Automated signal peptide screening workflow for secretion of heterologous proteins in *C. glutamicum*

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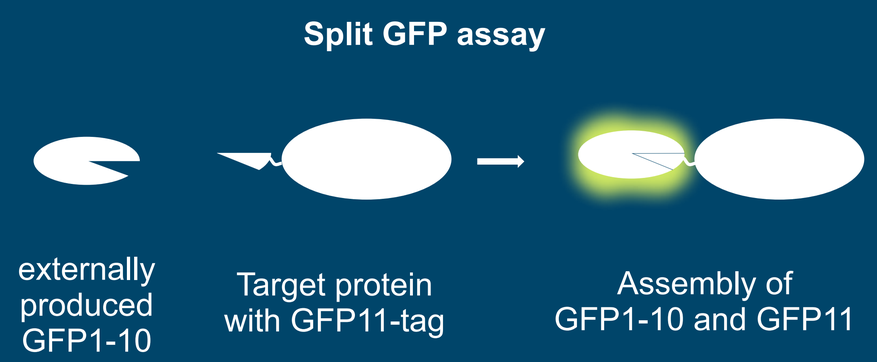
Challenge: Still no successful *a priori* prediction of suitable signal peptides for secretion of heterologous proteins possible

Aim: High throughput screening for Sec-type secretion of heterologous proteins in the industrially relevant platform organism *Corynebacterium glutamicum*

Solution: Transferable automated workflows

- Semi-automatic construction of a plasmid library with *Bacillus subtilis* Sec-type signal peptides
- Use of a microscale cultivation platform supported by laboratory automation for secretion screening
- Split GFP assay for activity-independent detection of secretion performance and easy adaptation to different target proteins [1, 2]



Workflow

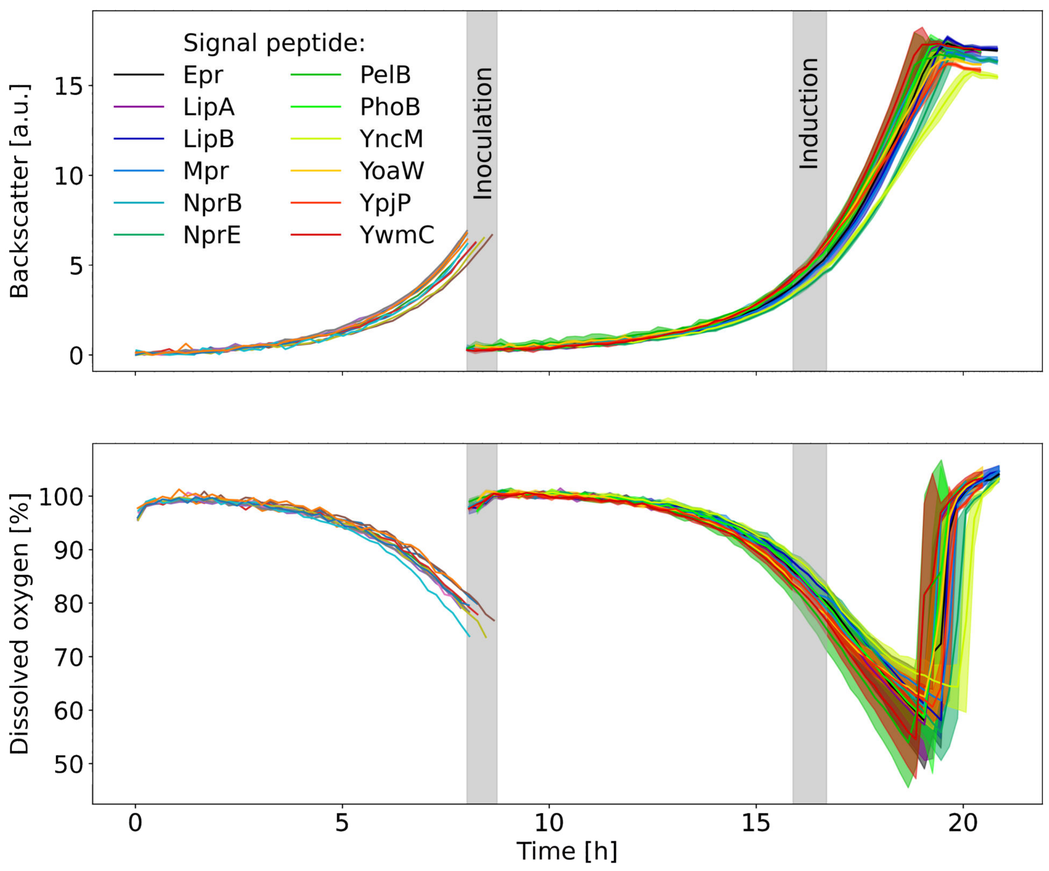
- Pre-cultures in BioLector®, inoculated from cryo stocks
- Backscatter-triggered inoculation of three main cultures from pre-culture
- Triggered induction with 200 µM IPTG if backscatter signal correlates to 4 g/l cell dry weight
- Individual harvest 4 h after induction and cooled storage of supernatant
- Automated split GFP (and activity) assay for target protein detection

Signal peptide screening

Cultivation:

