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Use of sigma factors from *Bacillus subtilis* in the development of an orthogonal expression system in *Escherichia coli*

Indra Bervoets¹, Katleen Van Nerom¹, Marjan De Mey², Daniel Charlier¹

¹Research group of Microbiology, Vrije Universiteit Brussel, Belgium; ²Centre of Industrial Biotechnology and Biocatalysis, UGent, Belgium

**Introduction**

Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning expression of synthesis pathways in specific conditions is mandatory.

Here we develop a new genetic circuit for regulated expression specifically in stationary phase due to clear advantages during this period (reduction of toxicity, competition).

This circuit consists of a heterologous sigma factor (σ) recognizing specific promoter sequences, which are not recognised by the native σ factors of *E. coli*, creating an orthogonal system. Our aim is to make this system condition specific by regulating the expression of the novel factor allowing the selective expression in the stationary phase. In combination a constitutive promoter library linked to this specific σ factor will be constructed.

**Methods & Results**

Several σ factors of *B. subtilis* were tested for their orthogonality in *E. coli* on the level of promoter recognition, by using a red-fluorescent reporter system. In addition, the potential of these σ factors from *B. subtilis* to work together with the *E. coli* core RNA polymerase could be tested, by expressing these proteins together with their promoters in a so-called ‘α expression system’. Simultaneously the influence on cell growth due to the presence of the heterologous factor could be assessed.

Condition specific expression was experimented with by cloning the mKate2 gene in the σ3 factor operon of *E. coli*. As some upregulation was observed entering the early stationary growth phase, that position was chosen to insert the heterologous factors into the genome. However, leaky expression during exponential phase is still observed and further research is needed to gain a strict controlled expression.

First results of different heterologous σ factors from *B. subtilis* show great potential for orthogonal expression in *E. coli* with limited burden on cell growth. Moreover, promoter banks that cover a wide range of promoter strengths while maintaining their orthogonal features in *E. coli* are feasible.

**Conclusion**

Combining all these elements should allow us to create an orthogonal genetic circuit that is able to transcribe specific genes under stationary phase with a limited influence on the host cell’s metabolism.