Biotechnology

A Laboratory Course

Second Edition

Jeffrey M. Becker
Department of Microbiology
Department of Biochemistry, Cellular, and Molecular Biology
The University of Tennessee
Knoxville, Tennessee

Guy A. Caldwell
Department of Biological Sciences
Columbia University
New York, New York

Eve Ann Zachgo
Department of Molecular Biology
Massachusetts General Hospital
Boston, Massachusetts

Academic Press
San Diego New York Boston London Sydney Tokyo Toronto

PARSE EX.1014.001

Front cover photograph: Bacteria and yeast, the work horses of biotechnology and molecular biology, are depicted as a bacterial colony and one budding yeast cell containing plasmid DNA and the DNA double helix. This illustration represents the manipulation of living organisms to produce material useful for humans – a definition of biotechnology in the broadest sense. The recent revolution in this field of endeavor has been made possible by the explosion of knowledge in molecular biology and by the rapid proliferation of techniques using microorganisms and recombinant DNA.

This book is printed on acid-free paper. ♾

Copyright © 1996, 1990 by ACADEMIC PRESS

All Rights Reserved.
No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without permission in writing from the publisher.

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone: (+44) 1865 843830, fax: (+44) 1865 853333, e-mail: permissions@elsevier.com.uk. You may also complete your request on-line via the Elsevier Science homepage (http://elsevier.com), by selecting "Customer Support" and then "Obtaining Permissions."

Academic Press
An imprint of Elsevier Science
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Academic Press
84 Theobald's Road, London WC1X 8RR, UK
http://www.academicpress.com

Library of Congress Catalog Card Number: 95-41427

International Standard Book Number: 0-12-084562-8

PRINTED IN THE UNITED STATES OF AMERICA
03 04 05 06 07 9 8 7 6

PARSE EX.1014.002

Exercise

15

Determination of β-Galactosidase in Permeabilized Yeast Cells

Introduction

Cell permeabilization techniques are often useful for many applications relating to enzyme technology. For example, permeabilization procedures are usually rapid and do not destroy cellular enzymes. Thus, the total amount of an enzyme associated with a cell can be assayed after permeabilization. A number of permeabilization methods for yeast have been developed, such as use of detergents, organic solvents, and desiccation.

In this exercise, *Saccharomyces cerevisiae* cells will be treated with a combination of an organic solvent (toluene) and a detergent (sarkosyl or sodium lauroyl sulfate), which effectively dissolves the permeability barriers (membrane lipids), allowing free access of added substrates to intracellular proteins. Because the cell wall barrier is not broken down by this treatment, most proteins remain entrapped and associated with the cell.

Reagents/Supplies

N-Lauroylsarcosine (5%, w/v)
Microcentrifuge tubes

PARSE EX.1014.003

Micropipettor
o-Nitrophenyl-β-D-galactopyranoside (ONPG), 4 mg/ml of distilled H_2O
Overnight cultures of *Saccharomyces cerevisiae* YNN281 (pRY121) and *Saccharomyces cerevisiae* YNN281
SF solution: 0.85% (w/v) NaCl, 3.7% (v/v) formaldehyde
Sodium carbonate (Na_2CO_3): 1 *M*
Toluene
YNB without amino acids plus supplements, as described below (Step 1), plus galactose (see Exercise 2 for preparation of YNB)
Z buffer (see Exercise 14)

Equipment

Incubator set at 37°C
Incubator set at 28°C
Microcentrifuge
Spectrophotometer
Vortex

Procedure

1. Grow *S. cerevisiae* YNN281 (pRY121) and *S. cerevisiae* YNN281 at 30°C in 50 ml of YNB, plus tryptophan (30 μg/ml), lysine (30 μg/ml), adenine sulfate (30 μg/ml), histidine (30 μg/ml), galactose (3%, w/v), and raffinose (2%, w/v) to mid-log (50 Klett units or OD_{600} of 0.7, which is about 1.7×10^7 cells/ml). Be sure to add uracil (30 μg/ml) to the YNN281 culture, but omit this supplement from the YNN281 (pRY121) culture.
2. Harvest (microcentrifuge for 1 minute at top speed at room temperature) 3.0 ml of each culture in microcentrifuge tubes, discard the supernatant, combine like pellets, and resuspend the pellets in 1 ml of Z buffer. Wash the pellets by centrifugation again at top speed at room temperature and resuspend in 300 μl of Z buffer.

PARSE EX.1014.004

3. Remove 100 μl of resuspended cells and add to 900 μl of SF solution in cuvette. Read the OD_{600} with a spectrophotometer.
4. Place 150 μl of the remaining cell suspension in a fresh microcentrifuge tube. Add 1.5 μl of toluene and 1.5 μl of *N*-lauroylsarcosine (5%, w/v). Vortex at top speed several times.
5. Evaporate the toluene from the solution for 30 minutes at 37°C on a shaker with caps off the tubes.
6. Add 20 μl of toluenized cells to 1.5 ml of a prewarmed (28°C) reaction mixture of 1 ml of ONPG (4 mg/ml) and 0.5 ml of Z buffer. More cells can be added if equivalent amounts of Z buffer are left out. Vortex and start timing. When the color is yellow (or after 120 minutes), add 0.5 ml of Na_2CO_3 (1 *M*) to stop the reaction.
7. Read the optical density at 420 and 550 nm. The OD_{550} corrects for light scattering by the cell suspension. Alternatively, the reaction mixture can be spun to pellet the cells and the OD_{420} of supernatant read.
8. The units of activity can be calculated:

$$\text{Units} = \frac{1000 \times (OD_{420} - 1.75\ OD_{550})}{t \times v \times OD_{600}}$$

where t is the assay time (minutes), v is the volume of cells used in the assay (milliliters), OD_{600} is the cell density at the start of the assay, OD_{420} is the combination of absorbance by *o*-nitrophenol and light scattering by cells, and OD_{550} is the light scattering by cells.

References

Cordeiro, C. A. A., and Freire, A. P. (1994). Protein determination in permeabilized yeast cells using the Coomassie Brilliant Blue dye binding assay. *Anal. Biochem.* **223**, 321–323. [The paper presents a

PARSE EX.1014.005

method to assay proteins in permeabilized cells in order to compare enzyme activities obtained with permeabilization techniques to those with homogenates or purified enzymes.]

Gowda, L. R., Joshi, M. S., and Bhat, S. G. (1988). *In situ* assay of intracellular enzymes of yeast *(Kluyveromyces fragilis)* by digitonin permeabilization of cell membrane. *Anal. Biochem.* **175**, 531–536. [This paper presents an alternative method for yeast permeabilization, using the detergent digitonin.]

Reynolds, A., and Lundblad, V. (1989). Yeast vectors and assays for expression of cloned genes. *In* "Current Protocols in Molecular Biology" (Ausubel, F. M., *et al.*, eds.). John Wiley & Sons, New York, pp 13.6.2–13.6.4. [A description of yeast cell permeabilization by organic solvent/detergent mixture is presented.]

Questions

1. What are the advantages and disadvantages of enzyme assays on permeabilized cells versus cell extracts?
2. Why is galactose the major carbon source in the growth medium for this experiment?

PARSE EX.1014.006