

Hamel R. ¹, Neumann A. ¹, Bienstein M. ², Contreras F. ², Schwaneberg U. ², Wiechert W. ¹, Oldiges M. ¹

¹Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany

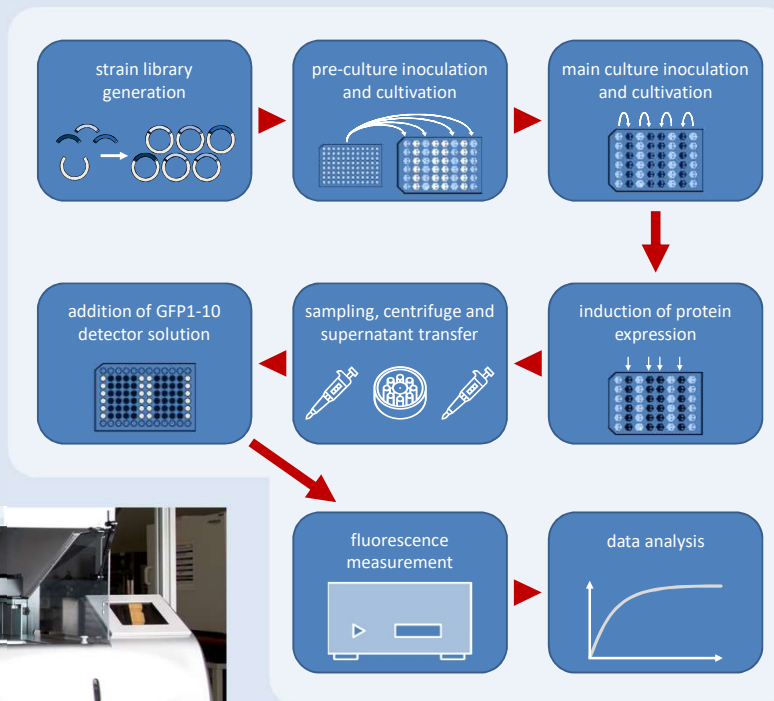
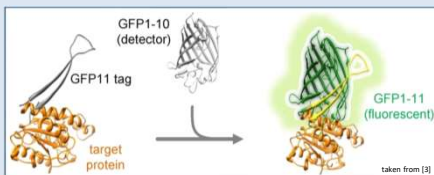
²Institute of Biotechnology, RWTH Aachen, Aachen

Micro- and nanoplastic particles (MP/NP) in food and beverages have been associated with health risks. An approach is needed by which MP/NP can be detected in and ultimately removed from foodstuffs to ensure human health and monitor environmental contaminations. Adhesion promoting peptides (APs) present a solution by binding specifically to different types of polar and nonpolar MP/NP. They can be used to label MP/NP with fluorescent dyes for specific detection [1]. Their secretory production in *Corynebacterium glutamicum* facilitates easy recovery and purification.

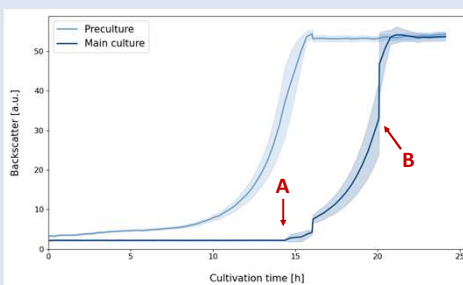
Protein secretion in *C. glutamicum* can be utilized in heterologous production by expressing a fusion protein consisting of a secretion signal peptide (SP) and the target protein. After establishing an **automated high-throughput screening workflow** [2], different SPs are investigated regarding their secretion efficiency for a chosen AP.

Methods

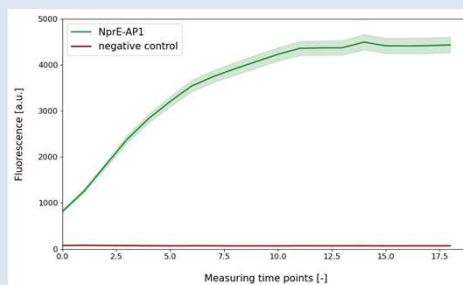
- Automated generation of *C. glutamicum* secretion strain library with different SPs from *B. subtilis*
- Establishment of high-throughput workflow for strain characterization using automated liquid handling
- splitGFP assay: fluorescence mediated by assembly of secreted GFP11-fusion protein and GFP1-10 detector to form GFP holoprotein



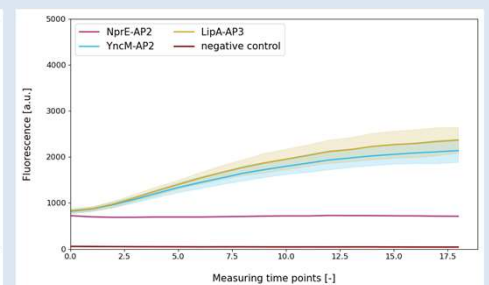
Results



Microbial growth curve for automated *C. glutamicum* cultivation using BioLector®. **A:** Main culture inoculation, **B:** Induction of protein expression



splitGFP assay results of one AP with the SP NprE: proof of feasibility for selected AP.



splitGFP assay results of two APs with different SPs: first results towards extensive candidate screening.

Conclusion

- Fully automated molecular biology workflows for strain library generation
- Successfully established splitGFP assay for automated high-

throughput screenings of AP secretion

- Proof of concept experiments with selected APs
- Next steps: include up to 24 SPs per AP in screening experiments supported by model-based decision making