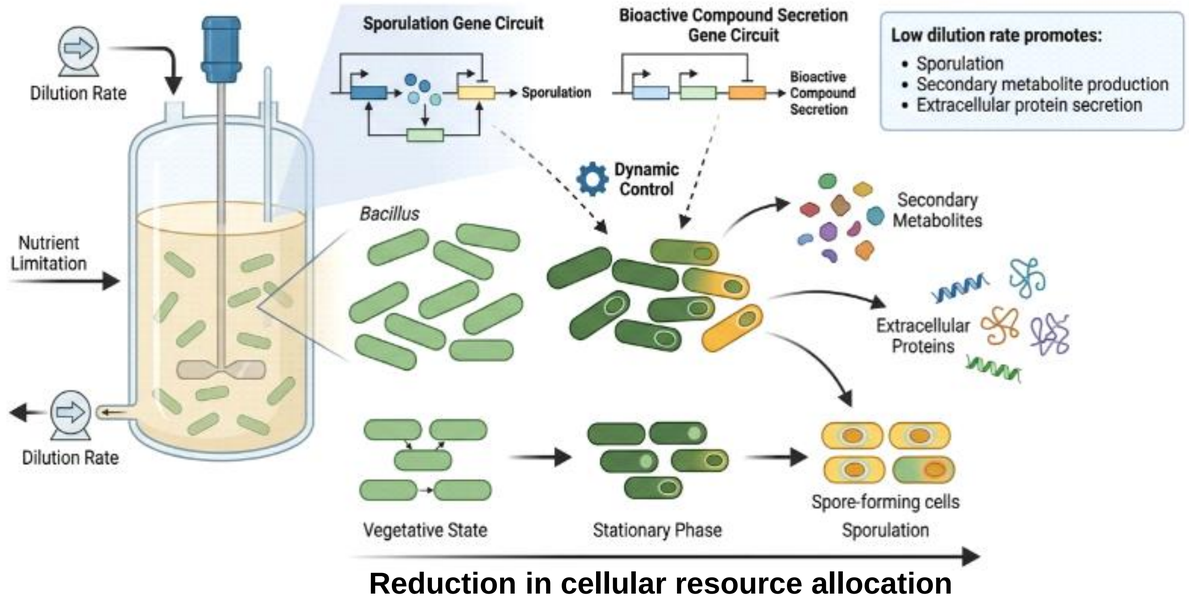


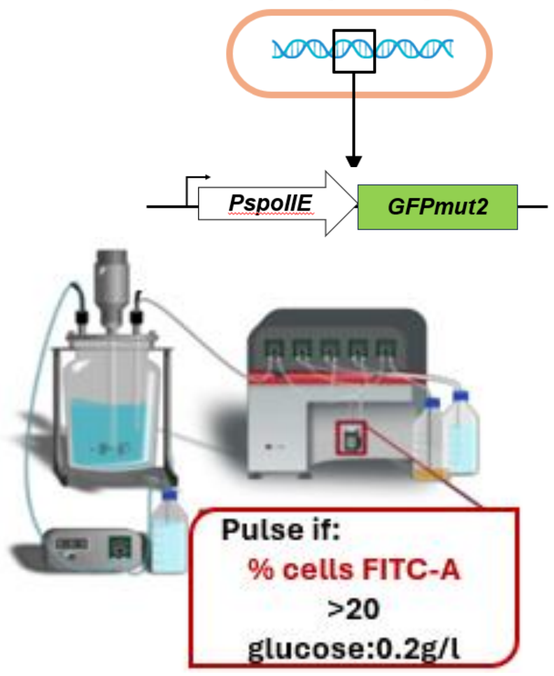
Objectives

1. Dynamic control of sporulation based on automatic flow cytometry

2. Effects of protein overexpression and secretion stress on sporulation.

1 Dynamic control of sporulation based on automated and reactive flow cytometry

B.Subtilis 168 ::PspoIIE_gfpmut2



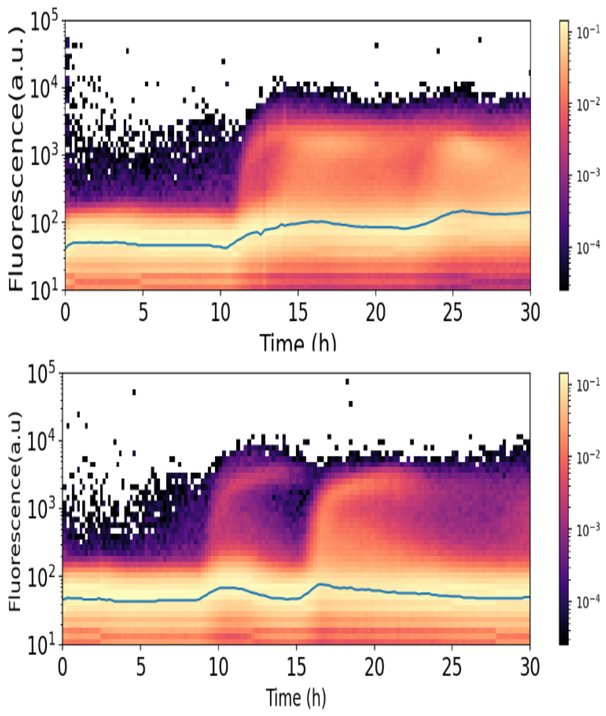


