PROMOTER AND PROCESS ENGINEERING FOR RECOMBINANT PROTEIN PRODUCTION IN PICHIA PASTORIS TOWARDS SIMPLE, FAST AND METHANOL-FREE CULTIVATION REGIMES AND HIGH PRODUCT TITERS

Roland Prielhofer, Austrian Centre of Industrial Biotechnology, Vienna, Austria
roland.prielhofer@boku.ac.at
Michaela Reichinger, Lonza AG, Rottenstraße 6, Visp 3930, Switzerland
Nina Wagner, Lonza AG, Rottenstraße 6, Visp 3930, Switzerland
Katrien Claes, Lonza AG, Rottenstraße 6, Visp 3930, Switzerland
Christoph Kiziak, Lonza AG, Rottenstraße 6, Visp 3930, Switzerland
Brigitte Gasser, University of Natural Resources and Life Sciences Vienna, Austria
Diethard Mattanovich, University of Natural Resources and Life Sciences Vienna, Austria

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Protein production in *Pichia pastoris* often applies methanol-induced gene promoters such as P_{AOX1} to drive the expression of the target gene. The use of methanol has major drawbacks, so there is a demand for alternative promoters with good induction properties independent of methanol such as the P_{GTH1} promoter which we reported recently [1]. In order to further increase its potential, we investigated its regulation in more detail by screening of promoter variants harbouring deletions and mutations. Thereby we could identify the main regulatory region and important transcription factor binding sites of P_{GTH1}. We also created a P_{GTH1} variant, called $P_{G1\text{-}3}$, with greatly enhanced induction properties compared to the wild type promoter.

Model based process engineering could successfully be implemented for $P_{G1\text{-}3}$ to outperform the P_{AOX1}-driven production in a simple feed regime, and to establish a speed fermentation with high titers after only two days total fermentation time.

[1] Prielhofer, R.; Maurer, M.; Klein, J.; Wenger, J.; Kiziak, C.; Gasser, B.; Mattanovich, D., Induction without methanol: novel regulated promoters enable high-level expression in *Pichia pastoris*. Microb Cell Fact 2013, 12 (1), 5.