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Synthetic Promoter Library for modulation of actinorhodin production in *Streptomyces coelicolor* A3(2)

Sujata Vijay Sohoni[I], Ivan Mijakovic[I,2], Anna E. Lantz[I]

[1]*Center for Microbial Biotechnology, Department of Systems Biology, Denmark Technical University, 2800 Kgs. Lyngby DENMARK.*
[2]*Microbiologie et Génétique Moléculaire, AgroParisTech-INRA-CNRS, Route de Thiverval, F-78850 Thiverval-Grignon FRANCE*
Email: svs@bio.dtu.dk, Ivan.Mijakovic@grignon.inra.fr, ael@bio.dtu.dk

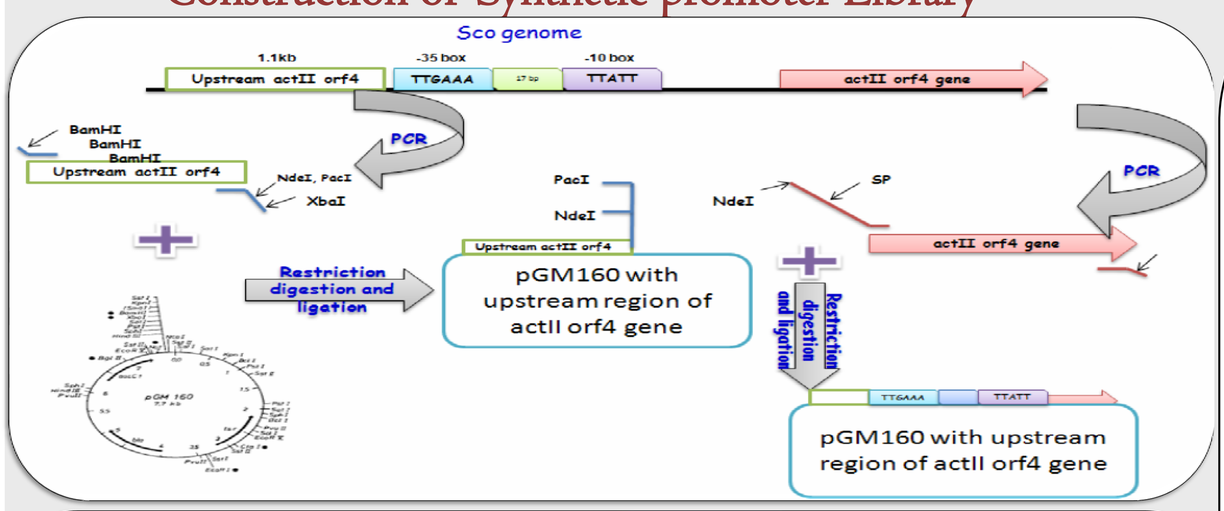
Introduction:

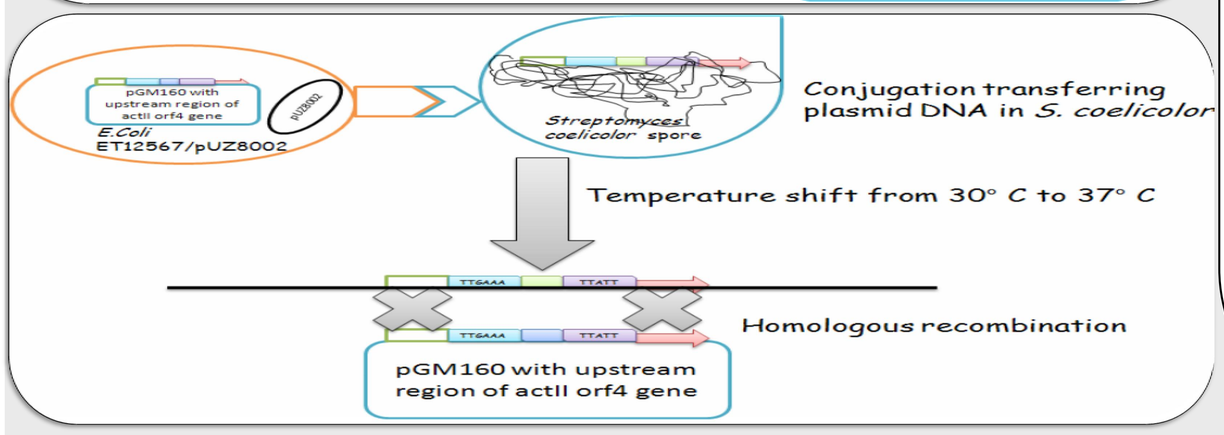
Genetic manipulation tools have been successfully used for improving properties of industrial microorganisms. Most of these approaches involve over-expression of a gene for the rate limiting enzyme or deletion of a gene situated at a branching point in case of branched pathways. These simplistic all-or-nothing approaches have been fruitful in some cases, but can fall short when careful optimization of gene expression is needed in order to tune the modified pathway with the rest of the cellular metabolism. Promoter strength plays an important role in the resulting levels of gene expression. The synthetic promoter technology, based on randomization of the promoter sequences, has been successfully employed to construct promoter libraries in order to optimize levels of gene expression.

