Graz University of Technology

Identification and isolation of GPI anchored proteins from *Pichia pastoris* and their potential for surface display of recombinant proteins

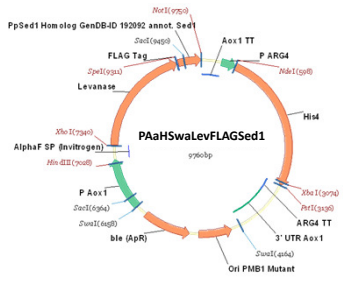
Rosales Rodriguez I. [a], Ahmad M. [a], Nudl T. [b], Pichler H. [a, c], Schwab H. [a, c]

[a] Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, 8010 Graz, Austria
[b] Baxter AG, 1221 Vienna, Austria
[c] Austrian Centre of Industrial Biotechnology (ACIB), Petersgasse 14, 8010 Graz, Austria

INTRODUCTION

Cell surface display of proteins is of big advantage for a variety of biotechnological applications, for instance directed evolution, screening of antibody libraries or use as a whole cell biocatalyst. Recently, the methylotrophic yeast *Pichia pastoris* has gained importance as host for immobilized heterologous enzyme expression on the surface of the cell. So far, mostly well established *Saccharomyces cerevisiae* surface anchors have been adapted for the use in *Pichia*. The aim of the study was to investigate new anchors originated from *Pichia pastoris* and their suitability for surface display by means of *Bacillus subtilis* levanase reporter system.

To identify putative new anchor proteins for surface display BLAST searches of the recently published *P.pastoris* CBS7435 *wt* strain (GenDB) were performed based on patented sequences of GPI anchor proteins used for surface display in *Pichia angusta*. Eight possible candidates (Fig.1) were chosen for cloning into pAaHSwa vector, all of them comprising predicted C-terminal GPI anchors. Surface expression of *B. subtilis* levanase *(sacC)* reporter protein was driven by AOX1 promoter and *S. cerevisiae* alpha mating factor secretion signal. A FLAG-tag was placed between the protein-anchor fusion for immunofluorescence detection. Expression constructs were sequenced for confirmation and transformed into *P. pastoris* CBS7435ΔHis strain. Histidin prototroph transformants were finally screened for MutS and Mut+ phenotype and solely MutS clones were chosen for deepwell fermentation. Figure 2 shows levanase activity of the hits of several prescreens tested by glucose-UV hexokinase assay (Dipromed). Supernatant and washed pellet fractions were assayed in quadruplicates over several timepoints. Immunofluorescence microscopy was performed to confirm the localization of the levanase protein on the *Pichia* surface (Fig. 3).



Anchor	GenDB annotation	GenDB ID
A	gas 1	148978
B	gas 1	148979
C	cwp 1	153002
D	wsc 2	191035
E	sed 1	192092
F	cwp 1	199427
G	gas 2	199999
I	tir 3	208766

Figure 1: Different levanase-FLAG-anchor constructs for surface display.

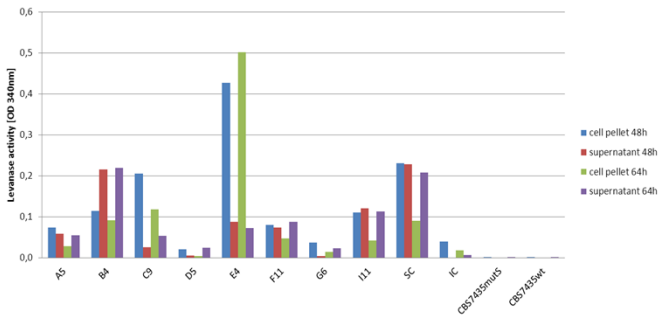


Figure 2: Comparison of levanase activity levels.
A5, B4, C9, D5, E4, F11, G8, I11: different clones named according to the anchor (see Fig. 1). SC: secretory control; IC: intracellular control. Fermentations were done with single clones („hits" of prescreens) in quadruplicates in 96 deepwell plates. Pellets were washed twice with ddH_2O. Measurements were done according to the Glucose-UV Hexokinase Assay manual (Dipromed). Data were normalized to OD_{600} values.

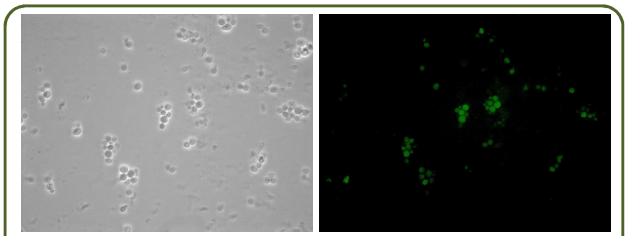


Figure 3: Immunofluorescence of clone E4 (Sed1 anchor) for confirmation of surface display. 1st ab: Sigma Anti-FLAG #F1804 1:500; 2nd ab: Thermo Scientific Dylight 488 #35503 1:500; Exposure Time 540ms; Enhancement 7,2x; Pseudocolors 529nm

CONCLUSION

The results of this study clearly show the identification and isolation of at least one efficient new *P. pastoris* anchor, namely Sed1 (anchor E). Excellent levanase activity was detectable in the washed pellet fraction whereas almost no measurable activity was found in the supernatant. Results were confirmed by immunofluorescence microscopy. Nevertheless, it is important to explore the utility of this anchor with other proteins as surface display depends on interplay of secretion signal, protein of interest and anchor.

REFERENCES

Küberl A, Schneider J, Thallinger GG, Anderl I, Wibberg D, Hajek T, Jaenicke S, Brinkrolf K, Goesmann A, Szczepanowski R, Pühler A, Schwab H, Glieder A, Pichler H:High-quality genome sequence of Pichia pastoris CBS7435. J Biotechnol. Epub 2011 May 6

Choi E-S, Sohn J-H, Kim S-Y: Novel cell wall anchor proteins derived from yeast, genes thereof and cell surface expression systems using the same. International Patent application number: PCT/KR00/00819; 2002 February 14

Schörgendorfer K, Schwab H, Lafferty RM: Molecular characterization of *Bacillus subtilis* levanase and a C-terminal deleted derivative. J.Biotechnol., **7**, 247-258, 1988