

Improvement of recombinant protein producing yeast hosts by rational design and random screening

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The methylotrophic yeast *Pichia pastoris* is known as a highly efficient expression system, but there is only little knowledge about the physiology and the genetics lying underneath, and no genome sequence has been published at the time of writing this abstract. During the recent years, it has become evident that a variety of intrinsic, metabolic and environmental stresses may have a strong impact on recombinant protein production.

We have focused on the analysis of physiological reactions of *P. pastoris* to different stress situations in lab-scale production processes including environmental factors such as extracellular pH, intrinsic factors like product processing and unfolded protein response, and heterologous gene copy number. Apart from classical biochemical methods we employed flow cytometry (immunofluorescence, intracellular pH determination, viability) and heterologous DNA microarrays to study host cell physiology.

Data characterizing physiological constraints for overexpression can be employed to design strategies for the targeted opening of bottlenecks to enhance protein production. As an example, folding limitation will be discussed in more detail. Alternatively, screening of large libraries with FACS has been proven to be a powerful tool for the isolation of rare, yet unidentified traits enhancing expression. Examples demonstrating both strategies will be presented.