

Maximising levels of secreted recombinant proteins in *P. pastoris* by optimization of microscale as well as bioreactor cultivation

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Pichia pastoris is being increasingly embraced by the biopharmaceutical industry as an expression system for therapeutic proteins, next to its usage as highly efficient producer of enzymes. For both applications, key-to-success is the rapid and reliable generation of high-level production strains matching all requirements with respect to protein quantity and quality attributes. The application of a versatile promoter library for fine-tuned expression of both, the target gene(s) as well as specific or general auxiliary gene(s) creates a diversity of expression strains that can only be analyzed in depth with a solid high throughput cultivation and screening system.

A highly reliable 96-well scale cultivation and screening platform was established for the analysis of up to ~25,000 individual *Pichia* strains which drive the expression of (multiple) recombinant genes with variants of the strong methanol-inducible AOX1-promoter. Thereby, the wide diversity of differently regulated expression patterns enables the quick identification of the best performing genetic constellation, i.e. the one strain secreting the target protein(s) with highest quality and/or quantity. Not only methanol-dependent, but also methanol-free high-level protein production is now possible by the creation of novel AOX1-promoter variants with unique expression characteristics in glycerol- or glucose-fed processes, fully devoid of methanol. The outstanding performance of strains generated by microscale screening is proven in high-cell density fermentations in controlled bioreactors. A large number of model proteins were subjected to this high-throughput screening platform, often yielding multi g/L levels of secreted recombinant protein, with peak concentrations of over 20 g/L for human serum albumin.

Biography

Roland Weis completed his Ph.D. in molecular biotechnology at Graz University of Technology, Austria in 2004. After a post doctorate at Graz University of Technology in a bilateral project with Pasadena, US-based BioCatalytics, Inc., he acted as authorized representative and scientific director of BioCatalytics Europe GmbH in Grambach, Austria. In 2007, he joined VTU Technology to head the operational team for protein production with *Pichia pastoris*

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