- Target protein: *Fusarium solani* f. sp. *pisi* cutinase with GFP11-tag
- 12 different Sec-dependent signal peptides from *B. subtilis*
- BioLector® cultivation (FlowerPlate®, 30 °C, 1400 rpm, CGXII medium, backscatter and dissolved oxygen measurement)

Results:

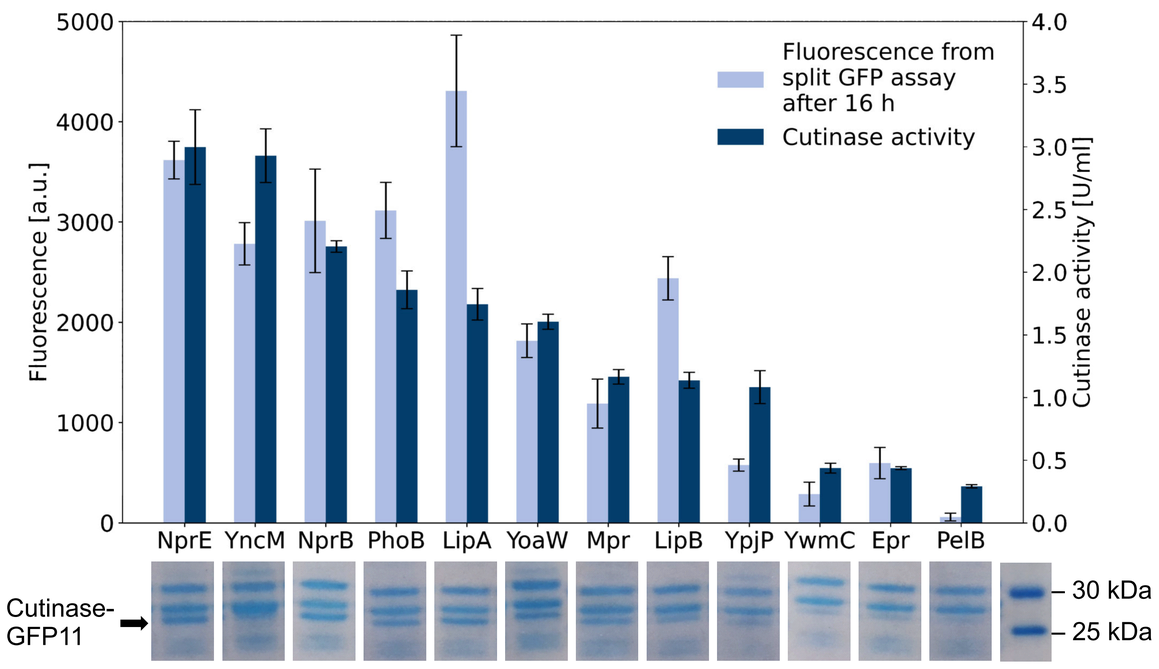


- Less work due to automated inoculation, induction and harvest
- Comparable growth regardless of the signal peptide
- Duration of main cultures: 12.0 - 12.8 h

Target protein detection:

- Secreted cutinase-GFP11 detected by automated cutinase activity and split GFP assay
- Proteins in supernatant were precipitated by trichloroacetic acid [3] and analyzed by SDS-PAGE (Theoretical M_W of Cutinase-GFP11: 25.09 kDa [4])

Results:



- The top five performers are identified with split GFP and cutinase activity assay
- Split GFP assay cannot differentiate between active and inactive proteins
  E.g., similar cutinase activity for secretion with signal peptides LipB and YpjP, but higher fluorescence in the split GFP assay and higher amounts of cutinase-GFP11 on SDS-PAGE with LipB
- The signal peptide affects the ratio of active to inactive protein after secretion, presumably in the protein folding step

Resources:

[1] Cabantous, S., Terwilliger, T. C., & Waldo, G. S. (2005). Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. Nature biotechnology, 23(1), 102-107. doi: 10.1038/nbt1044

[2] Knapp, A., Ripphahn, M., Volkenborn, K., Skoczinski, P., & Jaeger, K. E. (2017). Activity-independent screening of secreted proteins using split GFP. Journal of biotechnology, 258, 110-116. doi: 10.1016/j.jbiotec.2017.05.024

[3] Bakkes, P. J., Ramp, P., Bida, A., Dohmen-Olma, D., Bott, M., & Freudl, R. (2020). Improved pEKEx2-derived expression vectors for tightly controlled production of recombinant proteins in *Corynebacterium glutamicum*. Plasmid, 112, 102540. doi: 10.1016/j.plasmid.2020.102540

[4] Compute pI/Mw tool, ExPASy, Swiss Institute of Bioinformatics

- **Depending on the signal peptide, the ratio of active to inactive protein may change, which is not monitored by split GFP assay**
- **Split GFP assay is nevertheless suitable to select signal peptides for secretion of a new target protein without changes in the automated screening workflow**
- **Only selected signal peptides could then be used for activity assays which are usually more complex and in some cases cannot be automated**

➥ **Overall hands-on time for signal peptide screening can be reduced**
➥ **Further studies are needed to clarify how the signal peptide affects activity**

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This project was funded by the European Regional Development Fund (ERDF)