Pichia pastoris Protein Expression Excellence

METHANOL-FREE with VALIDOGEN´s 2nd generation AOX1 promoter variants

Key benefits

- Productivity records of 20 g/L of secreted protein in supernatant for methanol-free protein expression in Pichia
- Improved process safety by abolishing toxic and explosive methanol
- Robust processes with low cost of goods
- Decreased demand for cooling and aeration
- Glycerol or glucose as the sole carbon source
- Potential to significantly reduce process time

VALIDOGEN´s highly proven **1st generation methanol-inducible promoter variants** form the core of VALIDOGEN´s cutting-edge *Pichia pastoris* toolbox known as **UNLOCK PICHIA,** enabling fine-tuned high level protein expression of your protein with peak productivities of 35 g/L of secreted protein in the culture supernatant.

This library has been complemented with groundbreaking and unique **methanol-free 2nd generation PAOX1 promoter variants,** facilitating safe and economically viable protein production in glycerol- or glucose-fed processes without any need for induction with methanol.

Aside from improving safety by abolishing toxic and explosive methanol as a substrate, a major advantage of this technology includes decreased oxygen consumption which results in significantly reduced heat formation and cooling effort in bioreactor cultivations. Additionally, there is a high potential to reduce process time and cost of goods.

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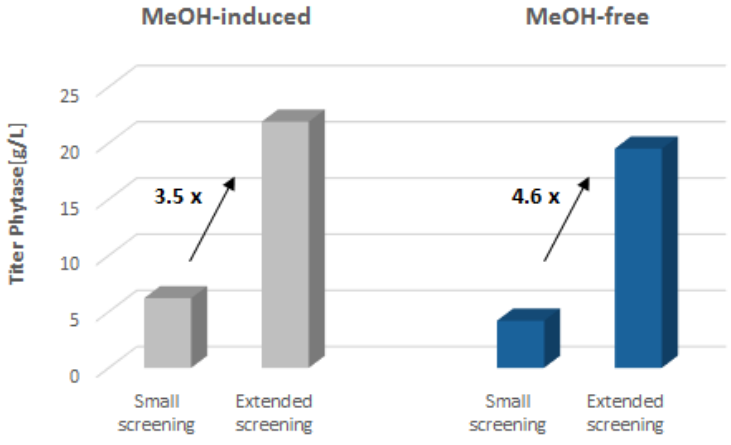
CASE STUDY - High level methanol-free phytase production in *Pichia pastoris*

Harnessing VALIDOGEN's *Pichia pastoris* protein production toolbox and screening know-how in a case study, the production of an engineered variant of *Butiauxella* sp. phytase yielded 22 g/L of enzyme in a methanol-induced process, and 20 g/L under methanol-free conditions, constituting the highest amounts of yeast-produced recombinant phytase reported so far.

This case study reveals that the appealing features of PAOX1 driven expression such as tight regulation of the production process while facilitating high protein yield are equally effective with VALIDOGEN's MeOH-free AOX1 promoter variants.

UNLOCK PICHIA by increasing genetic diversity

Compared to initial small screening, in extended screening a higher number of VALIDOGEN's promoter variants are applied by simultaneous transformation, thereby generating a broad diversity of genetic arrangements and expression profiles. In this setting protein production in methanol-induced and methanol-free screens was boosted significantly (3.5 and 4.6-fold respectively). Following VALIDOGEN´s reliable and uniform cultivation and screening protocol, thousands of clones can be screened in parallel allowing for the time-saving generation and identification of high-performance expression strains.



These results clearly underline the usefulness of VALIDOGEN's AOX1 promoter library and our entire expression toolbox, in addition to strain development and optimized cultivation protocols to unlock the capabilities of *Pichia pastoris* as a powerful host for powerful host for recombinant protein production. Using our approach, we demonstrate that increasing genetic diversity leads to a significant improvement of product yields (see figure).