

Poster Presentation

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Disruption of the *GAS1* gene of *Pichia pastoris* confers a supersecretory phenotype for *Rhizopus oryzae* lipase, but not for human trypsinogen

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Background

The methylotrophic yeast *Pichia pastoris* is widely used for the heterologous production of proteins due to an advantageous secretory potential [1]. To further improve the capacity of this yeast to synthesise and secrete high levels of protein the limitations of the host have to be unravelled and overcome. Folding and disulfide bridge formation have been studied in detail and some approaches have been described to modulate these processes. However, after release from the secretory organelles like endoplasmic reticulum and the golgi apparatus a protein still has to penetrate and cross the cell wall. This can be a major obstacle for heterologous protein secretion, but these events are less well studied up to now.

While for many heterologous proteins the cell wall is apparently not a limiting barrier, there are some indications of significant protein retention in the cell wall of *S.cerevisiae* [2,3]. In one case it was shown that deletion of the cell wall cross linking enzyme glycosylphospholipid-anchored surface protein 1 (Gas1) leads to an almost 7 fold increase of the secreted level of human insulin like growth factor 1 in *S. cerevisiae* [4].

Gas1p is a glycoprotein anchored to the outer layer of the plasma membrane through a glycosylphosphatidylinositol (GPI) anchor. Gas1p shows beta-1,3-glucanosyltrans-

ferase activity, required for the cell wall assembly. Disruption of *GAS1* results in several morphological defects: yeast cells lose their typical ellipsoidal shape, become larger than wild type cells, are defective in bud maturation and in cell separation. The cell wall of *Gas1* null mutants is highly resistant to zymolyase, more sensitive to cell wall perturbing agents such as congo red, and the cells are less protected against osmotic destabilizing agents as sodium dodecyl sulphate.

In this work we set out to clone and delete the *GAS1* homologue of *P. pastoris* and to analyse the secretory capacity of the yeast cells on the basis of two proteins studied in our labs for heterologous protein production, namely human trypsinogen and *Rhizopus oryzae* lipase (ROL).

Results

The coding sequences of the *GAS1* homologues of nine yeast species were aligned to identify regions of high homology. Within these regions PCR primers were designed to amplify a partial sequence of the *P. pastoris* *GAS1* gene. Subsequently, it was possible to amplify the full length gene from a cDNA library. Expression of the cloned gene in *S. cerevisiae* fully complements the phenotype of a *GAS1* deletion, indicating that the protein has the expected localization and enzymatic activity. Sequence

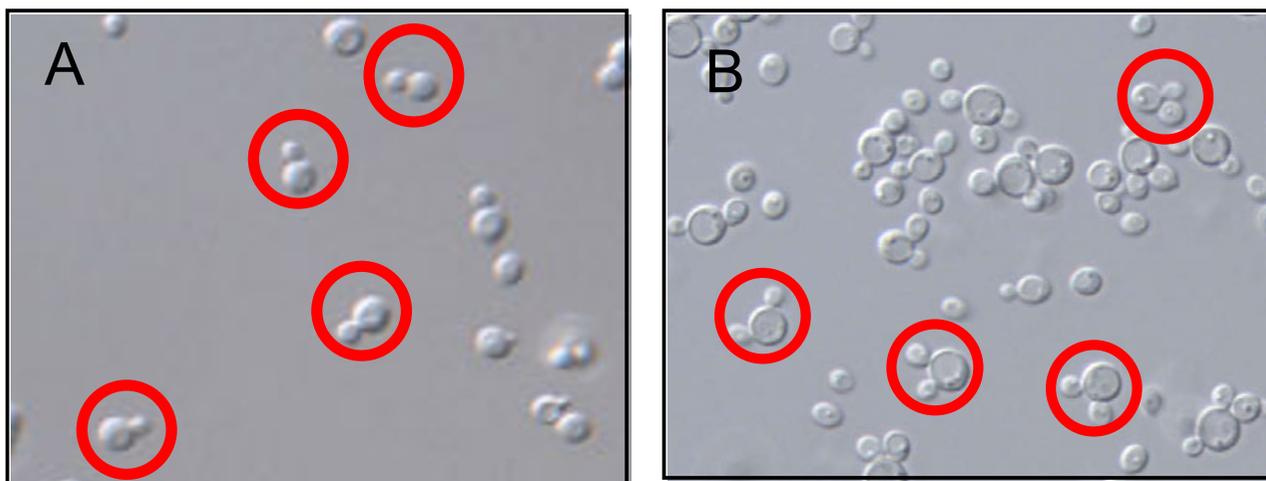


Figure 1

A. *P. pastoris* GS115 wt cells showing the normal budding phenotype. **B.** *P. pastoris* GS115 GAS1::kanMX4. The GAS1 deleted *P. pastoris* strain shows frequently two buds attached to the mother cell (Mickey Mouse-like appearance) as reported for GAS1 deficient *S. cerevisiae*. The typical appearance is indicated by red circles.

comparison revealed the anticipated similarities with other Gas1 like proteins, such as a cysteine rich domain and a serine rich domain and a number of conserved amino acid patches within the amino terminal catalytic domain.

Targeted gene disruption of the GAS1 gene in *P. pastoris* was successful and led to the particular phenotype, e.g. a budding defect resulting in the accumulation of Mickey Mouse like shaped yeast aggregates (see figure 1) and sensitivity against congo red and SDS.

Finally, we tested the secretion of two model proteins (human trypsinogen and ROL) in the GAS1 deleted background in comparison to the wt *P. pastoris* strain. Secretion of human trypsinogen was not altered, while the specific secretion rate of ROL was increased approximately 2 fold.

Conclusion

As has been previously shown, different heterologous proteins encounter different bottlenecks upon expression in yeast. In any case we could show that the cell wall should not be neglected for improvement of heterologous protein secretion. We could demonstrate for the first time that the deletion of a cell wall cross linking enzyme can have a beneficial effect on secretion of a heterologous protein in *P. pastoris*.

Further understanding of cell wall construction and the means by which secreted proteins can cross the cell wall are therefore highly desirable

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