Figure 1.a: Heat map of sporulating cell distributions under non controlled and AFC controlled.

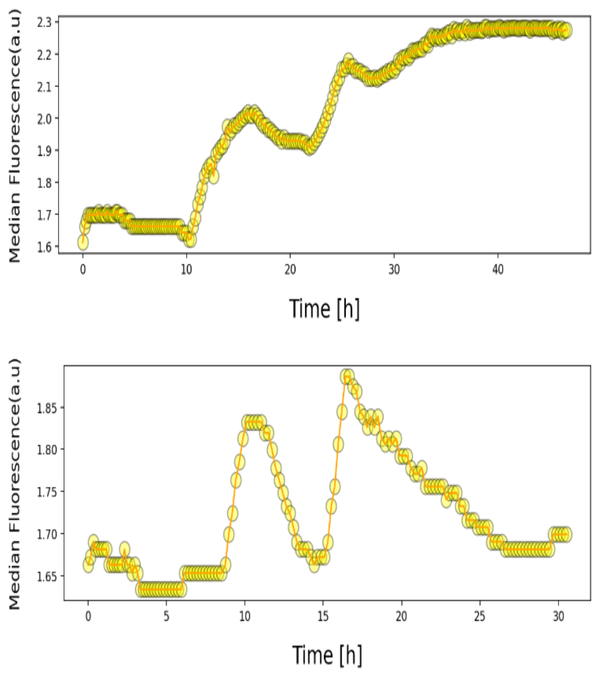


Figure 1.b: Trend of median fluorescence under non controlled and AFC controlled

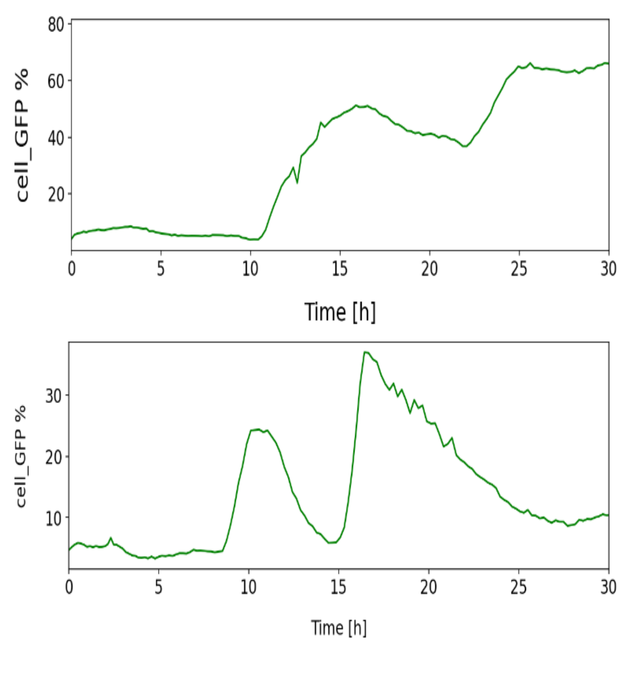
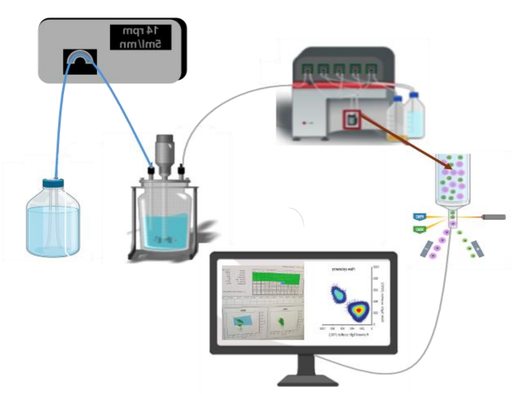


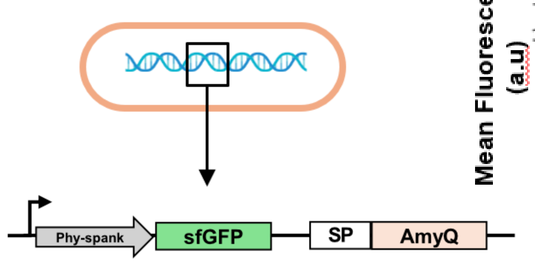
Figure 1.c : Evolutions of percentage of sporulating cell under non controlled and AFC controlled.

