

TENTH INTERNATIONAL SYMPOSIUM ON YEASTS

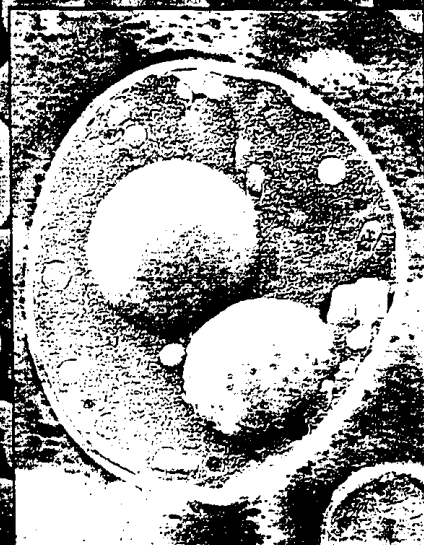
27 August - 1 September 2000

Papenhof, Arnhem, The Netherlands

BOOKS



THE RISING POWER OF YEASTS  
IN SCIENCE AND INDUSTRY



SYMPOSIUM BOOK

# **ISY 2000**

**Tenth International Symposium on Yeasts**

## **The rising power of yeasts in science and industry**

**27 August - 1 September 2000  
Papendal, Arnhem, The Netherlands**

### **Symposium Book**

*Under the auspices and guarantee of The Netherlands Foundation for Biotechnology and  
Delft University of Technology.*

**Abstracts of the Tenth International Symposium on Yeasts (ISY 2000)**  
The rising power of yeasts in science and industry  
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Papendal, Arnhem, The Netherlands

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**Lay-out:** Jenny Boks-Zondervan, Maria H.P. Komen-Zimmerman  
**Cover:** Coach Consult, Zuidhorn  
Cover photographs are kindly provided by Marten Veenhuis

**Published and distributed by:**

Delft University Press  
P.O. Box 98  
2600 MG Delft  
The Netherlands  
Telephone: +31 15 2785121  
Telefax: +31 15 2781661  
E-mail: [dup@library.tudelft.nl](mailto:dup@library.tudelft.nl)

ISBN 90-407-2067-3

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Kluyver Laboratory for Biotechnology  
Delft, The Netherlands

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## Rising power of yeasts

### SECRETION OF *ASPERGILLUS NIGER* $\beta$ -GALACTOSIDASE BY FLOCCULENT YEAST CELLS

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The culture of flocculent yeast cells allows for the continuous operation at a high cell density. With an appropriate bioreactor design the cell aggregates are retained inside the bioreactor allowing for the operation at higher dilution rates leading to higher productivity than the one obtained in conventional continuous systems (chemostat). Moreover, the downstream processing is greatly facilitated as the aggregates are easily separated from the product. A flocculent continuous system has been successfully employed for alcoholic fermentation (Domingues et al., 1999).

The secretion of heterologous protein by *S. cerevisiae* is attractive by several reasons (e.g. GRAS organism, eukaryote organism, cultures are easy to handle and scale-up, few proteins are naturally secreted by yeast) but is hampered by its low secretion rate. Due to its industrial importance,  $\beta$ -galactosidase production has been largely studied. The overall productivity of bioreactors for  $\beta$ -galactosidase secretion can be enhanced using continuous high cell density systems operating with flocculent yeast cells. As in other high cell density systems, diffusional mass transfer limitations may be a main concern. However, in a previous study, it has been shown that this is not significant for brewer's yeast flocculating cells secreting *A. niger*  $\beta$ -galactosidase (Domingues et al., 2000).

In this work, the secretion of *A. niger*  $\beta$ -galactosidase by a flocculent yeast cell has been studied. For that, the flocculent *S. cerevisiae* strain NCYC869-A3 has been transformed with the plasmid pVK1.1 (Kumar et al., 1992). Higher levels of  $\beta$ -galactosidase were detected in the supernatant and overall enzyme productivity was clearly better than the productivity obtained in the previous work (Domingues et al., 2000). Biochemical characterization of  $\beta$ -galactosidase secreted to the supernatant was done. The recombinant  $\beta$ -galactosidase is seen as a diffuse high molecular weight band (>250 KDa) in SDS-Page gels. The identity of the referred band was confirmed by an enzymatic gel assay. Treatment with EndoH resulted in a sharp band around 116 KDa, clearly indicating heterogeneous glycosylation in some of the N-linked sites in the cloned fungal  $\beta$ -galactosidase. This results agree with the ones previously obtained with non-flocculent *S. cerevisiae* cells (Kumar et al., 1992). Batch fermentations aiming at the  $\beta$ -galactosidase production optimization are also presented. The effect of lactose concentration, aeration rate and yeast extract concentration on the recombinant  $\beta$ -galactosidase production were considered.

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The authors acknowledge the financial support provided by the IBQF, Portugal. Lucilia Domingues was supported by a grant (PRAXIS XXI/BD/11306/97) from FCT, Portugal.