Synthetic *Pichia pastoris* promoters

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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. Expression of hundreds of different proteins has been reported so far, including a large number of biocatalysts. Several processes were implemented on industrial scale. One major reason for the success of this yeast relies on the inducible alcohol oxidase I (*AOX1*) promoter. In addition to its exceptional expression strength it is also stongly repressed by glucose and only marginally activated by absence of glucose (derepression). Methanol is necessary to reach the full strength of the wild type *AOX1* promoter.

Based on sequence analyses, we modified the *AOX1* promoter. Deletion studies were performed. We identified both, a high number of positively and negatively acting promoter elements. This information is used to generate a set of promoter variants with altered expression levels and regulatory properties for protein expression. Finally we created a synthetic promoter library, in order to facilitate the identification of the perfectly- matching promoter/target gene combination.

This particular promoter library represents a toolbox for optimized expression of industrial and therapeutic enzymes as well as for metabolic engineering. In order to demonstrate some of its advantages compared to standard expression vectors we employed some of our new synthetic promoters for the expression of several industrial enzymes (HNL, HRP and CalB).

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