Methanol free protein expression using methanol inducible promoters in *Pichia pastoris*

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Oral Presentation

During the last decade, the methylotrophic yeast, *Pichia pastoris*, has become a major eukaryotic host for recombinant protein production [1]. Expression of hundreds of different proteins has been reported so far. Several processes, employing *P. pastoris* expressed biocatalysts (enzymes), were implemented on industrial scale [2, 3]. One major reason for the success of this yeast is the inducible alcohol oxidase I (*AOX1*) promoter, which is tightly repressed in presence of glucose, glycerol and other carbon sources and strongly induced by methanol in absence of repressing carbon sources [1]. However methanol is toxic, flammable and its presence in growth media might also be unfavourable for some sensitive secreted products (e.g. biopharmaceuticals).

Based on sequence analyses, we modified the *AOX1* promoter to generate promoter variants with altered expression levels and regulatory properties. Finally we created a synthetic promoter library, in order to facilitate the identification of the perfectly matching promoter/target gene combination. Several reporter proteins like the diagnostic reporter HRP (Horseraddish peroxidase), the industrial enzyme CALB *(Candida antarctica* lipase B) and porcine Trypsinogen, which is used in processing of biopharmaceuticals, were expressed using the modified promoter variants. The properties of the generated libraries were studied and resulted in some promoters with more efficient expression in *Pichia pastoris* and some new promoter variants with high expression upon glucose depletion, providing the opportunity of methanol free protein expression using a methanol inducible promoter [4, 5, 6, 7].

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