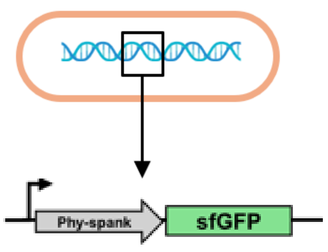
2 Low dilution rate increases proteins expressions and delays sporulation



Experimental Setup

***B.Subtilis* 168 ::Phy-spank_sfGFP_SPAmyQ**





***B.Subtilis* 168 ::Phy-spank_sfGFP**

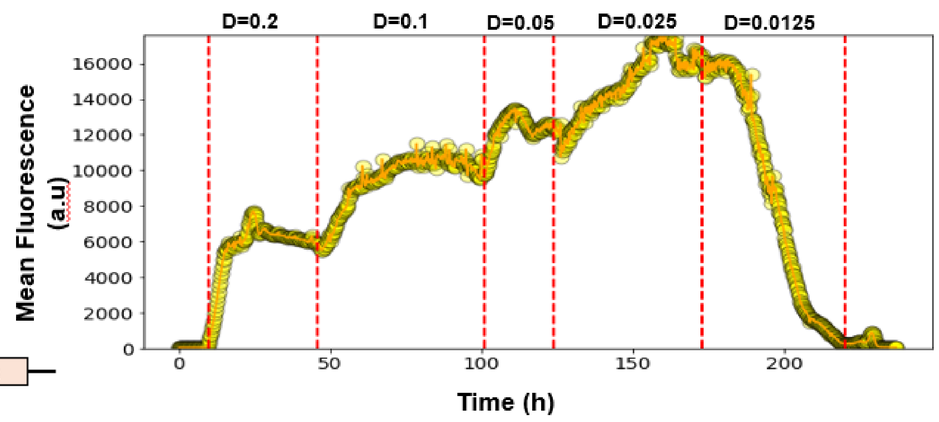


Figure 2:

Fluorescence increased as the dilution rate decreased and collapsed at a dilution rate of D = 0.0125 h^{-1}.

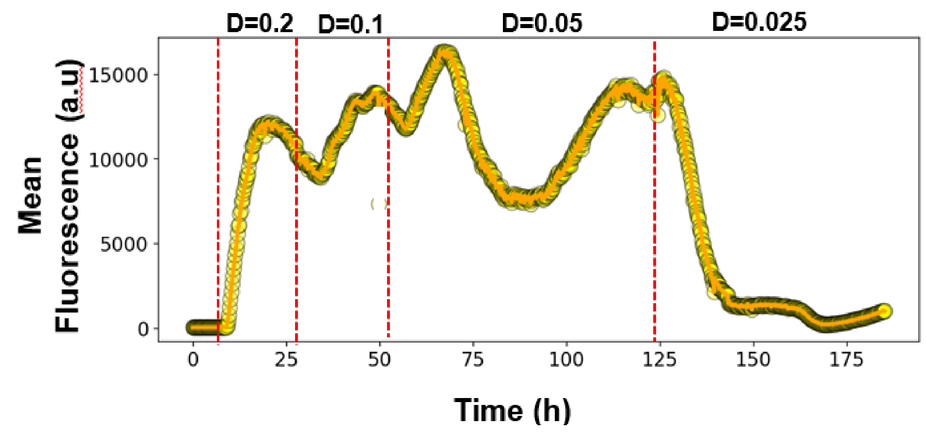


Figure 3:

Fluorescence oscillations were observed due to early sporulation, followed by a collapse at a dilution rate of D = 0.025 h^{-1}.

Take home messages

Automated and reactive flow cytometry enables dynamic control of sporulation and can be used to silence sporulation genes during the growth phase.

Overexpression, secretion stress, and dilution rate are key parameters that can be used to control or suppress sporulation.

Perspectives

- ❑ **Quantify spore formation to identify the optimal dilution rate that maximizes production while inhibiting sporulation.**
- ❑ **Evaluate the stability of the expression system after sporulation.**

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