Identification of the alpha mating factor from the improved draft genome sequence of *Pichia pastoris* CBS7435 and investigating its ability for secretory heterologous protein expression

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INTRODUCTION

The methylotrophic yeast *Pichia pastoris* has become one of the most important microbial hosts for heterologous protein expression. It is able to use methanol as the sole carbon source and as a eukaryote *Pichia pastoris* has many advantages of higher eukaryotic systems like protein processing, protein folding and post-translational modifications. An important asset of the *P. pastoris* protein expression system besides the strong, methanol inducible promoter is the option to secrete recombinant proteins into the culture medium. The aim of this study was to show the ability of the recently identified *Pichia pastoris* alpha mating factor for secretory homologous protein expression by the use of *Bacillus subtilis* levanase (*sacC*) as a model system.

RESULTS

The draft genome sequence of *Pichia pastoris* CBS7435 strain was generated by the use of two different next generation sequencing techniques, namely the pyrosequencing (454 Life Sciences, Roche) and the sequencing-by-synthesis (Illumina) method. The alpha mating factor protein of *P. pastoris* was identified by BLAST search with tetra- and hexa-peptide fragments comprising the Kex2p and Ste13p processing sites (KREA/EA) that link the pro-sequence and the mature alpha factor peptide. After PCR amplification from *P.pastoris* CBS7435 genomic DNA and Sanger sequencing, it became obvious that the protein architecture is more complex than the one already known from *Saccharomyces* (Figure 1). Different expression constructs based on our own vector system were made using *B.subtilis* levanase to investigate the *P.pastoris* alpha factor secretion capacity (Figure 2). Levanase activity of the fermentation supernatants of the single copy strains was determined at different time points by use of the Glucose-UV Hexokinase assay (Figure 3). Aliquots of the 36h supernatants were loaded onto NuPAGE gels before and after EndoH treatment (Figure 4).

Figure 1: Comparison of alpha mating factor proteins of *Pichia pastoris* (A) and *Saccharomyces cerevisiae* (B). Mature alpha factor peptide (violet), Glu(Asp)-Ala repeats (turquoise) and pre- (light grey) as well as pro- (grey) parts of secretion signaling sequences are indicated. Pre- and pro-sequences governing ER membrane translocation and ER-Golgi transport efficiency, respectively, were predicted using Phobius, PrediSI and SIG-Pred: Signal Peptide Prediction with identical results.

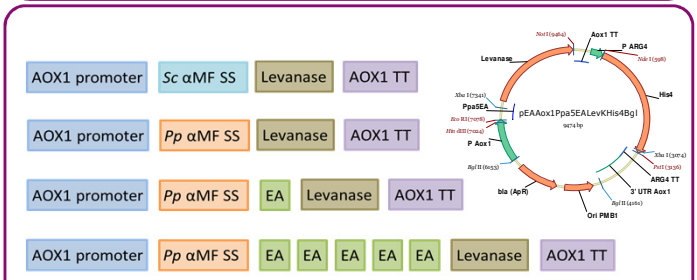

Figure 2: Different levanase constructs for secretion.

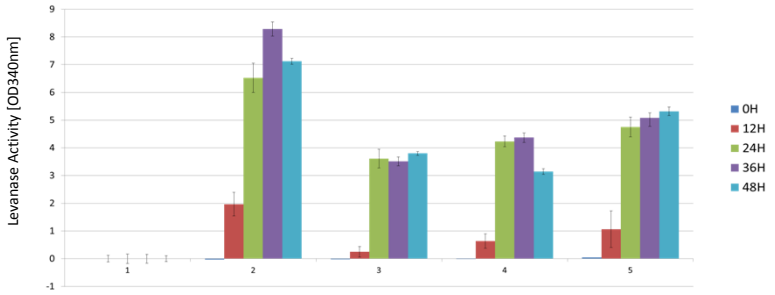

Figure 3: Comparison of secreted levanase activity levels.
1 Intracellular levanase expression, 2 *S. cerevisiae* αMF SS without EA repeats, 3 *P. pastoris* αMF SS without EA repeats, 4 *P. pastoris* αMF SS with 1 EA repeat, 5 *P. pastoris* αMF SS with 5 EA repeats. Fermentations were done with single clones („hits" of prescreens) in 12 replicates in 96 deepwell plates. Measurements were done according to the Glucose-UV Hexokinase Assay manual (Dipromed). Data were normalized to OD_{600} values.

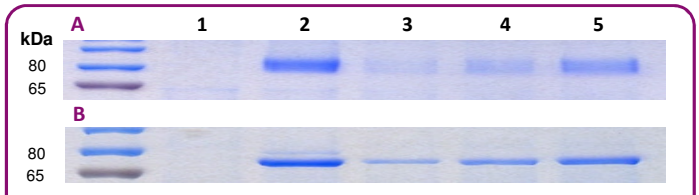

Figure 4: Coomassie stained NuPAGE 4-12% Bis-Tris SDS gels of secreted levanase without (A) and with Endo H treatment (B) 1 Intracellular levanase expression, 2 *S. cerevisiae* αMF SS without EA repeats, 3 *P. pastoris* αMF SS without EA repeats, 4 *P. pastoris* αMF SS with 1 EA repeat, 5 *P. pastoris* αMF SS with 5 EA repeats. Fermentations were done with single clones in 96 deepwell plates. The calculated MW of levanase is 75,8 kDa.

CONCLUSION

In this study it was clearly shown that the *Pichia pastoris* alpha mating factor has a more complex structure than its *Saccharomyces* counterpart including more EA repeats indicating the Ste13p processing sites. The *Pichia pastoris* alpha mating factor is able to drive secretion for heterologous protein expression although to a weaker extent than the *Saccharomyces* one, at least in case of levanase.

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