Synthetic promoter technology is based on the fact that the spacer sequences surrounding the consensus -35 and -10 regions of bacterial promoters contribute significantly to promoter strength. Randomizing these spacer sequences results in Synthetic Promoter Libraries.

In the current study the native promoter of *actII orf4* was modified by randomizing spacer sequence between -35 box and -10 box and 5 nucleotide before and 5 nucleotides after -35 box and -10 box respectively. The resulting library was screened and characterized for production of actinorhodin.

Construction of Synthetic promoter Library

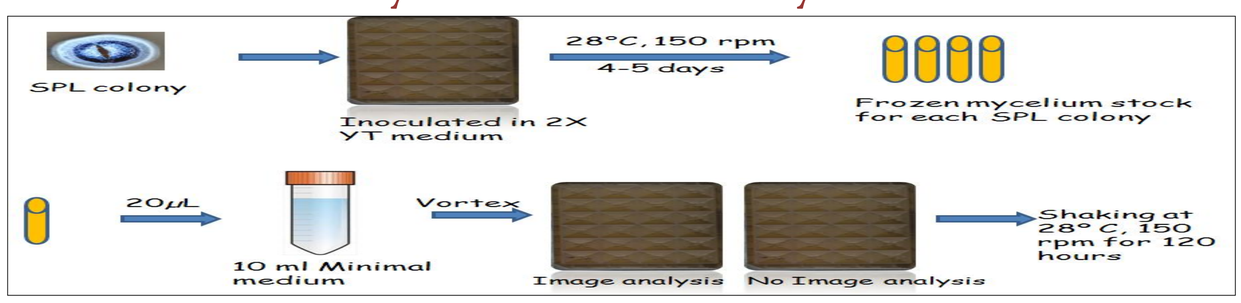


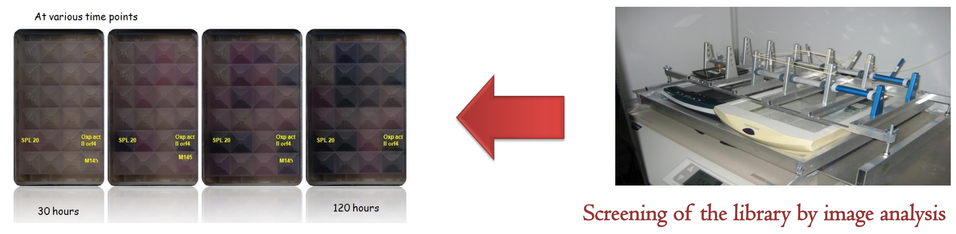


Screening of Synthetic Promoter Library

- Around 10,000 colonies were screened by visual screening
- 200 colonies having a blue actinorhodin hallow were selected for characterization
- Out of the 200 colonies, 12 colonies were subjected to detailed physiology studies

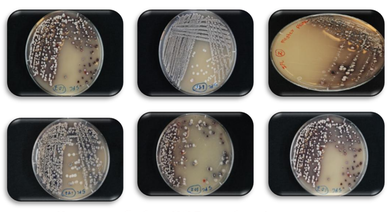
Characterization of Synthetic Promoter Library



