MOL231: Benchmarking an Expression System in *Bacillus subtilis*

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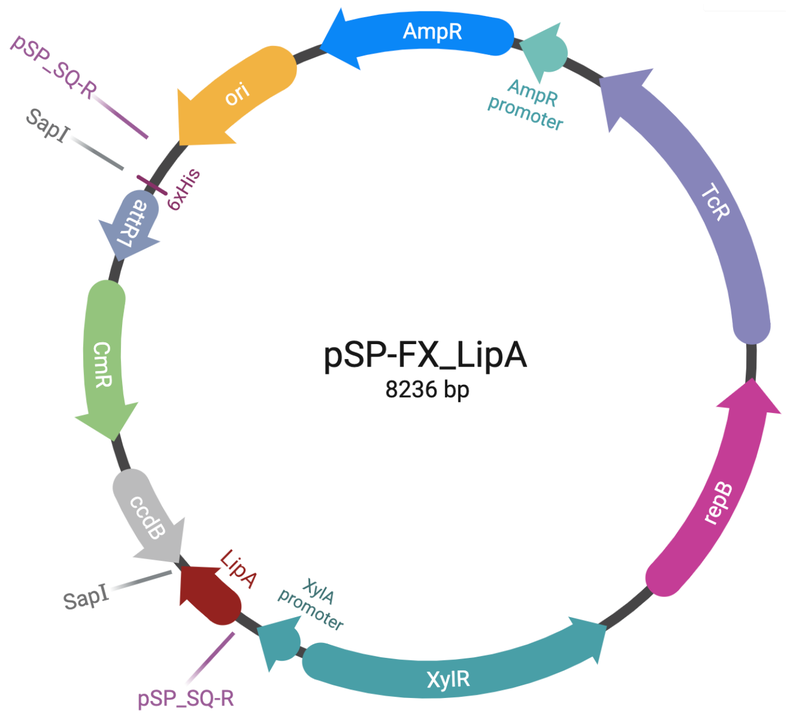
Introduction

Bacillus subtilis is well-known for its important role in the industrial production of proteins and is thus an ideal organism to study mechanisms like protein expression (1). The aim of this experiment was to benchmark an expression system in our *B. subtilis strain* KO8, by utilizing six different enzymes, table 1. For this purpose, the plasmid pSP-FX_LipA, fig. 1, was used, as well as *B. subtilis strain* KO8 (2).



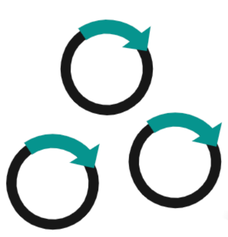
SapI
5' ... G C T C T T C N ▼ ... 3'
3' ... C G A G A A G N N N N ▲ ... 5'

Fig. 1 Shuttle vector for cloning in E. coli and expression in B. subtilis. Expression is under control of the inducible xylose promoter. Plasmid confirms resistance to tetracycline. Gene is cloned in frame with leader sequence that directs protein product to the secretion route in B. subtilis.

