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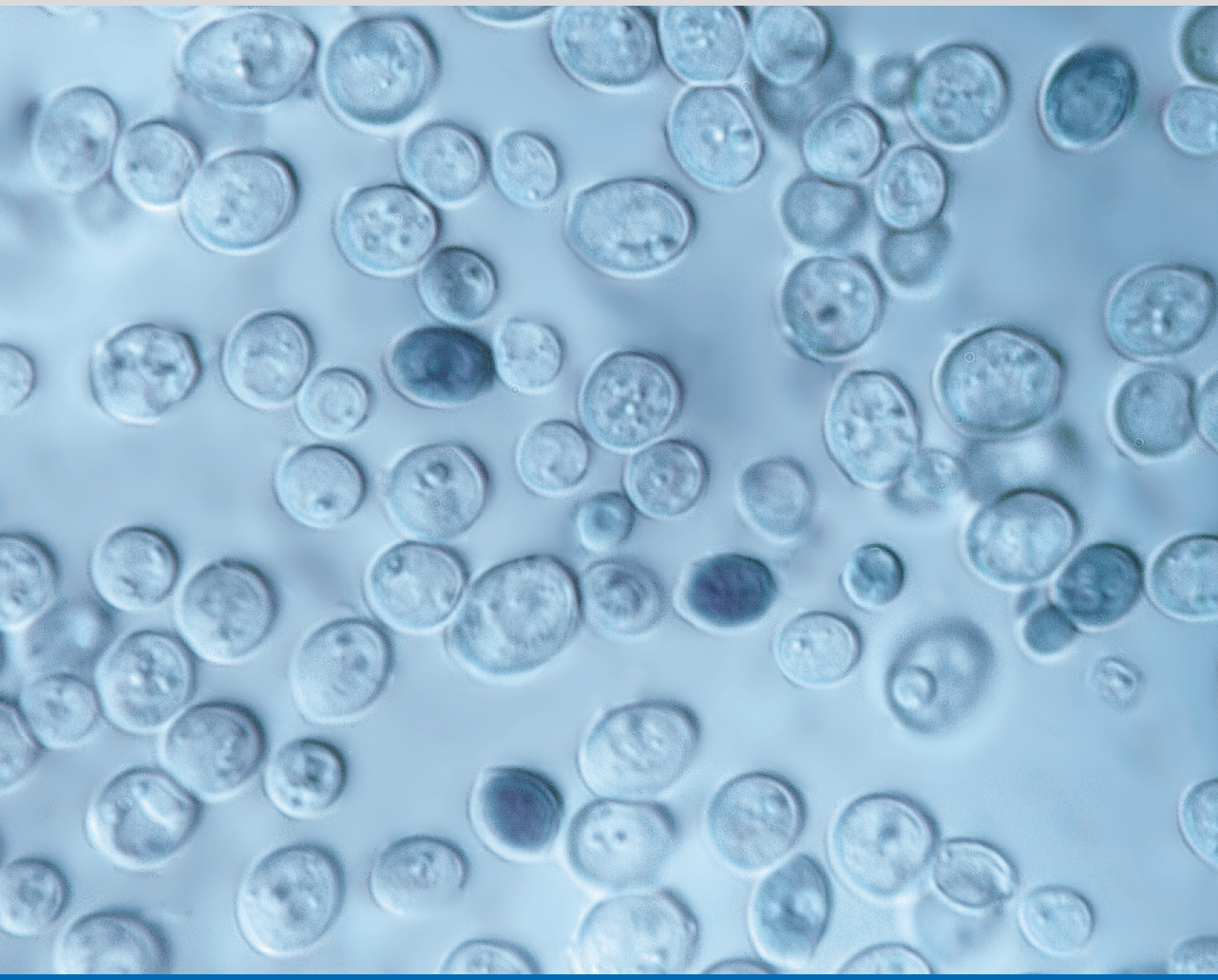
Yeasts as Production Hosts for Biocatalysts

Kamila Morka



Bioorganische Chemie an der Heinrich-Heine-Universität
im Forschungszentrum Jülich

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Herausgegeben von Jörg Pietruszka

Enzymes, as natural catalysts, are becoming more widely applied in organic chemistry due to their selectivity and availability. High yield expression of a desired enzyme in a heterologous host is a prerequisite for its successful application in biocatalysis. Yeasts are an especially attractive expression platform, as they combine many advantages of producing proteins in microbes with the eukaryotic ability to modify and secrete the produced protein.

In this thesis, two main topics were addressed. Firstly, possible influence of host-specific changes on the properties of the produced protein was investigated. For this purpose, three industrially relevant yeast strains: *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Hansenula polymorpha* were selected. Comparative expression of the model enzyme in the yeasts revealed significant difference in the biochemical properties of the produced protein. The results exemplify how the selection of the host even within one taxonomic family (*Saccharomycetaceae*) affects the produced enzyme's characteristics.

The second part of this thesis focused on the application of the yeast expression systems for expression and modification of rhamnosidases as a prerequisite for their potential application in the synthesis of glycoside containing natural products. Three published rhamnosidases – Rha_{Ba} from *Bacillus* sp, Rha_{La} from *Lactobacillus acidophilus* and Rha_{Hp} from *H. polymorpha* – were targeted during this study and four major aspects: gene identification, expression, creation of mutants with altered activity and crystallisation were investigated. The protocols for protein expression, purification and crystallisation established in this study, as well as created rhamnosidase variants are important step towards future application of this enzyme class in biocatalysis.

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