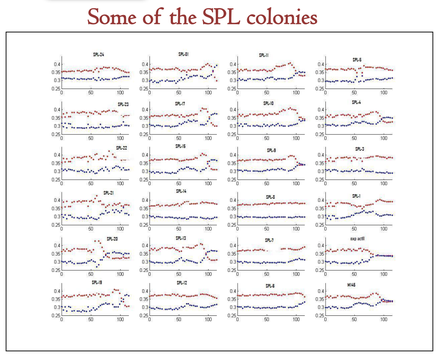


Screening of the library by image analysis

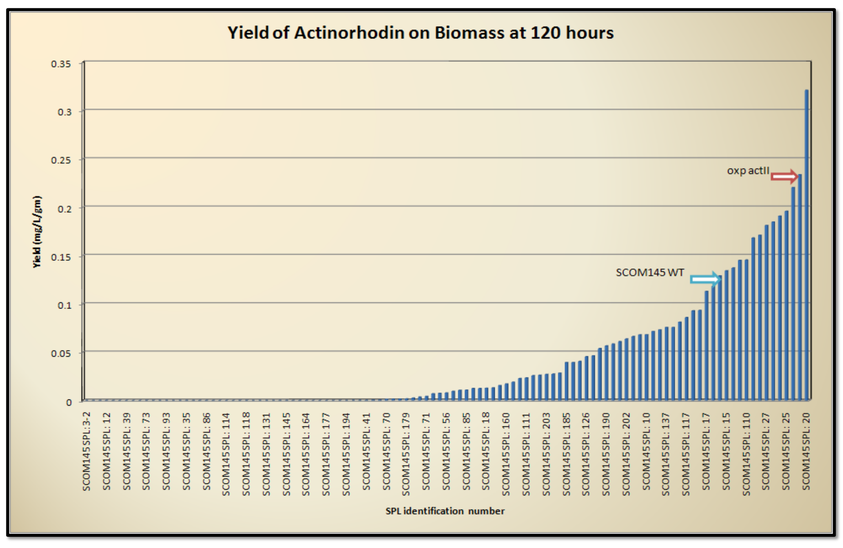
Results and Discussion



Some of the SPL colonies



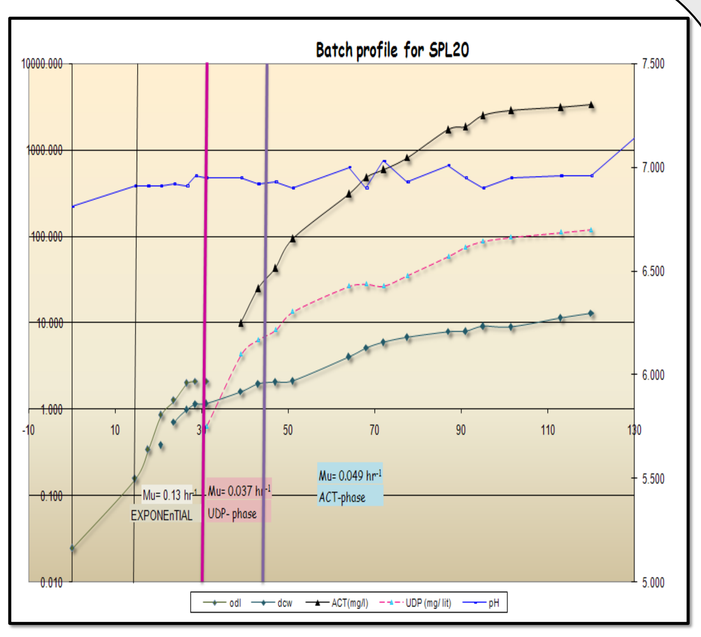
Output of image analysis showing onset of antibiotics UDP (red) and Act (Blue)



Representation of the library based on yields of actinorhodin on biomass

SPL 20 strain in reactor

Strain	µexp (h-1)	µUDP (h-1)	µACT (h-1)	r P UDP (mg/L/hr)	r P ACT (mg/L/hr)	-rS (g/g)	Y P/X ACT (g/g)
Sco M145	0.1±0.01	0.06	-	1.5	15.1±1.4	1.8±0.01	0.13
Sco M145 oxp act II orf4	0.12	-	0.12	-	38.1	2.2±0.01	0.23
Sco SPL20	0.13	0.04	0.05	1.6±0.1	70.9±1.5	2.5±0.01	0.32



- A promoter library producing different yields of actinorhodin was constructed. SPL 20 was found to be very interesting and hence studied in details in bioreactor.
- 12 strains showing higher and lower yields than that of the wild type strain were also studied in more details and the promoter sequnces are being obtained.
- SPL 20 is a very good example of the principle of promoter library. Hence the library was successfully constructed. The promoters thus obtained could be generalized for various expression levels of different genes according to the requirements

References

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(2) Arias, P., Fernandez-Moreno, M. A., Malpartida, F. 1999. Characterization of the pathway-specific positive transcriptional regulator for actinorhodin biosynthesis in *Streptomyces coelicolor* A3(2) as a DNA-binding protein. Journal of Bacteriology, 181(22), 6958-6968

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