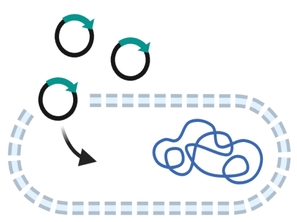
Figure created with BioRender.com

Methods

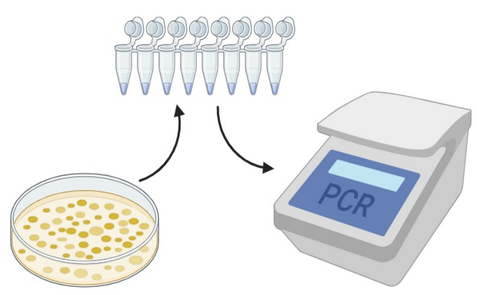
1. Fragment Exchange Cloning



2. Subcloning and Transformation to E. coli

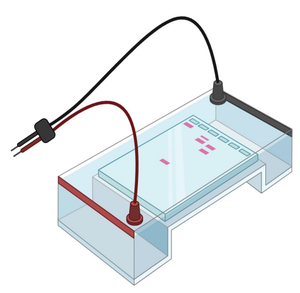


3. Colony PCR

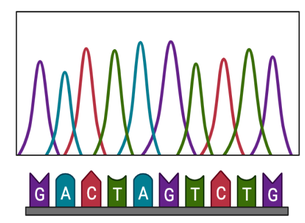


Control point

4. Agarose Gel Electrophoresis

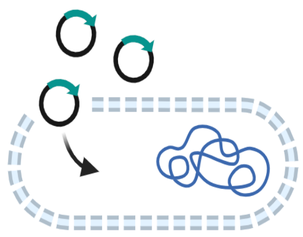


5. Sequencing Verification

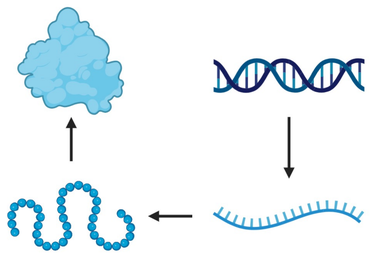


Control point

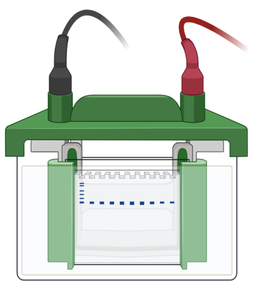
6. Transformation to *Bacillus subtilis*



7. Expression



8. SDS-PAGE



Figures created with BioRender.com

Table 1 The benchmarked enzymes in the study

Target	Source	PCR-product (bp)	Pre-protein/mature (kDa)
T0307B	Tyrosinase	2096	72.7 / 69.0
T0308	Alpha-amylase	1583	54.4 / 50.7
T0309	Mannose isomerase	1523	52.7 /49.0
T0310	Catalase	1733	60.1 / 56.4
T0311	Cutinase	929	27.7 / 24.0
T0312	C1-peptidase	1373	46.5 / 42.5

Results and Discussion

Successful analyzing of spent media using Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). T0311 was confirmed to be expressed in the medium, as shown in fig. 2. The yield was measured to be 11.37%. The expression of the other samples could not be definitively confirmed. In future studies it should be considered to include analytical techniques such as Western blotting, RNA sequencing and codon optimization.

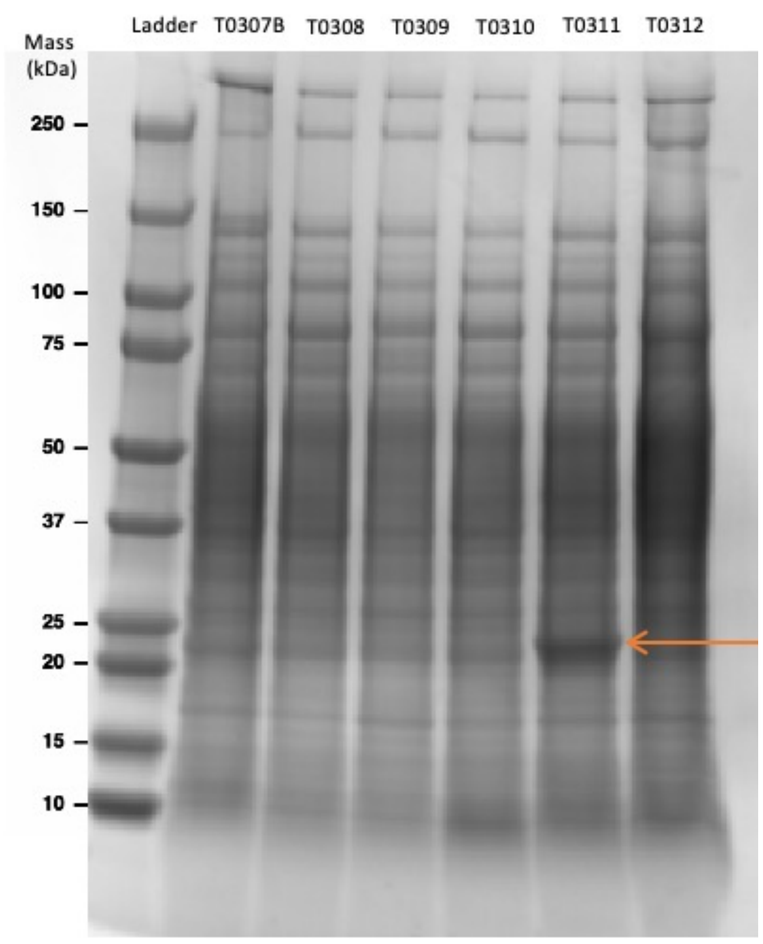


Fig. 2 SDS-PAGE of TCA concentrated spent media

SCAN ME

References:
1. Cui W. *et al*. [2018] Exploitation of Bacillus subtilis as a robust workhorse for production of heterologous proteins and beyond. World J Microbiol Biotechnol.
2. García-Moyano A. et al [2020] Fragment Exchange Plasmid Tools for CRISPR/Cas9-Mediated Gene Integration and Protease Production in *Bacillus subtilis.* Appl Environ Microbiol.

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